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Article

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Design, Synthesis, and Evaluation of the Highly Selective and Potent G-protein-Coupled Receptor Kinase 2 (GRK2) Inhibitor for the Potential Treatment of Heart Failure

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Abstract

A novel class of therapeutic drug candidates for heart failure, highly potent and selective GRK2 inhibitors, exhibit potentiation of β-adrenergic signaling *in vitro* studies. Hydrazone derivative **5** and

1,2,4-triazole derivative **24a** were identified as hit compounds by HTS. New scaffold generation and SAR studies of all parts resulted in a 4-methyl-1,2,4-triazole derivative with an *N*-benzylcarboxamide moiety with highly potent activity towards GRK2, and selectivity over other kinases. In terms of subtype selectivity, these compounds showed enough selectivity against GRK1, 5, 6, 7 with almost equipotent inhibition to GRK3. Our medicinal chemistry efforts led to the discovery of **115h** (GRK2 IC_{50} = 18 nM) which was obtained the co-crystal structure with human GRK2 and an inhibitor of GRK2 that potentiates β-adrenergic receptor (βAR)-mediated cAMP accumulation and prevents internalization of βARs in β2AR-expressing HEK293 cells treated with isoproterenol. Therefore, **115h** appears to be a novel class of therapeutic for heart failure treatment.

Introduction

Heart failure (HF) is the most common disease for hospitalization in the elderly, with approximately 10 % of men and 8 % of women over the age of 60 affected.[1] The prevalence of HF is growing with the rise of an aging population in developed countries. There remains an intense need for novel beneficial HF therapies with more than 3 million people in the United States diagnosed per year, and HF related mortality and rehospitalization rates remaining high despite the modest improvement in survival rates[2] seen from advances in device therapy[3] and pharmacological therapy (angiotensin II receptor blockers,[4] angiotensin converting enzyme inhibitors,[5] and β-blockers[6]). A

plethora of research into HF has revealed it to be a complex disease associated with various pathogenetic mechanisms, including ventricular remodeling, excessive neurohormonal stimulation, abnormal Ca^{2+} handling, and proliferation of the extracellular matrix.[7,8] Although an overstimulation of the sympathetic nervous system (SNS) initially compensates for cardiac dysfunction, the subsequent release of catecholamine ultimately promotes disease progression via long-term exposure.[9] Activation of the SNS is mediated by adrenergic receptors (AR), and chronic β-AR activation induces β-AR desensitization and downregulation, subsequently leading to the reduction of β-AR signaling.[10] G-protein receptor kinase (GRK) 2 phosphorylates agonist-occupied β-AR, promotes the binding of β-AR arrestin to the Gβγ subunit of the G-protein, facilitates the G-protein uncoupling from β-AR, and results in β-AR desensitization and downregulation.[11] In the hearts of HF patients, GRK2 expression levels and activity were elevated, accompanied by lowered β-AR density and signaling.[12-14] Moreover, GRK2 inhibition by overexpression of the βARKct, the peptide inhibitor of GRK2, or cardiac specific GRK2 gene ablation, improved cardiac function and survival with the increases in β-AR density and β-AR responses in several HF models.[15-17] These results suggest that GRK2 has a strong relationship with HF, and inhibition of GRK2 is a promising mechanism for the treatment of HF. Although there are no small molecular GRK2 inhibitors currently in clinical trials, there are several small molecules[18-26] reported in preclinical studies. Furthermore, several papers to discover the other chemotypes of highly selective GRK2 inhibitors were reported by use of the information of **115h** and **139d**.[18-20] Moreover, **115h** and the related

compounds were introduced by reveiws.[27,28]

Here we describe the design, synthesis, structure-activity relationships (SAR), and discovery of (4-pyridyl)-1,2,4-triazole derivatives and the novel, selective, GRK2 inhibitor **115h**. Furthermore, we describe the acquisition and utilization of crystal structures of human GRK2 with the inhibitors as well as relevant biological studies.

Design and Synthetic Plans

A high throughput screen testing inhibition of GRK2 mediated phosphorylation of Ulight TopoIIα as an artificial substrate yielded a 4-pyridylhydrazone derivative (hit compound **5**) and a 4-pyridyl-1,2,4-triazole derivative (hit compound **24a**) as hit compounds. These compounds act by competitive inhibition of ATP, binding to the kinase active site. Chemical modifications of compounds **5** (IC_{50} = 1800 nM) and **24a** (IC_{50} = 1200 nM) were carried out to develop novel GRK2 inhibitors with potent activity, as well as good selectivity against Rho-associated protein kinase 2 (ROCK2) and Protein kinase C alpha (PKCα), with minimal effects on blood pressure *in vivo*. First, a series of compounds related to hit compounds **5** (acyclic hydrazone derivative) is described as follows (Figure 1). i) Introduction of substituent(s) on the benzene ring at the right hand side (RHS), ii) Cyclization to produce patentable ring systems, iii) Optimization of the substituent at the 3-position of the benzene ring at the RHS. Other strategies from acyclic hydrazone derivatives to

discover isoquinoline derivatives (for examples, *N*-isoquinolin-6-yl-*N*-2-(3-methoxyphenyl)glycinamide, IC_{50} (nM): GRK2 / ROCK2 = 12 / 1.3) and 3-(4-pyridyl)pyrazoline derivatives (for examples, *N*-(2,3-dihydro-1*H*-inden-1-yl)-3-((2-oxo-2-(3-(pyridin-4-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethyl)amino)benzamide, IC_{50} (nM): GRK2 / ROCK2 = 4.9 / 250) are detailed as well.

[Figure 1]

Second, a series of compounds related to hit compound **24a** (1,2,4-triazole derivative) is described as follows (Figure 2). i) Modification at the linker moiety, ii) Modification at the benzene ring at the RHS, iii) Transformation into the compounds bearing a 5-membered heterocyclic ring other than 1*H*-1,2,4-triazole ring, and iv) Modification of the pyridine ring. These two plans were designed, and the compounds were synthesized, to investigate their biological activities.

[Figure 2]

Chemistry

Acyclic Hydrazone Derivatives

Acyclic hydrazone derivatives **5a–f** were prepared as described in Scheme 1. Reaction of anilines

1a–f with TFAA activated the reactivity of the nitrogen atom and protected, then the alkylation with ethyl bromoacetate gave **3a–f** in near quantitative yield. Formation of acid hydrazides **4a–f** with hydrazine hydrate was achieved with simultaneous removal of trifluoroacetyl. The 4-Pyridyl part, as a hinge, was introduced by use of 4-pyridinecarboxaldehyde *via* dehydration reaction resulting in the final compounds **5a–f**.

[Scheme 1]

Cyclic Hydrazone Derivatives

Cyclic hydrazone derivatives **12a–e** were prepared as described in Scheme 2. Acid hydrazides **11a–e** were prepared in a similar way from anilines **6d–e** shown in scheme 1. Cyclic hydrazones **12a–e** were produced by use of 7,8-dihydroisoquinolin-5(6*H*)-one, instead of 4-pyridinecarboaldehyde, in good yields.

[Scheme 2]

Incorporation of the amide at the 3-position of the benzene ring at the RHS was performed as shown in Scheme 3. When alkylation of 3-trifluoroacetoamide-benzoate **14** was finished, the crude product was treated with TFAA again because a part of the trifluoroacetyl group was removed during

the alkylation reaction. Hydrolysis of the corresponding ester was differentiated *via* hydrogenation by use of palladium – on activated carbon and gave **16**. Condensation with Boc-hydrazine followed by hydrolysis of the ester group gave the key intermediate benzoic acid **18** having *N*-Boc acid hydrazide. Finally, cyclic hydrazone derivatives **12f** and **12 g** with amide were produced by WSC and HOBt amidation, removal of trifluoroacetyl, and dehydration with 7,8-dihydroisoquinolin-5(6*H*)-one.

[Scheme 3]

Modification of the Linker Moiety of 1,2,4-triazole derivatives

Synthesis of **24c**, **24g** and **24h**

The typical methods of preparation of 1*H*-1,2,4-triazole derivatives are shown in Scheme 4. Reaction of 4-cyanopyridine **21** with hydrazine and the following acylation of **22** with chloroacetyl chloride in the presence of DBU and heating in acetic acid gave the useful 1*H*-1,2,4-triazole intermediate **23a** in moderate yield. 1*H*-1,2,4-Triazole intermediate having 2-chloroethyl group at C(5) position **23b** was synthesized in a similar method in low yield. Compounds **23a–b** were treated with appropriate anilines or benzylamine to give **24c**, **24g** and **24h**, respectively.

[Scheme 4]

Synthesis of **24d**, **24f** and **24e**

Reaction of isonicotinic hydrazide **25** with ammonium thiocyanate, and subsequent treatment with aqueous sodium hydroxide afforded 3-mercapto-5-(4-pyridyl)-1*H*-1,2,4-triazole **27a** in 79% yield. Alkylation of the mercapto group with benzyl halide in the presence of potassium hydroxide gave **24d** in 66% yield in Scheme 5. Treatment of isonicotinic acid **26** with aminoguanidine hydrochloride yielded 3-amino-5-(4-pyridyl)-1*H*-1,2,4-triazole **27b** in 92 % yield. Acylation of the nitrogen atom on the 1,2,4-triazole of **27b,** and the subsequent rearrangement of the acyl group to the amino group by heating, gave the amide derivative **24f** in 91% yield. Reduction of the amide with BH_3 afforded **24e** in 69 % yield, as shown in Scheme 5.

[Scheme 5]

Introduction of Substituents into Phenyl Moiety

The synthetic routes to prepare 4-pyridyl-1*H*-1,2,4-triazole derivatives are shown in Schemes 6-10. Several anilines were purchased and the other anilines were prepared as follows. Treatment of **23a** with various anilines gave desired products exhibited in Table 4, **28a–i** in 22 – 74% yield, respectively, as shown in Scheme 6.

[Scheme 6]

Furthermore, treatment of **23a** with the other various anilines **29** gave desired products **30a–q** in 29 – 63% yield, respectively, shown in Scheme 7.

[Scheme 7]

Because **30i** demonstrated potent GRK2 inhibitory activity (*vide infra*) and was required for several further studies, an alternative synthetic route to 4-pyridyl-1*H*-1,2,4-triazole derivatives, having an amide group on the RHS, was investigated. Carboxylic acid bearing benzoate **16,** which was utilized for synthesis of the other chemotype, was condensated with benzamidrazone **22** by use of WSC and HOBt to produce **31**. After removal of the trifluoroacetyl group, formation of the 1*H*-1,2,4-triazole ring, under acidic conditions, followed by hydrolysis of the ester group, gave the key intermediate **34,** having a carboxylic acid. Amide analogues **30h** and **30i** were prepared by condensation with WSC and HOBt in good yields.

[Scheme 8]

Several anilines which were not purchased were prepared as follows in Scheme 9.

3-Nitrobenzamides **39i**, **39j** and **39l** were prepared by the condensation of 3-nitrobenzoic acid **36** with the appropriate amines in 57% – quantitative yield. Alkylation of 3-nitrophenol **37** using phenylethylbromide and potassium carbonate gave 3-nitrobenzene bearing ether **39c** in 83% yield, and the treatment of 3-nitrophenylisocyanate **38** with benzylamine obtained the corresponding 3-nitrobenzene having a urea bond **39p** in 97% yield. Hydrogenation of the nitro group of these compounds **39** under mild conditions, by use of reduced iron in the presence of $CaCl_2$ in ethanol, afforded anilines **29** in 89% – quantitative yield. Alternatively, **29i**, **29k** and **29q** were directly obtained by use of WSC and HOBt from 3-aminobenzoic acid **35a–b** and various amines in 42 – 84% yield.

[Scheme 9]

The synthesis of **30n** and **30o** is shown in Scheme 10. The 1*H*-1,2,4-triazole derivative **40** was prepared in a similar fashion to that shown in Scheme 6 and 7. The nitro group of **40** was converted into an amino group under the above-mentioned mild reductive conditions, to maintain the chlorine atom, in 85% yield. Finally, the condensation of **41** with the corresponding carboxylic acids, and subsequent hydrolysis to remove the acyl group attached to the 1,2,4-triazole ring, gave **30n** and **30o** in 66 and 63% yield, respectively.

[Scheme 10]

Introduction of Substituent into 4-Pyridine Ring

Synthesis of **47a** and **47b**

The synthesis of **47a** and **47b**, containing 2-methylpyridin-4-yl and 3-methylpyridin-4-yl moieties, respectively, is shown in Scheme 11. The methyl group at the 4-position of 2,4-dimethylpyridine **42a** and 3,4-dimethylpiridine **42b** were lithiated selectively, and the lithiated products were subsequently treated with dimethylamine and DMF to give enamines **43a–b**. The treatment of the enamines **43a–b** with aqueous $NaIO_4$ solution afforded 2-methyl or 3-methylpyridine-4-carbaldehyde **44a–b** in 85 – 92% yield. The aldehyde intermediates **44a–b** were converted into 2-methyl or 3-methylpyridine-4-carbonitorile **45a–b** *via* oximes in two steps. **47a** and **47b** were prepared by similar methods to those shown in Scheme 6 and 7.

[Scheme 11]

Synthesis of **47c** and **47d**

The synthesis of **47c** having 3-hydroxymethylpyridin-4-yl, and **47d** having 3-hydroxyethylpyridin-4-yl moieties, is shown in Scheme 12. Esterification of pyridine-3,4-dicaroxylic acid **48** in 65% yield, and subsequent reduction using $NaBH_4$ – $CaCl_2$ gave

pyridine-3,4-dimethanol **50** in 85% yield. Each mono-protection of a hydroxyl group of **22** with TBSCl in the presence of imidazole afforded **51** (15% yield) and **52** (25% yield), respectively. Swern oxidation of **51**, condensation with hydroxylamine, and subsequent dehydration, gave the desired 3-substituted 4-pyridinecarbonitrile **53** in 81% yield. Finally, treatment of **53** with hydrazide **20g** in ethanol in the presence of sodium ethoxide, generated by sodium hydride, resulted in the formation of the 1*H*-1,2,4-triazole ring and removal of the TBS group, and affording **47c** in 81% yield. Using the Mitsunobu reaction, **52** was transformed into nitrile **54** in 85% yield. The 4-pyridinecarbonitorile **55** was prepared from **54** in 40% yield in 5 steps, and **47d** was prepared in a similar fashion to that of **47c** in 78% yield.

[Scheme 12]

Synthesis of **47e**

Selective protection of the nitrogen atom in the linker moiety of **47c** in two steps gave **56** in 87% yield. Subsequent carbamoylation of the hydroxyl function, and removal of the trifluoroacetyl group, afforded **47e** in 40% yield, as shown in Scheme 13.

[Scheme 13]

Synthesis of **68a**

The synthesis of **68a**, having 2-acetamidepyridin-4-yl, is shown in Scheme 14. After acetylation of **57** at the initial step, oxidation of the methyl group by $KMnO_4$ yielded the corresponding carboxylic acid **59**. Thereafter, acid hydrazide **61** was prepared by condensation with mono-Boc-protected hydrazine, followed by deprotection of the Boc group. On another front, the precursor of 4-methyl-1,2,4-triazole, thioamide **64**, was prepared from **62** in two steps. After treatment of **64** with Meerwein reagent, the alkylated intermediate was reacted with acid hydrazide **61** to construct the 4-methyl-1,2,4-triazole ring **65a**. Chloromethyl-4-methyl-1,2,4-triazole intermediate **67a** was prepared by hydrolysis of the acetate group and chlorination of the hydroxyl group. Finally, synthesis of target product **68a** was completed by reaction with the aniline containing 2-CF_3-benzyl amide **69a**, which was prepared from 3-aminobenzoic acid **35a**, and the corresponding substituted benzylamine, in good yield.

[Scheme 14]

Changing the 2-substitution of the pyridine ring was performed as follows in Scheme 15. At first, the acid hydrazide bearing 2-chloropyridine **71** was prepared from methyl 2-chloroisonicotinate **70**. The thioamide containing ethyl ester **74** was prepared from ethyl chloroglyoxylate **72** in two steps. The 4-Methyl-1,2,4-triazole intermediate bearing ethyl ester **75** was obtained as described previously

in Scheme 14, but with improved yield (63%). After reduction of the ester by use of $NaBH_4$ and $CaCl_2$ gave hydroxy methyl, introduction of various alkyl groups at the 2-position of the pyridine ring furnished the 2-alkylaminopyridine derivatives **66b–e** in moderate yield. The alternative synthesis of the target compounds is shown as follows. Reaction of the aldehyde **77b–e**, derived from the corresponding alcohol **66b–e**, and the aniline containing 2-F-6-F-benzyl amide **69b,** formed the imine intermediate, and subsequent reduction by $NaBH_4$ achieved the target products **68b–e**, with improved yield (68 – 94%).

[Scheme 15]

Replacement of 5-Membered Heterocyclic Ring System

Compounds bearing 5-membered heterocyclic aromatic rings other than the 1*H*-1,2,4-triazole ring were designed in order to investigate the effects of the 1*H*-1,2,4-triazole ring system on biological functions. Thus, compounds bearing thiazoles, 1,2,4- and 1,3,4-oxadiazoles, pyrazoles, isoxazoles, imidazoles, pyrroles and 4-methyl-1,2,4-triazoles were synthesized. These compounds were prepared from the corresponding chloromethyl or aldehyde intermediates and the appropriate aniline derivatives as follows.

Synthesis of **81a** and **81b**

Treatment of **78a–b** with LHMDS, followed by reaction with diethyl oxalate, gave 4-pyridyl-2,4-dioxopropionates, which were treated with hydrazine to afford pyrazole derivatives **79a–b** in 64 and 16% yield. Carboxylic acid **79a** was converted into alcohol **80a**, *via* the Weinreb amide, followed by reduction by two steps in 77% yield. Also, ester **79b** was converted into alcohol **80b** in low yield. These alcohols **80a–b** were converted into chloromethyl intermediates, and were treated with aniline **29i** to provide **81a** and **81b**, respectively, in 42 – 63% yield, as shown in Scheme 16.

[Scheme 16]

Synthesis of **81c**

The Suzuki coupling reaction between the borate **82** and 4-iodo-*N*-tritylimidazole (**83**) gave **84** in 83% yield. The 4-(4-pyridyl)-*N*-tritylimidazole **84** was lithiated, and subsequent reaction with DMF gave **85** in 49% yield. Reduction of the aldehyde **85** using $NaBH_4$ gave alcohol that was chlorinated to afford the chloromethyl intermediate. Finally, the chloromethyl intermediate was treated with aniline **29i** to provide **81c** in 54% yield, as shown in Scheme 17.

[Scheme 17]

Synthesis of **81d** and **81e**

4-Pyridyl-2,4-dioxopropionate prepared in Scheme 16 was treated with hydroxylamine to afford the mixture of two isoxazole isomers. Reduction of the ester function using $LiAlH_4$, and subsequent chlorination of resulting alcohols afforded the chloromethyl intermediates **86** and **87**, which were separated by column chromatography on silica gel. Finally, both chloromethyl intermediates **86** and **87** were treated with aniline **29i** to provide **81d** (42% yield) and **81e** (39% yield**)**, respectively, as shown in Scheme 18.

[Scheme 18]

Synthesis of **81f**

The imidazole ring, containing trifluoromethyl moiety **89**, was constructed by using 3,3-dibromo-1,1,1-trifluoroacetone (**88**) and 4-pyridinecarboaldehyde in 31% yield. Conversion of the trifluoromethyl moiety of **89** into a cyano group was carried out by treatment with 5% aqueous ammonia solution in 84% yield. Reduction of the nitrile **90**, using DIBAL and then $NaBH_4$ to the corresponding alcohol and subsequent chlorination afforded chloromethyl intermediate. Finally, the chloromethyl intermediate was treated with aniline **29i** to provide **81f** in 60% yield, as shown in Scheme 19.

[Scheme 19]

Synthesis of **81g**

3-(4-Pyridyl)pyrrole (**92**) was prepared from 4-vinylpyridine (**91**) and tosylmethyl isocyanide (TOSMIC) using a [2+3] cycloaddition reaction in 49% yield. Introduction of the formyl function into **57** was achieved by using the Vilsmeier reagent in low yield. Finally, reductive amination of **93** with **29i** produced the desired product **81g** in 21% yield, as shown in Scheme 20.

[Scheme 20]

Synthesis of **81h**

Treatment of 4-aminopyridine **94** with **95** in the presence of acetic acid afforded formylated 1-(4-pyridyl)pyrrole (**96**) in 22% yield. Reductive amination of **96** with **29i** yielded the desired product **81h** in 63% yield, as shown in Scheme 21.

[Scheme 21]

Synthesis of **81i**

The reaction of 4-cyanopyridine **21** with NaN_3 gave the corresponding tetrazole **97** in 34% yield,

which was converted into 1,3,4-oxadiazole **98** by treatment with ethyl oxalyl chloride in the presence of 2,4,6-collidine in 27% yield. Reduction of the ester, using $NaBH_4$ and $CaCl_2$, into alcohol, followed by chlorination, afforded chloromethyl intermediate. Finally, the chloromethyl intermediate was treated with aniline **29i** to provide **81i** in 57% yield, as shown in Scheme 22.

[Scheme 22]

Synthesis of **81j**

Compound **99** was prepared in 15% yield by the reacting 4-cyanopyridine **21** with hydroxylamine (98% yield), followed by treatment with chloroacetyl anhydride. Compound **99** was treated with aniline **29i** to provide **81j** in 71% yield, as shown in Scheme 23.

[Scheme 23]

Synthesis of **81k**

Compound **100** was prepared by condensation of 4-(bromoacetyl)pyridine with ethyl thiooxamate in 59% yield. Reduction of the ester function using DIBAL, and subsequent chlorination of alcohol using $SOCl_2$, afforded the chloromethyl intermediate **101**, containing a thiazole ring. Finally, compound **101** was treated with aniline **29i** to provide **81k**, as shown in Scheme 24.

[Scheme 24]

Synthesis of **81l**

Intermediate **103,** containing a thiazole ring, was prepared by condensation of thioamide **102** with 1,3-dichloroacetone in 35% yield. Compound **103** was treated with trifluoroacetoanilide **104** in the presence of potassium carbonate, and the trifluoroacetyl group was removed to provide **81l** in 48% yield, as shown in Scheme 25. Compound **104** was prepared from aniline **29i** with trifluoroacetic anhydride in 93% yield.

[Scheme 25]

Synthesis of **81m**

The treatment of Weinreb amide **105** (Intermediate **80a** in Scheme 16), with iodine in the presence of ceric ammonium nitrate (CAN), afforded the corresponding 4-iodopyrazole **106** in 82% yield. Suzuki coupling of **106** with phenylboronic acid gave **107** in 21% yield. Reduction of Weinreb amide **107** with $LiAlH_4$, and subsequent reductive amination with **29i**, afforded **81m** in 7% yield, as shown in Scheme 26.

[Scheme 26]

Introduction of Substituent into Phenyl Moiety of *N*-Benzylcarboxamide

The substituted aroyl thiosemicarbazide was prepared by the reaction of isonicotinic hydrazide **25** with methyl isothiocyanate, followed by heating the obtained thiosemicarbazide in the presence of sodium bicarbonate, giving 1,2,4-triazole-3-thiol **108** in quantitative yield. Compound **108** was treated with HNO_3 to take off the mercapto group, and afford **109** in 90% yield. 4-Methyl-1,2,4-triazole **109** was lithiated, and subsequent reaction with DMF gave **110** in 80% yield. Reductive amination with **13** yielded the desired intermediate, **113**, in 68% yield, as shown in Scheme 27. Compound **110** can also be prepared as follows. Treatment of ethyl oxalyl chloride (**72**) with methylamine and subsequent reaction of the resulting amide with Lawesson's reagent gave the thioamide. Alkylation of the thioamide with Meerwein reagent, followed by condensation with isonicotinic hydrazide (**25**), led to 4-methyl-1,2,4-triazole (**111**) in 60% yield. Reduction of the ester functionality using $NaBH_4$ and $CaCl_2$ in 80% yield and subsequent oxidation using MnO_2 afforded the above aldehyde **110** in 96% yield, as shown in Scheme 27. Hydrolysis of the ester of **113** in 99% yield and subsequent condensations with appropriate amines or anilines gave the desired compounds **115a–j** in 60 – 98% yield, except for **115e**, which was prepared from the aldehyde **110** by reductive amination in 2 steps.

[Scheme 27]

Because it was difficult to change the substitution of the 4-position of the 1,2,4-triazole after formation of this ring system, appropriate amines had to be used at the initial step in Scheme 28. The desired products **119a–f** were prepared *via* the same procedure as described in Scheme 27.

[Scheme 28]

A novel 3-ring system (**133**) was constructed as follows. 3-Hydroxyisonicotinate ethyl ester **123** was prepared from 3,4-pyridinecarboximide **120** in three steps. Homologation of **123** by the Mitsunobu reaction with Z-protected aminoethanol, and de-protection of the Z-group, gave the pyridine-fused 1,4-oxazepine derivative **125**. After thiocarbonylation by Lawesson's reagent, derivatives **133a–b** were produced by use of carboxamide containing acid hydrazides **132a–b**, as shown in Scheme 29.

[Scheme 29]

Synthesis of pyrimidine derivatives is shown in Scheme 30. Pyrimidine-4-carbohydrazide **136** was prepared from 4-methylpyrimidine **134** in 2 steps. Treatment of the corresponding thioimidates,

derived from ethyl oxalyl chloride **72** and appropriate amines, in 2 steps, as described in Schemes 26 and 27, gave **137a–b** in moderate yield. The desired products **139a–d** were synthesized by the same methods (*vide supra*).

[Scheme 30]

Results and Discussion

SAR for Benzene Ring Substitutions of Acyclic Hydrazone Derivatives

[Table 1]

Benzene ring substitution SAR for hit compound **5** (acyclic hydrazone derivative) is summarized in Table 1. These compounds were tested for inhibitory effects on kinase activity of GRK2, Rho-associated protein kinase 2 (ROCK2), and Protein kinase C alpha (PKCα). ROCK2 is one of the most significant kinases involved in vascular smooth muscle cell contraction. We found that our compounds with potent ROCK2 inhibition induced vasorelaxing effects in rat aorta rings (data not shown). PKCα is a member of the AGC family of kinases (PKA, PKG and PKC), regulating modulation of membrane structure and skeletal reorganization, receptor desensitization,

transcriptional control, cell growth and differentiation, and mediation of immune response, among others.

Although selectivity against ROCK2 was not achieved, introduction of a chlorine atom (**5a**) and a methoxy group (**5e**) on the *meta*-position of the benzene ring led to ~2.5-fold and ~35-fold increases in GRK2 inhibitory activity, respectively. However, introduction of substituents on the *ortho* and *para*-position of the benzene ring significantly decreased GRK2 inhibitory activity. Therefore, further optimization at the *meta*-position of the benzene ring to optimize the activity and selectivity was performed next.

SAR for Benzene Ring Substitutions of Cyclic Hydrazone Derivatives

[Table 2]

Effects of *meta*-position modifications on the RHS of benzene ring **12a** (cyclic hydrazone derivative) are summarized in Table 2. Novel cyclic hydrazone derivatives (Figure 1) displayed both GRK2 inhibitory activity and a desired trend in selectivity against ROCK2. Therefore, a variety of substituents on the *meta*-position of the benzene ring were introduced to increase GRK2 inhibitory activity utilizing the SAR information in Table 1. The resulting compounds, except for morpholino-amide derivative **12f**, show a dramatic increase in GRK2 inhibitory activity. In particular,

12e shows not only potent GRK2 inhibitory activity, but also excellent selectivity against ROCK2. Moreover, these cyclic hydrazone derivatives do not show affinity for PKCα. However, **12g** exhibits potent inhibitory activity towards GRK2, but does not show high selectivity over ROCK2.

Linker Moiety Modification SAR for 4-Pyridyl-1,2,4-Triazole Derivatives

[Table 3]

Linker moiety SAR for hit compound **24a** (4-pyridyl-1,2,4-triazole derivative) is summarized in Table 3. Displacement of the linker sulfur atom by an oxygen (hit compound **24b**) reduced GRK2 inhibitory activity, while changing it to nitrogen (**24c**) led to ~30-fold increase. Replacing carbon with sulfur (**24a** to **24d**) and nitrogen (**24c** to **24e**) resulted in drastic decreases in GRK2 inhibition. Amide derivative (**24f**) and methylene derivative (**24g** and **24h**) showed low activity. Next, based on this SAR information, further modification of the benzene ring of **24c** was implemented.

Benzene Ring Substitution SAR for 4-Pyridyl-1,2,4-Triazole Derivatives

[Table 4]

Benzene ring substitution SAR for **28a** is summarized in Table 4. Introduction of a methoxy group on the *meta*-position of benzene ring (**28c**) showed almost no change in GRK2 inhibitory activity, while a methoxy group in the *ortho* and *para*-position drastically decreased activity. Among chloro-derivatives, the p*ara*-position derivative **28g** showed the most potent activity. Furthermore, the 3-sulfonamide derivative **28h** showed stronger inhibitory activity than the corresponding 4-sulfonamide derivative **28i**. While all compounds showed high levels of selectivity against PKCα, we observed no improvement in ROCK2 selectivity. Next, further modifications of the 3-position of the benzene ring were implemented based on this SAR information.

[Table 5]

Initial SAR for benzene ring substitutions at the 3-position of **28a** are summarized in Table 5. Based on the SAR information in Table 4, introduction of 3-substitution (R^1) of the benzene ring on the RHS was preferable. Therefore, optimization of R^1 was implemented as follows. **28a**, **30a**, **30b** and **30c** introducing various alkoxy groups did not increase the inhibitory activity. Conversion of the benzyloxy of **30b** into benzylthio (**30d**), benzyl sulfonamide (**30e**) and benzylsulfone (**30f**) also did not increase the inhibitory activity. However, several secondary amide derivatives (**30g**, **30h**, **30i**, **30j**, and **30k**) undoubtedly gave rise to increases of inhibitory activity. In particular, the benzyl amide derivative (**30i**) demonstrated the most potent inhibitory activity in Table 5. The crystal structure of

human GRK2 in complex with **30i** obtained in Takeda California (TCAL) (Figure 3) revealed the compound formed the obligatory hinge interaction between the pyridine head group and the backbone amide of Met274. It also made significant interactions with the side chain of the DLG-motif residue Asp335, and the backbone amide of Phe202, in the P-loop. Additionally, the 1,2,4-triazole engaged in an interesting network of water-mediated interactions. These interactions contributed to increases in GRK2 inhibitory activity. This similar information has been already reported by use of co-crystallization between bovine GRK2 and **CMPD101** (*vide infra*, **115h**) by Thal D. M. *et. al*. in University of Michigan.[29] On the other hand, a tertiary amide (**30l**) evidently decreased the activity, compared with **30i**. These results indicate that the direction of the carbonyl group of the amide plays an important role and/or the orientation of the benzyl part is important in terms of pushing up the P-loop to enhance inhibition of GRK2. Revised amide derivatives (**30m**, **30n** and **30o**) and a urea derivative (**30p**) apparently maintained the potent activity. Judging from the SAR information between **30m** and **30n**, the chlorine atom at R2 does not affect GRK2 inhibitory activity. Furthermore, **30o** having reversed amide of **30i** which showed most potent activity in Table 5 maintained the potent activity. Introduction of a methylene (**30q**) between the amide and the central benzene ring markedly decreased the activity.

Figure 3. Section of the X-ray crystal structure of human GRK2 in complex with the small molecule inhibitor **30i**

[Figure 3]

SAR for Substituent on 4-Pyridine Ring

[Table 6]

SAR for substitutions on the 4-pyridine ring of **24c** or **30i** are summarized in Table 6. As shown in Tables 3, 4 and 5, a series of 4-pyridyl-1*H*-1,2,4-triazole derivatives showed potent GRK2 inhibitory activity, but also simultaneous inhibition of CYP3A4. As listed in Table 6, to reduce the inhibitory potency towards CYP3A4, a methyl group was introduced at the C(2) position of the pyridine ring of **24c** (**47a**). As a result, although the inhibition of CYP3A4 was decreased, the GRK2 inhibitory activity was also decreased. In further modifications, the introduction of methyl (**47b**), hydroxymethyl (**47c**), 2-hydroxyethyl (**47d**) and carbamoyloxymethyl (**47e**) groups at the C(3) position of the pyridine ring of **24c** or **30i** also resulted in decreases in GRK2 inhibitory potency. These compounds also still showed potent inhibition of CYP3A4.

[Table 7]

Further investigation to decrease the inhibition of CYP3A4 by introducing substitutions at the C(2) position of the pyridine ring by use of 4-methyl-1,2,4-triazole derivatives (*vide infra*: the SAR of 5 membered heterocyclic ring system including introduction of methyl group at the 4-position of 1,2,4-triazole) was performed as follows. These results were shown in Table 7. Introduction of acetamide (**68a**), methylamine (**68b**) and benzylamine (**68c**) decreased the inhibition of CYP3A4 while keeping moderate inhibitory activity towards GRK2. However, extending the alkyl portion of the benzylamine (**68d** and **68e**) showed a trend of increased inhibition of CYP3A4.

SAR for 5-Membered Heterocyclic Ring System Replacement

[Table 8]

SAR for 5-membered heterocyclic ring system replacement of **30i** are summarized in Table 8. Although replacement of the 1*H*-1,2,4-triazole ring with a pyrazole (**81a**), 4-methylpyrazole (**81b**), imidazole (X = N, Y = N, Z = C, **81c**) and two isoxazole isomers (**81d** and **81e**) resulted in equipotent inhibition of GRK2 as **30i**, the derivatives also showed potent inhibition of ROCK2 and CYP3A4. Interestingly, **81c**, containing a 4-(4-pyridyl)imidazole, showed more than 20-fold greater inhibition of GRK2 as **81f** (X = N, Y = C, Z = N), containing a 2-(4-pyridyl)imidazole. This result suggests that the location of the nitrogen atom plays a crucial role for inhibition of GRK2. It is

worthwhile to notice that introduction of a methyl group at the N(4) position of the 1,2,4-triazole ring (**115e**), which is described at a later point (Table 9), results in potent inhibition of GRK2 and lower activity against ROCK2, PKCα and CYP3A4. In addition to this result, introduction of a methyl group at the C(4) position of the pyrazole ring (**81b**) maintains potent inhibition of ROCK2 and CYP3A4. Based on the SAR information for the 1,2,4-triazole, pyrazole and imidazole derivatives, the nitrogen atom at the Y position of the 5-membered heterocyclic ring system might be important to show the activity. However, the pyrrole derivative bearing only a nitrogen atom at the Y position (**81g**) exhibited about a 20-fold loss in potency for GRK2. Moreover, *N*-linked 4-pyridyl-pyrrole derivative (**81h**) gave a dramatic decrease in potency for GRK2. Further investigation of the 5-membered heterocyclic ring system to generate two kinds of oxadiazoles and thiazoles did not maintain the same inhibitory activity (**81i**, **81j**, **81k** and **81l**). Finally, incorporation of a bulky group (phenyl group, **81m**) at the 4-position of the pyrazole ring of **81a** decreased the activity. Next, further modifications of the phenyl moiety of the *N*-benzylcarboxamide of the 1*H*-1,2,4-triazole derivative **30i** and the 4-methyl-1,2,4-triazole derivative **115e** were implemented based on this SAR information.

SAR for Phenyl Moiety Modification of *N*-Benzylcarboxamide

[Table 9]

SAR for phenyl moiety modification of the *N*-benzylcarboxamide of the 1*H*-1,2,4-triazole derivative **30i** and the 4-methyl-1,2,4-triazole derivative **115e** are summarized in Table 9. Further optimization of the compounds was focused on the synthesis of derivatives modified at the phenyl moiety of *N*-benzylcarboxamide. As listed in Table 9, introduction of a methoxy group on the *ortho* position of the phenyl moiety of *N*-benzylcarboxamide (**115c**) shows the most potent activity compared to the concomitant substituted compounds (vs **115a** and **115b**). Changing the methoxy group out for a chlorine atom (**115d**) shows equipotent activity. **115c**, having the 2-methoxy group slightly improved the selectivity against ROCK2, when compared with the corresponding 3- and 4-methoxy derivatives. However, **115c** was not improved in terms of the selectivity of PKCα and CYP3A4 inhibition. Introduction of a methyl group (**115e**) in the 4-position of the 1,2,4-triazole (**30i**) dramatically improves the issue of CYP3A4 and reasonably improves the selectivity against ROCK2 and PKCα. In particular, **115h** shows excellent potency for GRK2 and exhibits low activity against ROCK2, PKCα, and CYP3A4 compared with the corresponding 4- and 3-trifluoromethyl derivatives (**115f** and **115g**). Changing the trifluoromethyl group of **115h** into the methoxy version (**115i**) shows a nearly 2.5-fold decrease in potency for GRK2. However, **115j**, containing a chlorine atom, retains potency for GRK2. Furthermore, several 1,2,4-triazole derivatives potentiate β-signaling by cAMP accumulation assay in human embryonic kidney (HEK-B2) cells stimulated with isoproterenol. Among these compounds, **115h** demonstrates the strongest activity in HEK-B2 cells. In addition,

115h shows potentiation of β-signaling in rat neonatal cardiomyocytes in the presence of isoproterenol (data not shown).[30]

SAR for 4-Pyridyl 4-Alkyl Triazole Modification

[Table 10]

SAR for 4-position modifications of the 1,2,4-triazole ring are summarized in Table 10 and 11. As listed in Table 10, changing the methyl group at the 4-position of the 1,2,4-triazole ring to an ethyl group (**119a**) increases the GRK2 inhibitory activity . However, **119a** shows IC_{50} < 10 μM for PKCα and did not meet the criteria for CYP3A4 inhibition (less than 50% at 10 μM). The bigger the substituent at the 4-position of the 1,2,4-triazole ring (*n*-Pr: **119b**, *i*-Pr: **119c** and Bn: **119d**), the less inhibitory activity towards GRK2. Insertion of an oxygen atom into the alkyl substituent (**119e** and **119f**) slightly increases the GRK2 inhibitory activity, however CYP3A4 inhibition is not decreased. **119f**, which shows potent GRK2 inhibitory activity, demonstrates comparable activity with **115h** in HEK-B2 cell.

[Table 11]

Next, novel 3-ring system compounds by use of the substituent at the 4-position of the 1,2,4-triazole ring (**133a** and **133b**) shows the highest potency for GRK2 in the 1,2,4-triazole series and shows good selectivity against ROCK2. Moreover **133a** demonstrates the strongest activity in HEK-B2 cells and **133b** meets the criteria of CYP3A4 inhibition.

[Table 12]

Finally, 4-pyrimidine derivatives show more potent GRK2 inhibitory activity than 4-pyridine derivatives. In particular, 4-*n*-propyl-1,2,4-triazole derivatives (**139c** and **139d**) have tremendously potent GRK2 inhibitory activity. Additionally, **139a** and **139c** demonstrate potent activity in HEK-B2 cells. However these compounds do not decrease the inhibition of CYP3A4.

Selectivity of GRK2 inhibitors for GRK family

[Table 13]

Selectivity of GRK2 inhibitors for other GRK family proteins is summarized in Table 13. The lack of GRK4 inhibitory data is a limitation of the study design. Because of the high homology among GRK4-6, we predict that our compounds also show high selectivity against GRK4 and that it should

be confirmed in the future study. As listed in Table 13, several GRK2 inhibitors demonstrate high selectivity against other GRK family members, except for GRK3, due to the high homology. Novel 3-ring system compounds (**133a**), bearing highly potent GRK2 inhibitory activity, have decreased selectivity against GRK1, GRK5 and GRK7. The pyrazole derivative (**81b**) shows excellent selectivity against the other GRK family members except for GRK3.

Further information regarding 115h

Judging from the above-mentioned SAR studies, **115h** was selected for further investigation. Panlab[30] was used to run selectivity assays for cross-reactivity against other kinases, enzymes, receptors and ion channels. It was found that **115h** has no remarkable effects on any of the proteins surveyed. In particular, because IC_{50} of PKA of **115h** was > 1 μM (71% inhibition at 10 μM and 3% inhibition at 1 μM), **115h** showed the good selectivity toward GRK2 over PKA. In terms of cardiovascular risk, **115h** exhibited low activity against the HERG ion channel (9% at 1 μM and 31% at 10 μM). These results suggest that **115h** is a potent and highly selective inhibitor of GRK2.

The crystal structure of human GRK2 in complex with **115h** was solved at Takeda California, and described as follows (Figure 4). The structure, which contained clearly interpretable electron density for the ligand, was obtained by soaking the compound into apo crystals. The interactions between **115h** and human GRK2 were similar to those observed with **30i**. The ligand binding pose and

interactions with human GRK2 were also similar to those observed in the previous report[29] by Thal D. M. *et. al*.

Figure 4. Section of the X-ray crystal structure of human GRK2 in complex with the small molecule inhibitor **115h**

[Figure 4]

Retardation of the Internalization of β-Adrenergic Receptors[31-35]

Inhibitions of the internalization of βARs by use of small molecule GRK2 inhibitors (**30i** and **115h**) were visualized using a confocal microscope as follows (Figure 5). HEK-293 cells were engineered to overexpress a C-terminally fused human β2AR-green fluorescence protein (GFP) fusion protein, to establish a HEK-B2 cell line.

Figure 5. Retardation of the internalization of β-adrenergic receptors by using GRK2 inhibitors (**30i** and **115h**)

[Figure 5]

Without any stimulation, the β2AR-GFP fusion protein normally translocated on the HEK-B2 cell membrane. After stimulation of isoproterenol (0.1 μM), the β2AR-GFP fusion protein formed clathrin-coated vesicles in cytoplasm within about 5 minutes, showing the internalization of β2AR. In the presence of pre-incubation (20 minutes) of **30i** and **115h** (100 μM), the isoproterenol-induced formation of clathrin-coated vesicles were remarkably decreased and the β2AR-GFP fusion protein remained on the plasma membrane. These results suggest that **30i** and **115h** (100 μM) inhibit the internalization of β2AR. These effects of GRK2 inhibition were shown in a dose-dependent manner (30 μM and 10 μM, data were not shown). In these studies, we provide evidence that small molecule GRK2 inhibition reduces the internalization of β2ARs.

Conclusion

To develop novel GRK2 inhibitors with potent activity as well as good selectivity and improving cardiac β-adrenergic signaling with minimal effects on blood pressure *in vivo*, chemical modifications of the hit compound **24a** (IC_{50} = 1200 nM) were carried out. Thus, a series of compounds related to hit compound **24a** has been designed and synthesized to investigate their biological activities as follows. i) Modification at the linker moiety, ii) Modification at the benzene ring of the RHS, iii) Transformation into the compounds bearing a 5-membered heterocyclic ring

other than the 1*H*-1,2,4-triazole ring, and iv) Modification at the pyridine ring. Among these compounds, **115h** was found to possess potent and selective inhibition of GRK2 against ROCK2 and PKC. **115h** also showed potentiation of β-adrenergic signaling by a cAMP accumulation assay and an internalization assay of β-adrenergic receptors in isoproterenol-stimulated β2AR expressing 293 HEK-B2 cells. Furthermore, inhibition of GRK2 with **115h**, both in neonatal rat cardiomyocytes and in isolated perfused rat hearts, significantly prevented isoproterenol-induced desensitization of β-adrenergic receptors.[30] These results suggest that acute inhibition of cardiac GRK2 by small-molecule GRK2 inhibitors could improve cardiac β-adrenergic signaling for the first time. The specificity profile of **115h** obtained among various kinases looks very promising and also surprising in view of the close homology of the ATP binding site for a number of the enzymes. It was interesting to notice the high selectivity for GRK2 compared to ROCK2 for the test compounds, and **115h** was the most selective compound to our knowledge. These results strongly suggest that small molecule GRK2 inhibitors would be a novel class of therapeutic drug for the treatment of heart failure through improved cardiac β-adrenergic signaling. However, the pharmacokinetic profile of **115h** after oral administration was not good enough to show *in vivo* activity. Further efforts are needed to find inhibitors of GRK2 which are effective for heart failure by oral administration.

Experimental Section

Chemistry

General. Solvents and reagents were obtained from commercial suppliers and were used without purification. Reaction progress was determined by thin layer chromatography (TLC) analyses which were carried out on Merck Kieselgel 60 F_{254} plates or NH plates produced by Fuji Silysia, LTD. Visualization was done with UV light (254 nm). Chromatographic purification was carried out on silica gel columns [(Merck Kieselgel 60, 70−230 mesh size or 230−400 mesh size, Merck) or (Chromatorex NH-DM 1020, 100−200 mesh size)] or on Purif-Pack (particle size: SI 60 μM or NH 60 μM, Fuji Silysia, LTD.). The yields reported were not optimized. Melting points were determined on a Yanaco micro melting point apparatus and were uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian Murcury-300 (300 MHz) spectrometer. Chemical shifts are given in parts per million (ppm) downfield from tetramethysilane (δ) as the internal standard in deuterated solvent, and coupling constants (J values) are in Hertz (Hz). Data are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, td = triplet of doublets, dt = doublet of triplets, ddd = double doublet of doublets, tt = triplet of triplets, br = broad and brs = broad singlet), and coupling constants. Elemental analyses were carried out by Takeda Analytical Laboratories, LTD., and were within ±0.4% of the theoretical values unless otherwise noted. LC−MS analysis was performed on a Shimadzu liquid chromatography−mass spectrometer system, operating in APCI (+ or −) or ESI (+ or −) ionization mode. Analytes were eluted using a linear gradient of 0.05% TFA containing

H_2O/CH_3CN or 0.01% TFA containing H_2O/CH_3CN or 5 mM ammonium acetate containing H_2O/CH_3CN or 10 mM NH_4HCO_3 containing H_2O/CH_3CN or 0.01% heptafluorobutyric acid/1.0% isopropyl alcohol containing H_2O/CH_3CN mobile phase. Preparative HPLC was performed on a Waters 2525 separations module (L-column2 ODS (20 × 150 mm ID), CERI, Japan); MS spectra were recorded using a Waters ZQ2000 with electrospray ionization or on a GILSON system, equipped with a L-column2 ODS (20 × 150 mm ID, CERI, Japan) or on a Waters Deltaprep 300 system or on a Shimadzu 10A VP system. Samples were eluted using a linear gradient of 0.1% TFA in H_2O/CH_3CN or 10 mM NH_4HCO_3 in H_2O/CH_3CN, or H_2O/CH_3CN. Purity data were collected by a HPLC with Corona CAD (Charged Aerosol Detector), Nano quantity analyte detector (NQAD), or photo diode array detector. The column was a Capcell Pak C18AQ (50 mm × 3.0 mm ID, Shiseido, Japan) or L-column 2 ODS (30 mm × 2.0 mm ID, CERI, Japan) with a temperature of 50 ℃ and a flow rate of 0.5 mL/min. Mobile phases A and B under a neutral condition were a mixture of 50 mM ammonium acetate, H_2O, and CH_3CN (1:8:1, v/v/v) and a mixture of 50 mM ammonium acetate and CH_3CN (1:9, v/v), respectively. The ratio of mobile phase B was increased linearly from 5% to 95% over 3 min, 95% over the next 1 min. Mobile phases A and B under an acidic condition were a mixture of 0.2% formic acid in 10 mM ammonium formate and 0.2% formic acid in CH_3CN, respectively. The ratio of mobile phase B was increased linearly from 14% to 86% over 3 min, 86% over the next 1 min. The purity of all test compounds was assessed by analytical HPLC. Yields are not optimized.

General procedure for the preparation of *N*-(2-chlorophenyl)-2,2,2-trifluoroacetamide (2a). Trifluoroacetic anhydride (4.41 g) was added to a solution of 2-chloroaniline (**1a**, 2.55 g, 20.0 mmol) and Et_3N (4.2 mL) in THF (50 mL) at 0 °C. The mixture was stirred at room temperature for 45 min. The reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford 4.31 g (19.3 mmol, 96%) of **2a** as white solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19 (1H, td, *J* = 7.8, 1.5 Hz), 7.36 (1H, td, *J* = 8.0, 1.4 Hz), 7.45 (1H, dd, *J* = 8.1, 1.5 Hz), 8.34 (1H, dd, *J* = 8.3, 1.4 Hz), 8.43 (1H, br).

***N*-(3-Chlorophenyl)-2,2,2-trifluoroacetamide (2b).** This compound was prepared from 3-chloroaniline **1b** as described in the synthesis of **2a**, as white solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22 (1H, ddd, *J* = 8.1, 2.1, 1.2 Hz), 7.33 (1H, t, *J* = 8.0 Hz), 7.42 (1H, ddd, *J* = 8.1, 1.8, 1.2 Hz), 7.68 (1H, t, *J* = 2.0 Hz), 7.84 (1H, br).

***N*-(4-Chlorophenyl)-2,2,2-trifluoroacetamide (2c).** This compound was prepared from 4-chloroaniline **1c** as described in the synthesis of **2a**, as pale yellow solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34–7.39 (2H, m), 7.50–7.55 (2H, m), 7.90 (1H, br).

***N*-(2-Methoxyphenyl)-2,2,2-trifluoroacetamide (2d).** This compound was prepared from *o*-anisidine **1d** as described in the synthesis of **2a**, as pink solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.93 (3H, s), 6.94 (1H, dd, *J* = 8.1, 1.2 Hz), 7.01 (1H, td, *J* = 7.8, 1.4 Hz), 7.18 (1H, td, *J* = 7.7, 1.7 Hz), 8.32 (1H, dd, *J* = 8.0, 1.7 Hz), 8.57 (1H, br).

***N*-(3-Methoxyphenyl)-2,2,2-trifluoroacetamide (2e).** This compound was prepared from *m*-anisidine **1e** as described in the synthesis of **2a**, as pink solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.82 (3H, s), 6.78 (1H, ddd, *J* = 8.3, 2.4, 0.8 Hz), 7.04 (1H, ddd, *J* = 8.0, 1.9, 0.7 Hz), 7.27 (1H, t, *J* = 2.3 Hz), 7.28 (1H, t, *J* = 8.3 Hz), 7.82 (1H, br).

***N*-(4-Methoxyphenyl)-2,2,2-trifluoroacetamide (2f).** This compound was prepared from *p*-anisidine **1f** as described in the synthesis of **2a**, as pink solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.81 (3H, s), 6.88–6.93 (2H, m), 7.44–7.49 (2H, m), 7.77 (1H, br).

General procedure for the preparation of ethyl *N*-(2-chlorophenyl)-*N*-(trifluoroacetyl)glycinate (3a). A mixture of *N*-(2-chlorophenyl)-2,2,2-trifluoroacetamide (**2a**, 4.29 g, 19.2 mmol), ethyl bromoacetate (3.36 g) and potassium carbonate (3.98 g) in DMF (40 mL) was stirred at room temperature for overnight. The reaction mixture was concentrated under reduced pressure. The residue was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford 5.69 g (19.3 mmol, 96%) of **3a** as crude brown oil. This product was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (3H, t, *J* = 7.2 Hz), 3.74 (1H, d, *J* = 17.4 Hz), 4.14–4.30 (2H, m), 4.99 (1H, d, *J* = 17.1 Hz), 7.30–7.41 (2H, m), 7.51 (1H, dd, *J* = 7.5, 1.8 Hz), 7.64–7.68 (1H, m).

Ethyl *N*-(3-chlorophenyl)-*N*-(trifluoroacetyl)glycinate (3b). This compound was prepared from the corresponding trifluoroacetanilide **2b** as described in the synthesis of **3a**, as crude brown solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30 (3H, t, *J* = 7.2 Hz), 4.26 (2H, q, *J* = 7.0 Hz), 4.37 (2H, s),

7.31–7.44 (4H, m).

Ethyl *N*-(4-chlorophenyl)-*N*-(trifluoroacetyl)glycinate (3c). This compound was prepared from the corresponding trifluoroacetanilide **2c** as described in the synthesis of **3a**, as crude brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (3H, t, *J* = 7.1 Hz), 4.23 (2H, q, *J* = 7.2 Hz), 4.36 (2H, s), 7.33–7.41 (4H, m).

Ethyl *N*-(2-methoxyphenyl)-*N*-(trifluoroacetyl)glycinate (3d). This compound was prepared from the corresponding trifluoroacetanilide **2d** as described in the synthesis of **3a**, as crude brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.28 (3H, t, *J* = 7.4 Hz), 3.73 (1H, d, *J* = 17.1 Hz), 3.85 (3H, s), 4.12–4.28 (2H, m), 4.94 (1H, d, *J* = 17.1 Hz), 6.93–6.99 (2H, m), 7.38 (1H, ddd, *J* = 8.3, 7.7, 1.9 Hz), 7.46–7.49 (1H, m).

Ethyl *N*-(3-methoxyphenyl)-*N*-(trifluoroacetyl)glycinate (3e). This compound was prepared from the corresponding trifluoroacetanilide **2e** as described in the synthesis of **3a**, as crude brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (3H, t, *J* = 7.2 Hz), 3.82 (3H, s), 4.24 (2H, q, *J* = 7.2 Hz), 4.38 (2H, s), 6.94–6.98 (3H, m), 7.32 (1H, dd, *J* = 9.2, 7.7 Hz).

Ethyl *N*-(4-methoxyphenyl)-*N*-(trifluoroacetyl)glycinate (3f). This compound was prepared from the corresponding trifluoroacetanilide **2f** as described in the synthesis of **3a**, as crude brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (3H, t, *J* = 7.2 Hz), 3.82 (3H, s), 4.22 (2H, q, *J* = 7.2 Hz), 4.35 (2H, s), 6.87–6.92 (2H, m), 7.31 (2H, d, *J* = 8.4 Hz).

General procedure for the preparation of 2-((2-chlorophenyl)amino)acetohydrazide (4a). A

mixture of ethyl *N*-(2-chlorophenyl)-*N*-(trifluoroacetyl)glycinate (**3a**, 5.67 g, 18.3 mmol) and hydrazine hydrate (3.67 g) in ethanol (45 mL) was stirred at 90 °C for 16 h. The reaction mixture was concentrated under reduced pressure. The residue was partitioned between DCM and water. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The solid residue was washed with diethyl ether and hexane to afford 3.11 g of **4a** (15.6 mmol, 85%) as white solids. mp 82–83 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.72 (2H, d, J = 5.7 Hz), 4.26 (2H, brs), 5.57 (1H, t, J = 5.9 Hz), 6.52 (1H, dd, J = 8.1, 1.2 Hz), 6.61 (1H, td, J = 7.6, 1.3 Hz), 7.12 (1H, td, J = 7.7, 1.2 Hz), 7.25 (1H, dd, J = 7.8, 1.5 Hz), 9.15 (1H, brs). Anal. Calcd. for $C_8H_{10}ClN_3O$: C, 48.13; H, 5.05; N, 21.05. Found: C, 47.99; H, 4.88; N, 20.96.

2-((3-Chlorophenyl)amino)acetohydrazide (4b). This compound was prepared from the corresponding *N*-(trifluoroacetyl)glycinate **3b** as described in the synthesis of **4a**, as white solids. mp 82–84 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.62 (2H, d, J = 6.3 Hz), 4.26 (2H, brs), 6.23 (1H, t, J = 6.2 Hz), 6.50 (1H, ddd, J = 8.2, 2.2, 1.0 Hz), 6.54–6.57 (2H, m), 7.07 (1H, t, J = 8.3 Hz), 9.12 (1H, brs). Anal. Calcd. for $C_8H_{10}ClN_3O$: C, 48.13; H, 5.05; N, 21.05. Found: C, 47.97; H, 5.01; N, 20.89.

2-((4-Chlorophenyl)amino)acetohydrazide (4c). This compound was prepared from the corresponding *N*-(trifluoroacetyl)glycinate **3c** as described in the synthesis of **4a**, as white solids. mp 141–144 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.60 (2H, d, J = 6.0 Hz), 4.24 (2H, brs), 6.07 (1H, t, J = 6.0 Hz), 6.52–6.60 (2H, m), 7.05–7.10 (2H, m), 9.08 (1H, brs). Anal. Calcd. for $C_8H_{10}ClN_3O$•0.1H_2O: C, 47.70; H, 5.10; N, 20.86. Found: C, 47.94; H, 5.09; N, 20.60.

2-((2-Methoxyphenyl)amino)acetohydrazide (4d). This compound was prepared from the corresponding *N*-(trifluoroacetyl)glycinate **3d** as described in the synthesis of **4a**, as white solids. mp 103–105 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.64 (2H, d, J = 5.7 Hz), 3.79 (3H, s), 4.25 (2H, brs), 5.16 (1H, t, J = 5.9 Hz), 6.38 (1H, dd, J = 7.7, 1.4 Hz), 6.59 (1H, td, J = 7.7, 1.7 Hz), 6.76 (1H, td, J = 7.6, 1.3 Hz), 6.82 (1H, dd, J = 7.7, 1.4 Hz), 9.09 (1H, brs). Anal. Calcd. for $C_9H_{13}N_3O_2$: C, 55.37; H, 6.71; N, 21.52. Found: C, 55.14; H, 6.74; N, 21.24.

2-((3-Methoxyphenyl)amino)acetohydrazide (4e). This compound was prepared from the corresponding *N*-(trifluoroacetyl)glycinate **3e** as described in the synthesis of **4a**, as white solids. mp 106–108 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.59 (2H, d, J = 6.6 Hz), 3.66 (3H, s), 4.23 (2H, brs), 5.85 (1H, t, J = 6.3 Hz), 6.10–6.16 (3H, m), 6.96 (1H, t, J = 8.0 Hz), 9.05 (1H, brs). Anal. Calcd. for $C_9H_{13}N_3O_2$: C, 55.37; H, 6.71; N, 21.52. Found: C, 55.10; H, 6.56; N, 21.37.

2-((4-Methoxyphenyl)amino)acetohydrazide (4f). This compound was prepared from the corresponding *N*-(trifluoroacetyl)glycinate **3f** as described in the synthesis of **4a**, as pink solids. mp 104–106 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.55 (2H, d, J = 6.3 Hz), 3.62 (3H, s), 4.23 (2H, br), 5.42 (1H, t, J = 6.2 Hz), 6.46–6.52 (2H, m), 6.67–6.72 (2H, m), 9.00 (1H, brs). Anal. Calcd. for $C_9H_{13}N_3O_2$•0.25H_2O: C, 54.12; H, 6.81; N, 21.04. Found: C, 54.22; H, 6.65; N, 20.80.

Data of 2-anilino-*N*'-((*E*)-pyridin-4-ylmethylene)acetohydrazide (Hit compound 5). ^{1}H NMR (600 MHz, DMSO-d_6, ratio: 63:37, the minor signals are marked with an asterisk, mixture signals are marked with double asterisks) δ 3.83* (0.74H, d, J = 6.2 Hz), 4.26 (1.26H, d, J = 5.9 Hz), 5.72

(0.63H, t, *J* = 5.7 Hz), 6.00* (0.37H, t, *J* = 5.9 Hz), 6.54-6.61** (1.74H, m), 6.64 (1.26H, d, *J* = 8.1 Hz), 7.05-7.13** (2H, m), 7.61* (0.74H, d, *J* = 5.5 Hz), 7.69 (1.26H, d, *J* = 5.5 Hz), 8.00 (0.63H, s), 8.26* (0.37H, s), 8.59-8.68** (2H, m), 11.71* (0.37H, brs), 11.76 (0.63H, brs). ^{13}C NMR (151 MHz, DMSO-d_6, the minor signals are marked with an asterisk, mixture signals are marked with double asterisks) δ 43.72, 45.95*, 112.21, 112.24*, 115.98, 116.42*, 120.72, 120.83*, 128.70, 128.77*, 140.89, 141.14*, 141.34*, 144.34, 148.13*, 148.22, 150.12**, 167.42*, 171.81. MS (ESI/APCI) *m/z* 255.0 $[M + H]^+$. HPLC purity 100.0%.

General procedure for the preparation of 2-((2-chlorophenyl)amino)-*N*'-((*E*)-pyridin-4-ylmethylene)acetohydrazide (5a).

4-Pyridinecarboaldehyde (0.24 mL) was added to a solution of 2-((2-chlorophenyl)amino)acetohydrazide (**4a**, 500 mg, 2.50 mmol) in methanol (5.0 mL) at room temperature. The mixture was stirred at room temperature for 4 h. The solid was collected, washed with methanol and dried to afford 649 mg of **5a** (2.25 mmol, 90%) as white solids. mp 208–210 ºC. ^{1}H NMR (600 MHz, DMSO-d_6, ratio: 3:1, the minor signals are marked with an asterisk, mixture signals are marked with double asterisks) δ 3.97* (0.5H, d, *J* = 5.9 Hz), 4.37 (1.5H, d, *J* = 5.5 Hz), 5.55 (0.75H, t, *J* = 5.3 Hz), 5.73* (0.25H, t, *J* = 5.7 Hz), 6.59* (0.25H, d, *J* = 8.1 Hz), 6.61–6.66** (1H, m), 6.69–6.74 (0.75H, m), 7.12–7.19** (1H, m), 7.29** (1H, dd, *J* = 7.9, 1.3 Hz), 7.63* (0.5H, d, *J* = 5.5 Hz), 7.71 (1.5H, d, *J* = 5.9 Hz), 8.01 (0.75H, s), 8.25* (0.25H, s), 8.60–8.69** (2H, m), 11.88** (1H, brs). ^{13}C NMR (151 MHz, DMSO-d_6, ratio: 3:1, the minor signals are marked with an

asterisk, mixture signals are marked with double asterisks) δ 43.79, 45.34*, 111.35*, 111.68, 116.85, 117.14*, 117.78, 117.99*, 120.79, 120.85*, 127.95**, 128.73, 128.89*, 141.05, 141.28*, 141.32**, 143.48, 143.59*, 144.42*, 150.12, 166.69*, 171.16. Anal. Calcd. for $C_{14}H_{13}ClN_4O$: C, 58.24; H, 4.54; N, 19.40. Found: C, 58.14; H, 4.52; N, 19.43. MS (ESI/APCI) *m/z* 289.0 $[M + H]^+$. HPLC purity 100.0%.

2-((3-Chlorophenyl)amino)-*N*'-((*E*)-pyridin-4-ylmethylene)acetohydrazide (5b). This compound was prepared from the corresponding anilinoacetohydrazide **4b** as described in the synthesis of **5a**, as white solids. mp 180–183 ºC. ^{1}H NMR (600 MHz, DMSO-d_6, ratio: 65:35, the minor signals are marked with an asterisk, mixture signals are marked with double asterisks) δ 3.86* (0.70H, d, J = 6.2 Hz), 4.29 (1.30H, d, J = 5.9 Hz), 6.14 (0.65H, t, J = 5.9 Hz), 6.37* (0.35H, t, J = 5.9 Hz), 6.51–6.64** (2.35H, m), 6.67 (0.65H, s), 7.03–7.14** (1H, m), 7.62* (0.70H, d, J = 5.5 Hz), 7.69 (1.30H, d, J = 5.5 Hz), 8.00 (0.65H, s), 8.25* (0.35H, s), 8.64** (2H, d, J = 5.5 Hz), 11.77** (1H, s). ^{13}C NMR (151 MHz, DMSO-d_6, the minor signals are marked with an asterisk, mixture signals are marked with double asterisks) δ 43.55, 45.41*, 110.88**, 111.46**, 115.30, 115.71*, 120.76, 120.86*, 130.16, 130.27*, 133.43, 133.46*, 141.01, 141.11*, 141.29*, 144.42, 149.73*, 149.89, 150.11**, 166.92*, 171.42. Anal. Calcd. for $C_{14}H_{13}ClN_4O$: C, 58.24; H, 4.54; N, 19.40. Found: C, 57.90; H, 4.48; N, 19.24. MS (ESI/APCI) *m/z* 287.0 $[M – H]^+$. HPLC purity 100.0%.

2-((4-Chlorophenyl)amino)-*N*'-((*E*)-pyridin-4-ylmethylene)acetohydrazide (5c). This compound was prepared from the corresponding anilinoacetohydrazide **4c** as described in the synthesis of **5a**, as

white solids. mp 220–223 ºC. ^{1}H NMR (600 MHz, DMSO-d_6, ratio: 2:1, the minor signals are marked with an asterisk, mixture signals are marked with double asterisks) δ 3.84* (2/3H, d, J = 5.9 Hz), 4.26 (4/3H, d, J = 5.5 Hz), 5.99 (2/3H, t, J = 5.9 Hz), 6.23* (1/3H, t, J = 6.1 Hz), 6.60* (2/3H, d, J = 8.8 Hz), 6.65 (4/3H, d, J = 8.8 Hz), 7.10 (4/3H, d, J = 8.8 Hz), 7.12* (2/3H, d, J = 8.8 Hz), 7.62* (2/3H, d, J = 5.5 Hz), 7.68 (4/3H, d, J = 5.9 Hz), 7.99 (2/3H, s), 8.25* (1/3H, s), 8.64** (2H, d, J = 5.5 Hz), 11.73* (1/3H, s), 11.78 (2/3H, s). ^{13}C NMR (151 MHz, DMSO-d_6, the minor signals are marked with an asterisk, mixture signals are marked with double asterisks) δ 43.77, 45.76*, 113.59**, 119.19, 119.66*, 120.74, 120.84*, 128.36, 128.46*, 140.96, 141.11*, 141.31*, 144.39, 147.13*, 147.26, 150.11**, 167.05*, 171.50. Anal. Calcd. for $C_{14}H_{13}ClN_4O$: C, 58.24; H, 4.54; N, 19.40. Found: C, 58.19; H, 4.50; N, 19.35. MS (ESI/APCI) *m/z* 287.0 $[M - H]^+$. HPLC purity 100.0%.

2-((2-Methoxyphenyl)amino)-*N*'-((*E*)-pyridin-4-ylmethylene)acetohydrazide (5d). This compound was prepared from the corresponding anilinoacetohydrazide **4d** as described in the synthesis of **5a**, as white solids. mp 207–211 ºC. ^{1}H NMR (600 MHz, DMSO-d_6, ratio: 5:2, the minor signals are marked with an asterisk, mixture signals are marked with double asterisks) δ 3.81** (3H, s), 3.87* (0.57H, d, J = 5.9 Hz), 4.28 (1.43H, d, J = 5.5 Hz), 5.18 (0.71H, t, J = 5.5 Hz), 5.33* (0.29H, t, J = 5.9 Hz), 6.43* (0.29H, d, J = 7.7 Hz), 6.55–6.64** (1.71H, m), 6.75–6.82** (1H, m), 6.84** (1H, d, J = 8.1 Hz), 7.62* (0.57H, d, J = 5.9 Hz), 7.70 (1.43H, d, J = 5.9 Hz), 8.01 (0.71H, s), 8.24* (0.29H, s), 8.63* (0.57H, d, J = 5.9 Hz), 8.65 (1.43H, d, J = 5.9 Hz), 11.84** (1H, brs). ^{13}C NMR (151 MHz, DMSO-d_6, the minor signals are marked with an asterisk, mixture signals are

marked with double asterisks) δ 43.89, 45.77*, 55.23**, 109.38*, 109.58, 109.60, 109.76*, 116.03, 116.43*, 120.76, 120.84*, 120.91*, 120.95, 137.31, 137.34*, 141.07*, 141.21, 141.31*, 144.35, 146.31, 146.45*, 150.13**, 167.19*, 171.59. Anal. Calcd. for $C_{15}H_{16}N_4O_2$: C, 63.37; H, 5.67; N, 19.71. Found: C, 63.39; H, 5.67; N, 19.76. MS (ESI/APCI) *m/z* 285.1 $[M + H]^+$. HPLC purity 100.0%.

2-((3-Methoxyphenyl)amino)-*N*'-((*E*)-pyridin-4-ylmethylene)acetohydrazide (5e). This compound was prepared from the corresponding anilinoacetohydrazide **4e** as described in the synthesis of **5a**, as yellow solids. mp 137–139 ºC. ^{1}H NMR (600 MHz, DMSO-d_6, ratio: 3:2, the minor signals are marked with an asterisk, mixture signals are marked with double asterisks) δ 3.66 (1.8H, s), 3.67* (1.2H, brs), 3.82* (0.8H, d, *J* = 6.2 Hz), 4.24 (1.2H, d, *J* = 5.9 Hz), 5.76 (0.6H, t, *J* = 5.9 Hz), 6.02* (0.4H, t, *J* = 5.9 Hz), 6.13–6.27** (3H, m), 6.94–7.03** (1H, m), 7.61* (0.8H, d, *J* = 5.9 Hz), 7.68 (1.2H, d, *J* = 5.9 Hz), 8.00 (0.6H, s), 8.25* (0.4H, s), 8.61–8.67** (2H, m), 11.75** (1H, brs). ^{13}C NMR (151 MHz, DMSO-d_6, the minor signals are marked with an asterisk, mixture signals are marked with double asterisks) δ 43.77, 45.92*, 54.53, 54.58*, 98.17, 98.21*, 101.53, 101.88*, 105.20, 105.26*, 120.72, 120.84*, 129.43, 129.52*, 140.92, 141.12*, 141.33*, 144.32, 149.48*, 149.60*, 150.12**, 160.17**, 167.35*, 171.80. Anal. Calcd. for $C_{15}H_{16}N_4O_2$: C, 63.37; H, 5.67; N, 19.71. Found: C, 63.09; H, 5.63; N, 19.53. MS (ESI/APCI) *m/z* 285.1 $[M + H]^+$. HPLC purity 100.0%.

2-((4-Methoxyphenyl)amino)-*N*'-((*E*)-pyridin-4-ylmethylene)acetohydrazide (5f). This

compound was prepared from the corresponding anilinoacetohydrazide **4f** as described in the synthesis of **5a**, as white solids. mp 139–144 ºC. ^{1}H NMR (600 MHz, DMSO-d_6, ratio: 3:2, the minor signals are marked with an asterisk, mixture signals are marked with double asterisks) δ 3.64** (3H, s), 3.78* (0.8H, s), 4.21 (1.2H, s), 5.50** (1H, brs), 6.55* (0.8H, d, J = 8.8 Hz), 6.61 (1.2H, d, J = 8.8 Hz), 6.70–6.77** (2H, m), 7.61* (0.8H, d, J = 5.5 Hz), 7.68 (1.2H, d, J = 5.9 Hz), 7.99 (0.6H, s), 8.26* (0.4H, s), 8.59–8.69** (2H, m), 11.67* (0.4H, s), 11.74 (0.6H, s). ^{13}C NMR (151 MHz, DMSO-d_6, the minor signals are marked with an asterisk, mixture signals are marked with double asterisks) δ 44.52, 46.91*, 55.22**, 113.30, 113.33*, 114.45, 114.49*, 120.72, 120.83*, 140.83, 141.18, 141.38*, 142.26*, 142.44, 144.31*, 150.09**, 150.91, 151.20*, 167.67*, 172.08. Anal. Calcd. for $C_{15}H_{16}N_4O_2$•0.1H_2O: C, 62.97; H, 5.71; N, 19.58. Found: C, 62.74; H, 5.57; N, 19.68. MS (ESI/APCI) *m/z* 285.1 $[M + H]^+$. HPLC purity 93.8%.

2,2,2-Trifluoro-*N*-phenylacetamide (7a). This compound was prepared from aniline **6a** as described in the synthesis of **2a**, as pale brown solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20–7.29 (1H, m), 7.35–7.47 (2H, m), 7.51–7.62 (2H, m), 7.85 (1H, brs).

2,2,2-Trifluoro-*N*-(3-phenoxyphenyl)acetamide (7d). This compound was prepared from 3-phenoxyaniline **6d** as described in the synthesis of **2a**, as brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.88 (1H, ddd, J = 7.7, 2.3, 1.6 Hz), 7.02–7.06 (2H, m), 7.16 (1H, tt, J = 7.5, 1.3 Hz), 7.23 (1H, t, J = 2.3 Hz), 7.26–7.41 (4H, m), 7.80 (1H, br).

***N*-(3-(Benzyloxy)phenyl)-2,2,2-trifluoroacetamide (7e).** This compound was prepared from

3-benzyloxyaniline **6e** as described in the synthesis of **2a**, as white solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.08 (2H, s), 6.86 (1H, ddd, *J* = 8.6, 2.4, 0.9 Hz), 7.06 (1H, ddd, *J* = 8.0, 2.0, 0.7 Hz), 7.26–7.46 (7H, m), 7.81 (1H, br).

Ethyl 2-(2,2,2-trifluoro-*N*-phenylacetamido)acetate (8a). This compound was prepared from 2,2,2-Trifluoro-*N*-phenylacetamide **7a** as described in the synthesis of **2a**, as crude product. This product was used without further purification.

Ethyl *N*-(3-phenoxyphenyl)-*N*-(trifluoroacetyl)glycinate (8d). This compound was prepared from the corresponding trifluoroacetanilide **7d** as described in the synthesis of **3a**, as yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26 (3H, t, *J* = 7.2 Hz), 4.21 (2H, q, *J* = 7.1 Hz), 4.37 (2H, s), 7.00–7.08 (4H, m), 7.12–7.19 (2H, m), 7.35–7.40 (3H, m).

Ethyl *N*-(3-(benzyloxy)phenyl)-*N*-(trifluoroacetyl)glycinate (8e). This compound was prepared from the corresponding trifluoroacetanilide **7e** as described in the synthesis of **3a**, as brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (3H, t, *J* = 7.1 Hz), 4.23 (2H, q, *J* = 7.2 Hz), 4.37 (2H, s), 5.06 (2H, s), 6.97–7.05 (3H, m), 7.30–7.45 (6H, m).

Ethyl ((3-hydroxyphenyl)(trifluoroacetyl)amino)acetate (9). Ethyl *N*-(3-(benzyloxy)phenyl)-*N*-(trifluoroacetyl)glycinate (**8e**, 10.5 g, 27.5 mmol) and 10% palladium – on activated carbon (812 mg) in ethanol (100 mL) was stirred at room temperature for 2 h under hydrogen balloon. The reaction mixture was filtered and then washed with ethanol. The filtrate was concentrated under reduced pressure. The solid residue was purified by recrystallization from ethyl

acetate and hexane to afford 6.47 g of **9** as pale yellow solids. The mother liquor was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting hexane and ethyl acetate to afford 1.37 g of **9** as pale yellow solids. Total: 7.84 g (26.9 mmol, 98% yield). mp 97–100 ºC. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (3H, t, *J* = 7.2 Hz), 4.24 (2H, q, *J* = 7.2 Hz), 4.38 (2H, s), 5.31 (1H, s), 6.88–6.92 (2H, m), 6.95 (1H, d, *J* = 8.4 Hz), 7.28 (1H, td, *J* = 8.0, 0.8 Hz). Anal. Calcd. for $C_{12}H_{12}F_3NO_4$: C, 49.49; H, 4.15; N, 4.81. Found: C, 49.12; H, 4.16; N, 4.79.

Ethyl ((3-ethoxyphenyl)(trifluoroacetyl)amino)acetate (10c). A mixture of ethyl ((3-hydroxyphenyl)(trifluoroacetyl)amino)acetate (**9**, 2.09 g, 7.18 mmol), iodoethane (0.69 g) and potassium carbonate (1.19 g) in DMF (15 mL) was stirred at room temperature for overnight. The reaction mixture was diluted with ethyl acetate. The mixture was washed with 0.1 N sodium thiosulfate aqueous solution and then water. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford 2.25 g of **10c** (7.05 mmol, 98%) as crude brown oil. This product was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (3H, t, *J* = 7.2 Hz), 1.42 (3H, t, *J* = 6.9 Hz), 4.03 (2H, q, *J* = 7.0 Hz), 4.24 (2H, q, *J* = 7.2 Hz), 4.38 (2H, s), 6.94–6.96 (3H, m), 7.28–7.34 (1H, m).

2-(Phenylamino)acetohydrazide (11a). This compound was prepared from ethyl 2-(2,2,2-trifluoro-*N*-phenylacetamido)acetate **8a** as described in the synthesis of **4a**, as pale brown solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.88 (2H, s), 6.58–6.65 (2H, m), 6.79–6.87 (1H, m), 7.17–7.26 (2H, m), 7.75 (1H, brs).

2-((3-Ethoxyphenyl)amino)acetohydrazide (11c). This compound was prepared from the corresponding *N*-(trifluoroacetyl)glycinate **10c** as described in the synthesis of **4a**, as white solids. mp 96–98 ºC. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (3H, t, *J* = 7.1 Hz), 3.86 (2H, d, *J* = 5.4 Hz), 3.87 (2H, br), 3.99 (2H, q, *J* = 7.0 Hz), 4.14 (1H, t, *J* = 5.4 Hz), 6.15 (1H, t, *J* = 2.1 Hz), 6.21 (1H, dd, *J* = 8.1, 2.4 Hz), 6.37 (1H, dd, *J* = 8.1, 2.4 Hz), 7.11 (1H, t, *J* = 8.1 Hz), 7.72 (1H, brs). Anal. Calcd. for $C_{10}H_{15}N_3O_2$•0.1H_2O: C, 56.91; H, 7.26; N, 19.91. Found: C, 57.07; H, 7.11; N, 19.53.

2-((3-Phenoxyphenyl)amino)acetohydrazide (11d). This compound was prepared from the corresponding *N*-(trifluoroacetyl)glycinate **8d** as described in the synthesis of **4a**, as brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.84 (2H, d, *J* = 6.0 Hz), 3.85 (2H, br), 4.21 (1H, t, *J* = 5.6 Hz), 6.27 (1H, t, *J* = 2.3 Hz), 6.33 (1H, ddd, *J* = 8.2, 2.3, 0.8 Hz), 6.43 (1H, ddd, *J* = 8.2, 2.2, 0.8 Hz), 6.99–7.03 (2H, m), 7.11 (1H, tt, *J* = 7.4, 1.3 Hz), 7.14 (1H, t, *J* = 8.0 Hz), 7.30–7.37 (2H, m), 7.68 (1H, brs).

2-((3-(Benzyloxy)phenyl)amino)acetohydrazide (11e). This compound was prepared from the corresponding *N*-(trifluoroacetyl)glycinate **8e** as described in the synthesis of **4a**, as white solids. mp 111–112 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.59 (2H, d, *J* = 6.3 Hz), 4.25 (2H, brs), 5.00 (2H, s), 5.87 (1H, t, *J* = 6.3 Hz), 6.15–6.24 (3H, m), 6.96 (1H, t, *J* = 8.0 Hz), 7.29–7.44 (5H, m), 9.06 (1H, brs). Anal. Calcd. for $C_{15}H_{17}N_3O_2$•0.2H_2O: C, 65.53; H, 6.38; N, 15.28. Found: C, 65.42; H, 6.11; N, 15.23.

General procedure for the preparation of 2-((3-(benzyloxy)phenyl)amino)-*N*'-((5*E*)-7,8-dihydroisoquinolin-5(6*H*)-ylidene)acetohydrazide

(12e). A mixture of 2-((3-(benzyloxy)phenyl)amino)acetohydrazide (**11e**, 309 mg, 1.14 mmol) and 7,8-dihydroisoquinolin-5(6*H*)-one (168 mg) in ethanol (5.5 mL) was stirred at 90 ºC for 13 h. The reaction mixture was concentrated under reduced pressure. The residue was purified by recrystallization from ethanol to afford 345 mg of **10e** (0.86 mmol, 76%) as light yellow solids. mp 168–170 ºC. ^{1}H NMR (600 MHz, $CDCl_3$, ratio: 85:15, the minor signals are omitted) δ 1.99 (2H, quintet, *J* = 6.1 Hz), 2.56 (2H, t, *J* = 6.6 Hz), 2.79 (2H, t, *J* = 5.9 Hz), 4.38 (2H, d, *J* = 4.0 Hz), 4.67 (1H, brs), 5.04 (2H, s), 6.32–6.36 (2H, m), 6.37–6.41 (1H, m), 7.12 (1H, t, *J* = 7.9 Hz), 7.29–7.33 (1H, m), 7.37 (2H, t, *J* = 7.5 Hz), 7.41–7.46 (2H, m), 7.86 (1H, d, *J* = 5.1 Hz), 8.49 (1H, s), 8.53 (1H, d, *J* = 5.1 Hz), 9.24 (1H, s). ^{13}C NMR (151 MHz, $CDCl_3$, the minor signals are omitted) δ 21.08, 24.60, 26.03, 45.16, 69.94, 100.15, 103.52, 106.65, 117.66, 127.57, 127.95, 128.58, 130.08, 133.84, 137.17, 138.90, 146.23, 148.04, 148.76, 150.41, 160.14, 172.50. Anal. Calcd. for $C_{24}H_{24}N_4O_2$•0.1H_2O: C, 71.66; H, 6.06; N, 13.93. Found: C, 71.31; H, 5.99; N, 13.67. MS (ESI/APCI) *m/z* 401.2 $[M + H]^+$. HPLC purity 98.8%.

2-Anilino-*N*'-((5*E*)-7,8-dihydroisoquinolin-5(6*H*)-ylidene)acetohydrazide (12a). This compound was prepared from the corresponding anilinoacetohydrazide **11a** as described in the synthesis of **12e**, as white solids. mp 183 ºC. ^{1}H NMR (600 MHz, $CDCl_3$, ratio: 4:1, the minor signals are marked with an asterisk, mixture signals are marked with double asterisks) δ 1.85–1.93* (0.4H, m), 2.02 (1.6H, quintet, *J* = 6.3 Hz), 2.37* (0.4H, t, *J* = 6.4 Hz), 2.57 (1.6H, t, *J* = 6.4 Hz), 2.73* (0.4H, t, *J* = 5.9 Hz), 2.82 (1.6H, t, *J* = 6.1 Hz), 4.03* (0.4H, d, *J* = 5.5 Hz), 4.29* (0.2H, brs), 4.42 (1.6H, d, *J* = 4.8 Hz),

4.64 (0.8H, brs), 6.72** (2H, d, *J* = 8.1 Hz), 6.76 (0.8H, t, *J* = 7.3 Hz), 6.88* (0.2H, t, *J* = 7.3 Hz), 7.23** (2H, t, *J* = 7.9 Hz), 7.87 (0.8H, d, *J* = 5.1 Hz), 8.07* (0.2H, d, *J* = 5.1 Hz), 8.43* (0.2H, s), 8.46* (0.2H, d, *J* = 5.1 Hz), 8.50 (0.8H, s), 8.54 (0.8H, d, *J* = 5.5 Hz), 8.98 (0.8H, brs), 9.79* (0.2H, brs). ^{13}C NMR (151 MHz, $CDCl_3$, the minor signals are marked with an asterisk, mixture signals are marked with double asterisks) δ 21.07**, 24.49, 24.57*, 25.97, 26.03*, 45.27, 49.16*, 113.09, 113.70*, 117.59, 117.98, 118.58*, 120.11*, 129.34, 129.68*, 133.82**, 138.89**, 145.95**, 147.35**, 148.04**, 150.05*, 150.46, 172.40**. Anal. Calcd. for $C_{17}H_{18}N_4O$: C, 69.37; H, 6.16; N, 19.03. Found: C, 69.31; H, 6.31; N, 18.96. MS (ESI/APCI) *m/z* 295.1 $[M + H]^+$. HPLC purity 98.9%.

***N*'-((5*E*)-7,8-Dihydroisoquinolin-5(6*H*)-ylidene)-2-((3-methoxyphenyl)amino)acetohydrazide (12b).** This compound was prepared from the corresponding anilinoacetohydrazide **11b** as described in the synthesis of **12e**, as white solids. mp 140–141 ºC. ^{1}H NMR (600 MHz, $CDCl_3$, ratio: 4:1, the minor signals are marked with an asterisk, mixture signals are marked with double asterisks) δ 1.87-1.94* (0.4H, m), 2.01 (1.6H, quintet, *J* = 6.2 Hz), 2.39* (0.4H, t, *J* = 6.4 Hz), 2.57 (1.6H, t, *J* = 6.6 Hz), 2.74* (0.4H, t, *J* = 5.7 Hz), 2.81 (1.6H, t, *J* = 5.9 Hz), 3.78* (0.6H, brs), 3.79 (2.4H, s), 4.01* (0.4H, s), 4.40 (1.6H, s), 4.66** (1H, brs), 6.25–6.28** (1H, m), 6.33 (1.6H, td, *J* = 8.0, 2.0 Hz), 6.43* (0.4H, d, *J* = 8.1 Hz), 7.13 (0.8H, t, *J* = 8.1 Hz), 7.14-7.18* (0.2H, m), 7.87 (0.8H, d, *J* = 5.1 Hz), 8.07* (0.2H, d, *J* = 5.1 Hz), 8.44* (0.2H, s), 8.46* (0.2H, d, *J* = 5.1 Hz), 8.50 (0.8H, s), 8.53 (0.8H, d, *J* = 5.1 Hz), 9.12 (0.8H, s), 9.76* (0.2H, brs). ^{13}C NMR (151 MHz, $CDCl_3$, the minor signals are marked with an asterisk, mixture signals are marked with double asterisks) δ 21.07**,

24.55**, 25.98*, 26.04, 45.21, 49.01*, 55.15, 55.25*, 99.39, 100.08*, 102.70, 105.06*, 106.30**, 117.68, 118.66*, 130.11, 130.56*, 133.87**, 138.99**, 146.07**, 147.82*, 147.94, 148.19*, 148.73, 149.88*, 150.33, 160.87**, 172.42**. Anal. Calcd. for $C_{18}H_{20}N_4O_2$: C, 66.65; H, 6.21; N, 17.27. Found: C, 66.42; H, 6.21; N, 17.03. MS (ESI/APCI) *m/z* 325.2 $[M + H]^+$. HPLC purity 100.0%.

***N*'-((5*E*)-7,8-Dihydroisoquinolin-5(6*H*)-ylidene)-2-((3-ethoxyphenyl)amino)acetohydrazide (12c).** This compound was prepared from the corresponding anilinoacetohydrazide **11c** as described in the synthesis of **12e**, as white solids. mp 159–162 ºC. ^{1}H NMR (600 MHz, $CDCl_3$, ratio: 4:1, the minor signals are marked with an asterisk, mixture signals are marked with double asterisks) δ 1.40** (3H, t, *J* = 7.0 Hz), 1.90* (0.4H, d, *J* = 5.1 Hz), 1.96–2.05 (1.6H, m), 2.34–2.43* (0.4H, m), 2.57 (1.6H, t, *J* = 6.4 Hz), 2.73* (0.4H, brs), 2.81 (1.6H, t, *J* = 5.9 Hz), 4.01** (2H, q, *J* = 7.0 Hz + 0.4H, m), 4.31* (0.2H, brs), 4.40 (1.6H, s), 4.65 (0.8H, brs), 6.26** (1H, d, *J* = 1.8 Hz), 6.28–6.36** (1H + 0.8H, m), 6.42* (0.2H, d, *J* = 7.0 Hz), 7.06–7.17** (1H, m), 7.87 (0.8H, d, *J* = 5.1 Hz), 8.06* (0.2H, d, *J* = 4.8 Hz), 8.43* (0.2H, brs), 8.45* (0.2H, d, *J* = 5.1 Hz), 8.50 (0.8H, s), 8.54 (0.8H, d, *J* = 5.1 Hz), 9.25 (0.8H, brs), 9.77* (0.2H, brs). ^{13}C NMR (151 MHz, $CDCl_3$, the minor signals are marked with an asterisk, mixture signals are marked with double asterisks) δ 14.82*, 14.92, 21.09**, 24.60**, 25.98, 26.04*, 45.21, 49.01*, 63.25, 63.43*, 99.88, 100.53*, 103.29**, 105.63*, 106.26, 117.68, 118.58*, 130.05, 130.49*, 133.85**, 138.93**, 146.20**, 147.97*, 148.03, 148.17*, 148.75, 150.03*, 150.41, 160.21, 160.35*, 166.57*, 172.57. Anal. Calcd. for $C_{19}H_{22}N_4O_2$: C, 67.44; H, 6.55; N, 16.56. Found: C, 67.21; H, 6.37; N, 16.37. MS (ESI/APCI) *m/z* 339.2 $[M + H]^+$. HPLC purity

100.0%.

***N*'-((5*E*)-7,8-Dihydroisoquinolin-5(6*H*)-ylidene)-2-((3-phenoxyphenyl)amino)acetohydrazide (12d).** This compound was prepared from the corresponding anilinoacetohydrazide **11d** as described in the synthesis of **12e**, as white solids. mp 170–172 ºC. ^{1}H NMR (600 MHz, $CDCl_3$, ratio: 8:1, the minor signals are omitted) δ 2.00 (2H, quintet, *J* = 6.3 Hz), 2.57 (2H, t, *J* = 6.6 Hz), 2.80 (2H, t, *J* = 6.1 Hz), 4.38 (2H, d, *J* = 5.1 Hz), 4.71 (1H, t, *J* = 5.0 Hz), 6.35–6.39 (2H, m), 6.43–6.50 (1H, m), 7.03 (2H, d, *J* = 7.7 Hz), 7.07–7.11 (1H, m), 7.12–7.17 (1H, m), 7.30–7.34 (2H, m), 7.81 (1H, d, *J* = 5.1 Hz), 8.49 (1H, s), 8.52 (1H, d, *J* = 5.1 Hz), 9.24 (1H, s). ^{13}C NMR (151 MHz, $CDCl_3$, the minor signals are omitted) δ 21.09, 24.61, 26.03, 45.12, 103.37, 108.04, 108.33, 117.62, 119.03, 123.14, 129.62, 130.30, 133.84, 138.85, 146.28, 148.03, 148.94, 150.43, 157.13, 158.52, 172.35. Anal. Calcd. for $C_{23}H_{22}N_4O_2$•0.2H_2O: C, 70.82; H, 5.79; N, 14.36. Found: C, 70.84; H, 5.68; N, 14.02. MS (ESI/APCI) *m/z* 387.1 $[M + H]^+$. HPLC purity 100.0%.

***N*'-((5*E*)-7,8-Dihydroisoquinolin-5(6*H*)-ylidene)-2-((3-(morpholin-4-ylcarbonyl)phenyl)amino)acetohydrazide (12f).** This compound was prepared from the corresponding anilinoacetohydrazide **20f** as described in the synthesis of **12e**, as white foam. ^{1}H NMR (600 MHz, $CDCl_3$ ratio: 8:1, the minor signals are omitted) δ 2.01 (2H, quintet, *J* = 6.2 Hz), 2.59 (2H, t, *J* = 6.4 Hz), 2.82 (2H, t, *J* = 5.9 Hz), 3.47 (2H, brs), 3.63 (2H, brs), 3.76 (4H, brs), 4.41 (2H, d, *J* = 4.8 Hz), 4.81 (1H, t, *J* = 4.8 Hz), 6.70–6.77 (3H, m), 7.23 (1H, t, *J* = 7.9 Hz), 7.87 (1H, d, *J* = 5.1 Hz), 8.50 (1H, s), 8.55 (1H, d, *J* = 5.1 Hz), 9.21 (1H, s). ^{13}C NMR (151 MHz, $CDCl_3$, the minor signals are omitted) δ 21.10, 24.65,

26.03, 42.55, 45.02, 48.22, 66.96 (2C), 111.22, 114.49, 115.97, 117.65, 129.39, 133.88, 136.47, 138.82, 146.45, 147.63, 148.07, 150.46, 170.83, 172.07. MS (ESI/APCI) *m/z* 408.1 $[M + H]^+$. HPLC purity 100.0%.

***N*-Benzyl-3-((2-((2*E*)-2-(7,8-dihydroisoquinolin-5(6*H*)-ylidene)hydrazino)-2-oxoethyl)amino)benzamide (12g).** This compound was prepared from the corresponding anilinoacetohydrazide **20g** as described in the synthesis of **12e**, as white solids. mp 221–225 ºC. ^{1}H NMR (600 MHz, DMSO-d_6, ratio: 3:1, the minor signals are marked with an asterisk, mixture signals are marked with double asterisks) δ 1.83–1.88** (2H, m), 2.66** (2H, t, *J* = 6.4 Hz), 2.76** (2H, t, *J* = 5.9 Hz), 3.99* (0.5H, d, *J* = 4.0 Hz), 4.37 (1.5H, d, *J* = 5.9 Hz), 4.45** (2H, d, *J* = 5.9 Hz), 5.97 (0.75H, t, *J* = 5.3 Hz), 6.14* (0.25H, brs), 6.80** (1H, d, *J* = 7.7 Hz), 7.08–7.10 (0.75H, m), 7.12** (1.25H, brs), 7.16** (1H, t, *J* = 7.7 Hz), 7.21–7.26** (1H, m), 7.29–7.33** (4H, m), 7.81* (0.25H, brs), 7.92 (0.75H, d, *J* = 5.1 Hz), 8.41** (1H, d, *J* = 5.1 Hz), 8.46** (1H, s), 8.84** (1H, t, *J* = 6.1 Hz), 10.61* (0.25H, brs), 10.92 (0.75H, brs). ^{13}C NMR (151 MHz, DMSO-d_6, the minor signals are marked with an asterisk, mixture signals are marked with double asterisks) δ 20.69**, 24.98, 25.32**, 42.38**, 44.06, 45.34*, 110.80, 111.04*, 114.64, 114.91*, 115.10**, 117.37**, 126.51**, 126.98**, 128.10**, 128.54**, 133.93, 134.16* 135.09**, 139.09**, 139.77**, 145.40**, 147.26**, 148.22*, 148.43, 148.75*, 150.06, 166.75, 167.17*, 172.29**. Anal. Calcd. for $C_{25}H_{25}N_5O_2$: C, 70.24; H, 5.89; N, 16.38. Found: C, 70.30; H, 5.70; N, 16.28. MS (ESI/APCI) *m/z* 428.2 $[M + H]^+$. HPLC purity 99.1%

Ethyl 3-((trifluoroacetyl)amino)benzoate (14). This compound was prepared from ethyl

3-aminobenzoate **13** as described in the synthesis of **2a**, as white solids. mp 99–101 ºC. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (3H, t, *J* = 7.2 Hz), 4.39 (2H, q, *J* = 7.0 Hz), 7.50 (1H, t, *J* = 8.0 Hz), 7.91–7.98 (2H, m), 8.05 (1H, br), 8.10 (1H, t, *J* = 2.0 Hz). Anal. Calcd. for $C_{11}H_{10}F_3NO_3$: C, 50.58; H, 3.86; N, 5.36. Found: C, 50.46; H, 3.78; N, 5.25.

Ethyl 3-((2-(benzyloxy)-2-oxoethyl)(trifluoroacetyl)amino)benzoate (15). This compound was prepared from the corresponding trifluoroacetanilide **14** and benzyl bromoacetate as described in the synthesis of **3a**, as white solids. mp 110 ºC. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (3H, t, *J* = 7.1 Hz), 4.40 (2H, q, *J* = 7.1 Hz), 4.46 (2H, brs), 5.21 (2H, s), 7.32–7.39 (5H, m), 7.49 (1H, t, *J* = 7.7 Hz), 7.61 (1H, d, *J* = 8.1 Hz), 8.03 (1H, s), 8.10 (1H, dt, *J* = 7.5, 1.4 Hz). Anal. Calcd. for $C_{20}H_{18}F_3NO_5$: C, 58.68; H, 4.43; N, 3.42. Found: C, 58.72; H, 4.42; N, 3.42.

***N*-(3-(Ethoxycarbonyl)phenyl)-*N*-(trifluoroacetyl)glycine (16).** A mixture of ethyl 3-((2-(benzyloxy)-2-oxoethyl)(trifluoroacetyl)amino)benzoate (**15**, 25.0 g, 61.1 mmol) and 10% palladium – on activated carbon (2.50 g) in ethanol (800 mL) was stirred at room temperature for 2.5 h under hydrogen balloon. The reaction mixture was filtered and then washed with ethanol. The filtrate was concentrated under reduced pressure to afford 19.7 g of **16** (quantitative yield) as oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (3H, t, *J* = 7.1 Hz), 4.41 (2H, q, *J* = 7.1 Hz), 4.47 (2H, s), 7.53 (1H, t, *J* = 7.8 Hz), 7.63 (1H, d, *J* = 7.8 Hz), 8.05 (1H, s), 8.13 (1H, dt, *J* = 7.5, 1.4 Hz), CO_2H was not assigned.

***tert*-Butyl 2-(((3-(ethoxycarbonyl)phenyl)(trifluoroacetyl)amino)acetyl)hydrazinecarboxylate**

(17). A mixture of *N*-(3-(ethoxycarbonyl)phenyl)-*N*-(trifluoroacetyl)glycine (**16**, 9.86 g, 30.9 mmol), *tert*-butyl carbazate (4.49 g), WSC•HCl (8.88 g) and HOBt•H_2O (0.47 g) in DMF (100 mL) was stirred at room temperature for 16.5 h. Ethyl acetate was added to the reaction mixture and then the mixture was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting hexane and ethyl acetate to afford 13.4 g of **17** (quantitative yield) as white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (3H, t, *J* = 7.2 Hz), 1.47 (9H, s), 4.39 (2H, br), 4.40 (2H, q, *J* = 7.1 Hz), 6.54 (1H, br), 7.52 (1H, t, *J* = 7.8 Hz), 7.70 (1H, d, *J* = 8.4 Hz), 7.89 (1H, br), 8.04 (1H, s), 8.11 (1H, d, *J* = 7.8 Hz).

3-((2-(2-(*tert*-Butoxycarbonyl)hydrazino)-2-oxoethyl)amino)benzoic acid (18). 8 N NaOH aqueous solution (38.5 mL) was added to a solution of 2-(((3-(ethoxycarbonyl)phenyl)(trifluoroacetyl)amino)acetyl) hydrazinecarboxylate (**17**, 13.4 g, 30.9 mmol) in ethanol (40 mL) and THF (40 mL). The mixture was stirred at room temperature for 13 h. The reaction mixture was neutralized with acetic acid at 0 °C and then concentrated under reduced pressure. Water was added and the mixture was extracted with ethyl acetate and THF. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford 12.0 g of crude **18** (quantitative yield) as white foam. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.39 (9H, s), 3.71 (2H, d, *J* = 5.7 Hz), 6.17 (1H, br), 6.77–6.79 (1H, m), 7.16–7.29 (3H, m), 8.77 (1H, s), 9.71 (1H, s), 12.40 (1H, br).

General procedure for the preparation of *tert*-butyl 2-(((3-((benzylamino)carbonyl)phenyl)amino)acetyl)hydrazinecarboxylate (19g). A mixture of 3-((2-(2-(*tert*-butoxycarbonyl)hydrazino)-2-oxoethyl)amino)benzoic acid (**18**, 3.12 g, 10.1 mmol), benzylamine (1.3 mL), WSC•HCl (2.89 g) and HOBt•H_2O (0.15 g) in DMF (30 mL) was stirred at room temperature for 15 h. Ethyl acetate was added to the reaction mixture and then the mixture was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting hexane and ethyl acetate to afford 3.75 g of **19g** (9.41 mmol, 94%) as white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34 (9H, s), 3.91 (2H, d, *J* = 5.4 Hz), 4.42 (1H, t, *J* = 5.3 Hz), 4.64 (2H, d, *J* = 6.3 Hz), 6.38 (1H, brs), 6.79 (1H, dd, *J* = 8.0, 1.4 Hz), 7.09 (1H, s), 7.19–7.35 (7H, m), 7.54 (1H, br), 8.21 (1H, d, *J* = 2.7 Hz).

***tert*-Butyl 2-(((3-(4-morpholinylcarbonyl)phenyl)amino)acetyl)hydrazinecarboxylate (19f).** This compound was prepared from the corresponding aminobenzoic acid **18** and morpholine as described in the synthesis of **19g**, as white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46 (9H, s), 3.46 (2H, br), 3.64 (2H, br), 3.75 (4H, br), 3.87 (2H, d, *J* = 6.0 Hz), 4.56 (1H, t, *J* = 5.9 Hz), 6.45 (1H, br), 6.64–6.67 (2H, m), 6.77 (1H, dt, *J* = 7.5, 1.2 Hz), 7.22 (1H, dd, *J* = 8.7, 7.5 Hz), 8.21 (1H, d, *J* = 2.7 Hz).

General procedure for the preparation of *N*-benzyl-3-((2-hydrazino-2-oxoethyl)amino)benzamide (20g). A solution of 4 N HCl in ethyl

acetate (14 mL) was added to a solution of *tert*-butyl 2-(((3-((benzylamino)carbonyl)phenyl)amino)acetyl)hydrazinecarboxylate (**19g**, 3.74 g, 9.39 mmol) in ethyl acetate (40 mL) and ethanol (40 mL). The mixture was stirred at room temperature for overnight. The reaction mixture was concentrated under reduced pressure. Saturated $NaHCO_3$ aqueous solution was added to the residue. The mixture was extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by recrystallization from ethyl acetate and hexane to afford 2.58 g of **20g** (8.65 mmol, 92%) as white solids. mp 147–149 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.67 (2H, d, J = 6.0 Hz), 4.25 (2H, brs), 4.45 (2H, d, J = 6.0 Hz), 6.03 (1H, t, J = 5.9 Hz), 6.71 (1H, ddd, J = 8.0, 2.4, 1.2 Hz), 7.05–7.35 (8H, m), 8.85 (1H, t, J = 5.9 Hz), 9.09 (1H, s). Anal. Calcd. for $C_{16}H_{18}N_4O_2$: C, 64.41; H, 6.08; N, 18.78. Found: C, 64.25; H, 6.08; N, 18.57.

2-((3-(4-Morpholinylcarbonyl)phenyl)amino)acetohydrazide (20f). This compound was prepared from the corresponding Boc-hydrazide **19f** and morpholine as described in the synthesis of **20g**, as white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.44 (2H, br), 3.62 (2H, br), 3.75 (8H, br), 4.74 (1H, br), 6.60 (2H, br), 6.72–6.74 (1H, m), 7.17–7.23 (1H, m), 8.00 (1H, br).

General procedure for the preparation of pyridine-4-carboxyimidehydrazide (22). Anhydrous hydrazine (30.0 g) was added to a solution of 4-cyanopyridine (**4**, 15.0 g, 145 mmol) in diethyl ether (25 mL) and ethanol (20 mL) at 0 °C. The mixture was stirred at room temperature for overnight. After the reaction mixture was concentrated under reduced pressure, the precipitate was washed with

diethyl ether and ethanol to afford 18.9 g of **22** (96 %, 139 mmol) as white solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.34 (2H, br), 5.71 (2H, br), 7.62 (2H, d, J = 6.2 Hz), 8.50 (2H, d, J = 6.2 Hz).

General procedure for the preparation of 4-(3-(chloromethyl)-1*H*-1,2,4-triazol-5-yl)pyridine (23a). Pyridine-4-carboxyimidehydrazide (**22**, 6.00 g, 44.1 mmol) and DBU (5.0 mL) were dissolved with THF (250 mL) and DCM (400 mL). Chloroacetyl chloride (3.5 mL) was added at 0 °C. The mixture was stirred at room temperature for 1 h and then concentrated under reduced pressure. After water was added to the residue, the precipitate was collected by filtration, followed by washing with water to afford the corresponding intermediate as pale brown solids. After this product was dissolved with ethanol (500 mL), acetic acid (5.0 mL), the mixture was stirred at reflux for 5 h. After the reaction mixture was concentrated under reduced pressure, brine was added. The mixture was extracted with ethyl acetate. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to afford 4.00 g of **6a** (20.6 mmol, 47%) as white solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.80 (2H, s), 7.97 (2H, d, J = 6.0 Hz), 8.74 (2H, d, J = 6.0 Hz), NH was not assigned.

4-(5-(2-Chloroethyl)-1*H*-1,2,4-triazol-3-yl)pyridine (23b). This compound was prepared from 3-chloropropionyl chloride as described in the synthesis of **23a**, as white solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.37 (2H, t, J = 6.3 Hz), 3.95 (2H, t, J = 6.3 Hz), 7.96 (2H, dd, J = 4.4, 1.7 Hz), 8.71 (2H, dd, J = 4.2, 1.8 Hz), 11.55 (1H, br).

The data of 4-(3-(((4-chlorophenyl)sulfanyl)methyl)-1*H*-1,2,4-triazol-5-yl)pyridine (Hit

compound 24a). ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.41 (2H, s), 7.36–7.41 (2H, m), 7.42-7.46 (2H, m), 7.86–7.90 (2H, m), 8.68 (2H, d, J = 5.1 Hz), 14.41 (1H, brs). ^{13}C NMR (151 MHz, DMSO-d_6) δ 28.16, 119.82, 128.87, 130.37, 131.04, 133.98, 150.28, 150.32, 2 aromatic carbons were not assigned. MS (ESI/APCI) *m/z* 300.9 $[M - H]^+$. HPLC purity 98.7%.

The data of 4-(3-((3-chlorophenoxy)methyl)-1*H*-1,2,4-triazol-5-yl)pyridine (Hit compound 24b). ^{1}H NMR (600 MHz, DMSO-d_6) δ 5.32 (2H, s), 7.06 (2H, dt, J = 8.1, 2.4 Hz), 7.21 (1H, t, J = 2.2 Hz), 7.35 (1H, t, J = 8.3 Hz), 7.90–7.97 (2H, m), 8.66–8.77 (2H, m), 14.72 (1H, brs). ^{13}C NMR (151 MHz, DMSO-d_6) δ 62.07, 113.83, 114.83, 119.90, 121.21, 130.86, 133.66, 136.73, 150.39, 158.60, 2 aromatic carbons were not assigned. MS (ESI/APCI) *m/z* 285.0 $[M - H]^+$. HPLC purity 98.2%.

General procedure for the preparation of 4-chloro-*N*-((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)aniline (24c). A mixture of 4-(3-(chloromethyl)-1*H*-1,2,4-triazol-5-yl)pyridine (**23a**, 500 mg, 2.57 mmol) and 4-chloroaniline (**1c**, 2.00 g) in DMF (5.0 mL) was stirred at 80 °C for 1 h. After saturated $NaHCO_3$ aqueous solution was added, the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting ethyl acetate and methanol, and by recrystallization from ethyl acetate to afford 350 mg of **24c** (1.22 mmol, 48%) as white solids. mp 198–200 °C. ^{1}H NMR (600 MHz, CD_3OD) δ 4.53 (2H, s), 6.62 (2H, d, J = 9.2 Hz), 7.08 (2H, d, J = 8.8 Hz), 7.97–8.05 (2H, m), 8.59–8.66 (2H, m), 2NH were vanished. ^{13}C NMR (151 MHz, CD_3OD)

δ 43.17, 116.87, 123.72, 125.12, 131.67, 141.96, 149.79, 152.52, 161.32, 161.82. Anal. Calcd. for $C_{14}H_{12}ClN_5$•$0.2H_2O$: C, 58.12; H, 4.32; N, 24.21. Found: C, 58.48; H, 4.34; N, 23.84. MS (ESI/APCI) *m/z* 286.0 $[M + H]^+$. HPLC purity 100.0%.

4-(5-((4-Chlorobenzyl)thio)-1*H*-1,2,4-triazol-3-yl)pyridine (24d). A solution of 4-chlorobenzyl chloride (190 mg) in methanol (5.0 mL) was added to a solution of 5-(4-pyridyl)-1*H*-1,2,4-triazole-3-thiol (**27a**, 200 mg, 1.12 mmol) and potassium hydroxide (120 mg) in methanol (10 mL) at 0 °C. The mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated under reduced pressure and water was added to the residue. The pH was adjusted to 4 with acetic acid. The mixture was extracted with ethyl acetate and then the organic layer was washed with water and brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by recrystallization from ethyl acetate to afford 220 mg of **24d** (0.73 mmol, 66%) as white solids. ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.46 (2H, s), 7.32–7.40 (2H, m), 7.42–7.49 (2H, m), 7.86–7.94 (2H, m), 8.64–8.80 (2H, m), 14.61 (1H, brs). ^{13}C NMR (151 MHz, DMSO-d_6) δ 34.89, 119.81, 128.29, 130.65, 131.86, 136.71, 150.41, 3 aromatic carbons were not assigned. MS (ESI/APCI) *m/z* 301.0 $[M – H]^+$. HPLC purity 100.0%.

***N*-(4-Chlorobenzyl)-3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-amine (24e).** 4-Chloro-*N*-(5-(4-pyridyl)-1*H*-1,2,4-triazol-3-yl)benzamide (**24f**, 500 mg, 1.67 mmol) was added to a solution of borane in THF (1.0 M, 13.3 mL) and then the mixture was stirred at 90 °C for 1.5 h. After the reaction mixture was cooled to room temperature, 12 N HCl aqueous solution (2.0 mL) was

carefully added at 0 °C. The mixture was stirred at 65 °C for 30 min and then was adjusted to a basic solution with 8 N NaOH aqueous solution. After brine was added, the mixture was extracted with ethyl acetate. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The precipitate was washed with ethyl acetate to afford 330 mg of **24e** (1.15 mmol, 69%) as pale yellow solids. ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.42 (2H, d, J = 6.2 Hz), 7.32–7.47 (5H, m), 7.80 (2H, d, J = 5.9 Hz), 8.61 (2H, brs), 12.64 (1H, brs). ^{13}C NMR (151 MHz, DMSO-d_6) δ 45.40, 119.62, 128.09, 129.02, 131.31, 138.69, 139.12, 149.75, 156.49, 158.00. MS (ESI/APCI) *m/z* 284.0 $[M - H]^+$. HPLC purity 99.4%.

1-(4-Chlorobenzoyl)-3-(4-pyridyl)-1*H*-1,2,4-triazole-5-amine. 4-Chlorobenzoyl chloride (1.58 mL) was added to a solution of 5-(4-pyridyl)-1*H*-1,2,4-triazole-3-amine (**27b**, 2.00 g, 12.4 mmol) in pyridine (15 mL) at 0 °C. The mixture was stirred at room temperature for 24 h. Water was added and the precipitate was collected by filtration, followed by washing with hot ethyl acetate to afford 2.20 g of 1-(4-chlorobenzoyl)-3-(4-pyridyl)-1*H*-1,2,4-triazole-5-amine (7.34 mmol, 59%) as white solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.65–7.68 (2H, m), 7.83–7.85 (2H, m), 7.97 (2H, brs), 8.15–8.18 (2H, m), 8.68–8.70 (2H, m).

4-Chloro-*N*-(3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)benzamide (24f). 1-(4-Chlorobenzoyl)-3-(4-pyridyl)-1*H*-1,2,4- triazole-5-amine (1.00 g, 3.34 mmol) was heated at 250 °C for 2 h. After the reaction mixture was cooled to room temperature, the precipitate was collected by filtration, followed by washing with ethyl acetate to afford 910 mg of **24f** (3.04 mmol,

91%) as white solids. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.64 (2H, d, J = 8.4 Hz), 7.88–7.96 (2H, m), 8.12 (2H, d, J = 8.4 Hz), 8.70 (2H, d, J = 5.9 Hz), 12.30 (1H, brs), 14.05 (1H, brs). ^{13}C NMR (151 MHz, DMSO-d_6) δ 119.68, 128.62, 130.03, 130.80, 137.54, 138.17, 149.81, 150.26, 156.18, 164.54. MS (ESI/APCI) *m/z* 298.0 $[M - H]^+$. HPLC purity 98.4%.

1-Phenyl-*N*-((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)methanamine (24g). This compound was prepared from 4-(3-(chloromethyl)-1*H*-1,2,4-triazol-5-yl)pyridine **23a** and benzylamine as described in the synthesis of **24c**, as white solids. mp 159–161 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.87 (2H, s), 4.07 (2H, s), 7.24–7.36 (5H, m), 7.93–8.04 (2H, m), 8.61–8.80 (2H, m), 2NH was not assigned. ^{13}C NMR (151 MHz, $CDCl_3$) δ 44.80, 53.71, 120.60, 127.62, 128.22, 128.74, 138.42, 138.78, 150.21, 157.56, 160.30. Anal. Calcd. for $C_{15}H_{15}N_5$: C, 67.90; H, 5.70; N, 26.40; Found: C, 67.57; H, 5.66; N, 26.19. MS (ESI/APCI) *m/z* 266.1 $[M + H]^+$. HPLC purity 99.4%.

4-Chloro-*N*-(2-(3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)ethyl)aniline (24h). This compound was prepared from 4-(5-(2-chloroethyl)-1*H*-1,2,4-triazol-3-yl)pyridine **23b** and 4-chloroaniline **1c** as described in the synthesis of **24c**, as white solids. mp 193–196 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.16 (2H, t, J = 6.2 Hz), 3.62 (2H, t, J = 5.9 Hz), 4.16 (1H, brs), 6.57–6.64 (2H, m), 7.12–7.22 (2H, m), 7.90–8.00 (2H, m), 8.67–8.75 (2H, m), 10.88 (1H, brs). ^{13}C NMR (151 MHz, $CDCl_3$) δ 26.47, 42.03, 114.63, 120.50, 129.36, 139.23, 145.83, 150.41, 3 aromatic carbons were not assigned. Anal. Calcd. for $C_{15}H_{14}N_5Cl$: C, 60.10; H, 4.71; N, 23.36; Found: C, 60.00; H, 4.69; N, 23.13. MS (ESI/APCI) *m/z* 298.0 $[M - H]^+$. HPLC purity 99.6%.

5-(4-Pyridyl)-1*H*-1,2,4-triazole-3-thiol (27a). The mixture of isonicotinic hydrazide (**25**, 300 mg, 2.19 mmol) and ammonium thiocyanate (170 mg) was stirred at 170 ℃ for 1 h. After the reaction mixture was cooled to room temperature, water (1.0 mL) and 8 N NaOH aqueous solution (1.0 mL) were added. The mixture was stirred at reflux for 1.5 h. The reaction mixture was neutralized with 12 N HCl aqueous solution and then the pH was adjusted to 7. The precipitate was collected by filtration, followed by washing with water and diethyl ether to afford 320 mg of **27a** (1.80 mmol, 82%) as light yellow solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.78–7.82 (2H, m), 8.60–8.63 (2H, m), NH and SH were not assigned.

5-(4-Pyridyl)-1*H*-1,2,4-triazole-3-amine (27b). The mixture of isonicotinic acid (**26**, 10.7 g, 86.9 mmol) and aminoguanidine hydrochloride (19.2 g) was stirred at 190 ℃ for 9 h under argon atmosphere. After the reaction mixture was cooled to room temperature, water was added. The mixture was neutralized with 1 N NaOH aqueous solution and then the pH was adjusted to 7. The precipitate was collected by filtration, followed by washing with water and ethyl acetate to afford 12.9 g of **27b** (80.0 mmol, 92%) as white solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.26 (2H, brs), 7.78 (2H, d, J = 6.2 Hz), 8.60 (2H, d, J = 6.2 Hz), NH was not assigned.

***N*-((3-(Pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)aniline (28a).** This compound was prepared from 4-(3-(chloromethyl)-1*H*-1,2,4-triazol-5-yl)pyridine **23a** and aniline **6a** as described in the synthesis of **24c**, as white foam. ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.36 (1H, brs), 4.65 (2H, brs), 6.66 (2H, d, J = 8.1 Hz), 6.84 (1H, t, J = 7.3 Hz), 7.22 (2H, t, J = 8.1 Hz), 7.96 (2H, d, J = 5.9 Hz),

8.67–8.74 (2H, m), 11.15 (1H, brs). ^{13}C NMR (151 MHz, $CDCl_3$) δ 41.72, 113.30, 119.50, 120.53, 129.67, 138.07, 146.70, 150.35, 157.20, 1 aromatic carbon was not assigned. Anal. Calcd. for $C_{14}H_{13}N_5$•0.1H_2O: C, 66.44; H, 5.26; N, 27.67; Found: C, 66.55; H, 5.32; N, 27.27. MS (ESI/APCI) *m/z* 252.1 $[M + H]^+$. HPLC purity 99.1%.

2-Methoxy-*N*-((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)aniline (28b). This compound was prepared from 4-(3-(chloromethyl)-1*H*-1,2,4-triazol-5-yl)pyridine **23a** and *o*-anisidine **1d** as described in the synthesis of **24c**, as white foam. ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.90 (3H, s), 4.66 (2H, d, *J* = 4.8 Hz), 4.92 (1H, brs), 6.51 (1H, d, *J* = 7.7 Hz), 6.77–6.88 (3H, m), 7.97 (2H, d, *J* = 5.5 Hz), 8.70 (2H, d, *J* = 5.9 Hz), 11.10 (1H, brs). ^{13}C NMR (151 MHz, $CDCl_3$) δ 41.61, 55.54, 109.87, 110.59, 110.95, 114.88, 118.85, 120.52, 120.64, 121.01, 121.49, 147.14, 150.33. Anal. Calcd. for $C_{15}H_{15}N_5O$•0.15H_2O: C, 63.43; H, 5.43; N, 24.66; Found: C, 63.79; H, 5.46; N, 24.30. MS (ESI/APCI) *m/z* 282.1 $[M + H]^+$. HPLC purity 97.4%.

3-Methoxy-*N*-((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)aniline (28c). This compound was prepared from 4-(3-(chloromethyl)-1*H*-1,2,4-triazol-5-yl)pyridine **23a** and *m*-anisidine **1e** as described in the synthesis of **24c**, as white foam. ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.74 (3H, s), 4.37 (1H, brs), 4.62 (2H, brs), 6.20 (1H, t, *J* = 2.0 Hz), 6.26 (1H, dd, *J* = 7.9, 1.7 Hz), 6.38 (1H, dd, *J* = 8.1, 1.8 Hz), 7.12 (1H, t, *J* = 8.1 Hz), 7.96 (2H, d, *J* = 5.9 Hz), 8.62–8.76 (2H, m), 11.57 (1H, brs). ^{13}C NMR (151 MHz, $CDCl_3$) δ 41.57, 55.19, 99.69, 104.29, 106.13, 120.56, 130.50, 138.17, 148.15, 150.26, 161.00, 2 aromatic carbons were not assigned. Anal. Calcd. for $C_{15}H_{15}N_5O$•H_2O: C, 60.19; H,

5.72; N, 23.40; Found: C, 60.20; H, 5.63; N, 23.44. MS (ESI/APCI) *m/z* 282.2 $[M + H]^+$. HPLC purity 97.8%.

4-Methoxy-*N*-((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)aniline (28d). This compound was prepared from 4-(3-(chloromethyl)-1*H*-1,2,4-triazol-5-yl)pyridine **23a** and *p*-anisidine **1f** as described in the synthesis of **24c**, as white foam. ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.72 (3H, s), 4.55 (2H, s), 6.53–6.62 (2H, m), 6.71–6.82 (2H, m), 7.91–8.02 (2H, m), 8.62–8.70 (2H, m), 12.58 (1H, brs), NH was not assigned. ^{13}C NMR (151 MHz, $CDCl_3$) δ 42.42, 55.74, 114.66, 115.10, 120.67, 138.39, 140.81, 150.03, 153.28, 157.51, 160.18. Anal. Calcd. for $C_{15}H_{15}N_5O•0.1H_2O$: C, 63.64; H, 5.41; N, 24.74; Found: C, 63.64; H, 5.34; N, 24.47. MS (ESI/APCI) *m/z* 280.0 $[M - H]^+$. HPLC purity 97.8%.

2-Chloro-*N*-((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)aniline (28e). This compound was prepared from 4-(3-(chloromethyl)-1*H*-1,2,4-triazol-5-yl)pyridine **23a** and 2-chloroaniline **1a** as described in the synthesis of **24c**, as white solids. mp 177–178 ºC. ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.70 (2H, d, *J* = 5.5 Hz), 5.02 (1H, t, *J* = 5.3 Hz), 6.59 (1H, d, *J* = 7.7 Hz), 6.73 (1H, td, *J* = 7.6, 1.3 Hz), 7.09–7.16 (1H, m), 7.29 (1H, dd, *J* = 7.9, 1.3 Hz), 7.96 (2H, d, *J* = 5.9 Hz), 8.66 (2H, d, *J* = 5.9 Hz), 11.90–12.78 (1H, m). ^{13}C NMR (151 MHz, $CDCl_3$) δ 41.35, 111.61, 119.28, 119.87, 120.64, 128.16, 129.49, 138.18, 142.78, 150.04, 157.25, 160.38. Anal. Calcd. for $C_{14}H_{12}ClN_5$: C, 58.85; H, 4.23; N, 24.51. Found: C, 58.85; H, 4.15; N, 24.48. MS (ESI/APCI) *m/z* 286.1 $[M + H]^+$. HPLC purity 100.0%.

3-Chloro-*N*-((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)aniline (28f). This compound was prepared from 4-(3-(chloromethyl)-1*H*-1,2,4-triazol-5-yl)pyridine **23a** and 3-chloroaniline **1b** as described in the synthesis of **24c**, as white foam. ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.47 (1H, brs), 4.54–4.77 (2H, m), 6.54 (1H, d, *J* = 8.4 Hz), 6.67 (1H, d, *J* = 1.8 Hz), 6.80 (1H, d, *J* = 8.1 Hz), 7.13 (1H, d, *J* = 2.9 Hz), 7.88–8.02 (2H, m), 8.72 (2H, dt, *J* = 2.8, 1.7 Hz), 10.96 (1H, brs). ^{13}C NMR (151 MHz, $CDCl_3$) δ 41.41, 111.42, 113.27, 119.39, 120.49, 130.65, 135.43, 147.89, 150.43, 3 aromatic carbons were not assigned. Anal. Calcd. for $C_{14}H_{12}ClN_5$: C, 58.85; H, 4.23; N, 24.51; Found: C, 58.88; H, 4.42; N, 24.43. MS (ESI/APCI) *m/z* 286.1 $[M + H]^+$. HPLC purity 99.2%.

3-(((3-(Pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)amino)benzenesulfonamide (28h). This compound was prepared from 4-(3-(chloromethyl)-1*H*-1,2,4-triazol-5-yl)pyridine **23a** and 3-aminobenzenesulfonamide as described in the synthesis of **24c**, as white foam. ^{1}H NMR (600 MHz, CD_3OD) δ 4.60 (2H, s), 6.83–6.87 (1H, m), 7.16–7.20 (1H, m), 7.20–7.23 (1H, m), 7.25–7.30 (1H, m), 7.99–8.03 (2H, m), 8.60–8.64 (2H, m), 4NH were vanished. ^{13}C NMR (151 MHz, CD_3OD) δ 42.85, 112.49, 117.59, 118.99, 123.75, 132.51, 141.87, 147.43, 151.53, 152.54, 161.13, 161.76. MS (ESI/APCI) *m/z* 331.1 $[M + H]^+$. HPLC purity 95.3%.

4-(((3-(Pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)amino)benzenesulfonamide (28i). This compound was prepared from 4-(3-(chloromethyl)-1*H*-1,2,4-triazol-5-yl)pyridine **23a** and 4-aminobenzenesulfonamide as described in the synthesis of **24c**, as white foam. ^{1}H NMR (600 MHz, CD_3OD) δ 4.61 (2H, s), 6.69–6.77 (2H, m), 7.61–7.69 (2H, m), 7.99–8.02 (2H, m), 8.61–8.64 (2H,

m), 4NH were vanished. ^{13}C NMR (151 MHz, CD_3OD) δ 42.48, 114.64, 123.72, 130.72, 134.08, 141.87, 152.55, 154.30, 161.02, 161.72. MS (ESI/APCI) *m/z* 331.1 $[M + H]^+$. HPLC purity 94.3%.

General procedure for the preparation of 3-amino-*N*-benzylbenzamide (29i). Reduced iron (12.7 g) was added to a solution of *N*-benzyl-3-nitrobenzamide (**39i**, 9.72 g, 37.9 mmol) and calcium chloride (2.10 g) in 85% ethanol aqueous solution (190 mL) at room temperature. The mixture was stirred at 90 °C for 2 h. The reaction mixture was filtered with Celite and then washed with methanol. The filtrate was concentrated under reduced pressure. Ethyl acetate was added to the residue and then the mixture was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by recrystallization from ethyl acetate and IPE to afford 8.02 g of **29i** (35.4 mmol, 93%) as white solids. mp 85–87 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.78 (2H, brs), 4.62 (2H, d, *J* = 5.7 Hz), 6.39 (1H, br), 6.78 (1H, ddd, *J* = 8.0, 2.4, 0.9 Hz), 7.05–7.08 (1H, m), 7.15 (1H, t, *J* = 1.8 Hz), 7.18 (1H, t, *J* = 7.8 Hz), 7.27–7.35 (5H, m). Anal. Calcd. for $C_{14}H_{14}N_2O$: C, 74.31; H, 6.24; N, 12.38. Found: C, 74.08; H, 6.41; N, 12.07.

3-(2-Phenylethoxy)aniline (29c). This compound was prepared from **39c** as described in the synthesis of **29i**, as brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.08 (2H, t, *J* = 7.2 Hz), 3.63 (2H, brs), 4.13 (2H, t, *J* = 7.2 Hz), 6.24 (1H, t, *J* = 2.3 Hz), 6.28 (1H, ddd, *J* = 8.1, 2.3, 0.9 Hz), 6.32 (1H, ddd, *J* = 8.1, 2.4, 0.9 Hz), 7.04 (1H, t, *J* = 8.1 Hz), 7.20–7.35 (5H, m).

3-Amino-*N*-(2-phenylethyl)benzamide (29j). This compound was prepared from **39j** as described in the synthesis of **29i**, as white solids. mp 76 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.93 (2H, t, *J* = 6.9

Hz), 3.70 (2H, q, *J* = 6.5 Hz), 3.77 (2H, brs), 6.07 (1H, br), 6.77 (1H, dd, *J* = 8.0, 1.7 Hz), 6.94 (1H, d, *J* = 7.8 Hz), 7.08 (1H, t, *J* = 2.0 Hz), 7.16 (1H, t, *J* = 8.0 Hz), 7.23–7.36 (5H, m). Anal. Calcd. for $C_{15}H_{16}N_2O$: C, 74.97; H, 6.71; N, 11.66. Found: C, 74.67; H, 6.64; N, 11.73.

3-Amino-*N*-benzyl-*N*-methylbenzamide (29l). This compound was prepared from **39l** as described in the synthesis of **29i**, as yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) syn:anti = 1:1, δ 2.86 (1.5H, s), 3.00 (1.5H, s), 3.74 (2H, s), 4.53 (1H, s), 4.74 (1H, s), 6.70 (1H, br), 6.76 (1H, t, *J* = 2.1 Hz), 6.80 (1H, d, *J* = 7.5 Hz), 7.16 (2H, br), 7.29–7.34 (4H, m).

***N*-(3-Aminophenyl)-*N*'-benzylurea (29p).** This compound was prepared from **39p** as described in the synthesis of **29i**, as white solids. mp 151–153 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.26 (2H, d, *J* = 5.7 Hz), 4.93 (2H, s), 6.11 (1H, d, *J* = 9.0 Hz), 6.45–6.51 (2H, m), 6.70 (1H, t, *J* = 2.1 Hz), 6.82 (1H, t, *J* = 8.0 Hz), 7.20–7.34 (5H, m), 8.20 (1H, s). Anal. Calcd. for $C_{14}H_{15}N_3O$•$0.1H_2O$: C, 69.17; H, 6.30; N, 17.29. Found: C, 69.13; H, 6.40; N, 17.51.

General procedure for the preparation of 3-amino-*N*-benzylbenzamide (29i) from 3-aminobenzoic acid (35a). A mixture of 3-aminobenzoic acid (**35a**, 27.4 g, 200 mmol), benzylamine (24.0 mL), WSC•HCl (46.0 g) and HOBt•H_2O (3.06 g) in DMF (300 mL) was stirred at room temperature for 15 h. The reaction mixture was concentrated under reduced pressure. Ethyl acetate was added to the residue and then the mixture was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting hexane and ethyl acetate, and

by recrystallization from ethyl acetate and IPE to afford 43.6 g of **29i** (193 mmol, 96%) as white solids. The spectrum data of **29i** were described (*vide supra*).

3-Amino-*N*-(3-phenylpropyl)benzamide (29k). This compound was prepared from 3-aminobenzoic acid **35a** and 3-phenylpropylamine as described in the synthesis of **29i**, as pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.95 (2H, quintet, *J* = 7.3 Hz), 2.72 (2H, t, *J* = 7.7 Hz), 3.48 (2H, q, *J* = 6.6 Hz), 3.77 (2H, brs), 6.03 (1H, br), 6.77 (1H, ddd, *J* = 8.0, 2.3, 0.8 Hz), 6.94 (1H, ddd, *J* = 7.6, 1.6, 1.0 Hz), 7.04 (1H, t, *J* = 1.8 Hz), 7.16 (1H, t, *J* = 7.8 Hz), 7.18–7.33 (5H, m).

2-(3-Aminophenyl)-*N*-benzylacetamide (29q). This compound was prepared from 3-aminophenylacetic acid **35b** and benzylamine as described in the synthesis of **29i**, as pale yellow solids. mp 122–124 ºC. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.54 (2H, s), 3.69 (2H, brs), 4.41 (2H, d, *J* = 5.7 Hz), 5.76 (1H, br), 6.58–6.65 (3H, m), 7.10–7.33 (6H, m). Anal. Calcd. for $C_{15}H_{16}N_2O$: C, 74.97; H, 6.71; N, 11.66. Found: C, 74.83; H, 6.74; N, 11.65.

3-Phenoxy-*N*-((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)aniline (30a). This compound was prepared from 4-(3-(chloromethyl)-1*H*-1,2,4-triazol-5-yl)pyridine **23a** and 3-phenoxyaniline **6d** as described in the synthesis of **24c**, as white solids. mp 185–187 ºC. ^{1}H NMR (600 MHz, CD_3OD) δ 4.50 (2H, s), 6.22–6.25 (1H, m), 6.27 (1H, dt, *J* = 8.1, 1.1 Hz), 6.42 (1H, dd, *J* = 8.1, 1.5 Hz), 6.85–6.92 (2H, m), 6.97 (1H, t, *J* = 7.3 Hz), 7.08 (1H, t, *J* = 8.3 Hz), 7.23 (2H, dd, *J* = 8.4, 7.7 Hz), 7.97–8.01 (2H, m), 8.62 (2H, d, *J* = 6.2 Hz), 2NH were vanished. ^{13}C NMR (151 MHz, CD_3OD) δ 43.12, 106.10, 110.82, 111.07, 121.49, 123.75, 125.78, 132.38, 132.98, 142.10, 152.50, 152.63,

160.45, 161.46, 161.50, 161.84. Anal. Calcd. for $C_{20}H_{17}N_5O$: C, 69.96; H, 4.99; N, 20.40. Found: C, 69.59; H, 5.13; N, 20.20. MS (ESI/APCI) *m/z* 344.1 $[M + H]^+$. HPLC purity 99.6%.

3-(Benzyloxy)-*N*-((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)aniline (30b). This compound was prepared from 4-(3-(chloromethyl)-1*H*-1,2,4-triazol-5-yl)pyridine **23a** and 3-benzyloxyaniline **6e** as described in the synthesis of **24c**, as white foam. ^{1}H NMR (600 MHz, CD_3OD) δ 4.52 (2H, s), 4.99 (2H, s), 6.26 (1H, dd, *J* = 8.1, 1.5 Hz), 6.29 (1H, d, *J* = 1.8 Hz), 6.33 (1H, dd, *J* = 8.1, 1.5 Hz), 7.01 (1H, t, *J* = 8.1 Hz), 7.22–7.26 (1H, m), 7.30 (2H, t, *J* = 7.5 Hz), 7.35 (2H, d, *J* = 7.3 Hz), 8.00–8.04 (2H, m), 8.60-8.64 (2H, m), 2NH were vanished. ^{13}C NMR (151 MHz, CD_3OD) δ 43.25, 72.54, 102.69, 107.25, 108.99, 123.74, 130.17, 130.46, 131.16, 132.68, 140.69, 152.52, 163.11, 3 aromatic carbons were not assigned. MS (ESI/APCI) *m/z* 358.1 $[M + H]^+$. HPLC purity 97.5%.

3-(2-Phenylethoxy)-*N*-((5-(4-pyridyl)-1*H*-1,2,4-triazol-3-yl)methyl)aniline (30c). This compound was prepared from 4-(3-(chloromethyl)-1*H*-1,2,4-triazol-5-yl)pyridine **23a** and 3-(2-phenylethoxy)aniline **29c** as described in the synthesis of **24c**, as white solids. mp 157–158 °C. ^{1}H NMR (600 MHz, CD_3OD) δ 2.97 (2H, t, *J* = 7.0 Hz), 4.07 (2H, t, *J* = 7.0 Hz), 4.51 (2H, s), 6.20 (1H, brs), 6.24 (2H, d, *J* = 8.1 Hz), 6.99 (1H, t, *J* = 8.1 Hz), 7.10–7.18 (1H, m), 7.19–7.31 (4H, m), 7.96–8.08 (2H, m), 8.57–8.69 (2H, m), 2NH were vanished. ^{13}C NMR (151 MHz, CD_3OD) δ 38.47, 43.24, 71.39, 102.34, 106.91, 108.87, 123.72, 129.05, 131.11, 131.71, 132.66, 141.63, 142.11, 152.34, 152.51, 161.67, 161.87, 163.13. Anal. Calcd. for $C_{22}H_{21}N_5O$: C, 71.14; H, 5.70; N, 18.85. Found: C, 71.02; H, 5.69; N, 18.73. MS (ESI/APCI) *m/z* 372.2 $[M + H]^+$. HPLC purity 99.7%.

3-(Benzylsulfanyl)-*N*-((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)aniline (30d). This compound was prepared from 4-(3-(chloromethyl)-1*H*-1,2,4-triazol-5-yl)pyridine **23a** and 3-benzylsulfanylaniline as described in the synthesis of **24c**, as white foam. ^{1}H NMR (600 MHz, CD_3OD) δ 4.02 (2H, s), 4.50 (2H, s), 6.48 (1H, d, *J* = 8.1 Hz), 6.54–6.65 (2H, m), 7.00 (1H, t, *J* = 7.9 Hz), 7.09–7.15 (1H, m), 7.18 (4H, q, *J* = 7.8 Hz), 8.01 (2H, d, *J* = 5.1 Hz), 8.60 (2H, d, *J* = 5.1 Hz), 2NH were vanished. ^{13}C NMR (151 MHz, CD_3OD) δ 41.05, 43.07, 114.15, 116.24, 121.85, 123.72, 129.68, 131.04, 131.52, 132.19, 140.23, 141.05, 142.01, 151.38, 152.51, 161.48, 161.82. Exact Mass (FAB) $(M + H)^+$ for $C_{21}H_{20}N_5S$, Calcd: 374.1439. Found: 374.1446. MS (ESI/APCI) *m/z* 374.1 $[M + H]^+$. HPLC purity 94.0%.

***N*-Benzyl-3-(((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)amino)benzenesulfonamide (30e).** This compound was prepared from 4-(3-(chloromethyl)-1*H*-1,2,4-triazol-5-yl)pyridine **23a** and *N*-benzyl-3-aminobenzenesulfonamide as described in the synthesis of **24c**, as yellow foam. ^{1}H NMR (600 MHz, CD_3OD) δ 3.90 (2H, s), 4.59 (2H, s), 6.89 (1H, dd, *J* = 8.1, 1.5 Hz), 7.08–7.14 (4H, m), 7.15–7.22 (3H, m), 7.28 (1H, t, *J* = 7.9 Hz), 7.94–7.99 (2H, m), 8.57 (2H, d, *J* = 5.9 Hz), 3NH were vanished. ^{13}C NMR (151 MHz, CD_3OD) δ 42.75, 49.73, 112.92, 118.34, 119.81, 123.72, 130.13, 130.60, 131.10, 132.67, 140.35, 141.79, 144.27, 151.55, 152.49, 161.14, 161.74. Exact Mass (FAB) $[M + H]^+$ for $C_{21}H_{21}N_6O_2S$, Calcd: 421.1447. Found: 421.1488. MS (ESI/APCI) *m/z* 421.1 $[M + H]^+$. HPLC purity 100.0%.

3-(Benzylsulfonyl)-*N*-((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)aniline (30f). A mixture of

3-(benzylsulfanyl)-*N*-((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)aniline (**30d**, 423 mg, 1.13 mmol) and sodium periodate (485 mg) in MeOH–THF–H_2O (4:1:1, 15 mL) was stirred at 90 ºC for 1 h. The reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting ethyl acetate and methanol, and by HPLC followed by recrystallization from ethyl acetate and IPE to afford 89 mg of **30f** (0.22 mmol, 19%) as white solids. mp 118–120 ºC. ^{1}H NMR (600 MHz, CD_3OD) δ 4.37 (2H, s), 4.51 (2H, s), 6.90 (1H, s), 6.94 (2H, d, *J* = 7.7 Hz), 7.05 (2H, d, *J* = 7.3 Hz), 7.16–7.20 (2H, m), 7.22–7.27 (2H, m), 8.02 (2H, d, *J* = 5.5 Hz), 8.63 (2H, d, *J* = 2.9 Hz), 2NH were vanished. ^{13}C NMR (151 MHz, CD_3OD) δ 42.65, 64.75, 114.24, 119.81, 120.99, 123.75, 131.07, 131.32, 131.65, 132.53, 133.75, 141.81 (2C), 151.65, 152.57, 160.95, 161.72. Anal. Calcd. for $C_{21}H_{19}N_5O_2S•H_2O$: C, 59.56; H, 5.00; N, 16.54. Found: C, 59.95; H, 4.93; N, 16.58. MS (ESI/APCI) *m/z* 405.9 $[M + H]^+$. HPLC purity 97.7%.

In the above reaction, 3-(benzylsulfinyl)-*N*-((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)aniline was also afforded (230 mg, 0.59 mmol, 52%) as white solids. mp 194–197 ºC. ^{1}H NMR (300 MHz, CD_3OD) δ 4.09 (2H, s), 4.53 (2H, s), 6.74 (1H, d, *J* = 7.5 Hz), 6.75 (1H, s), 6.82 (1H, dd, *J* = 7.7, 2.0 Hz), 7.00–7.03 (2H, m), 7.17–7.26 (4H, m), 8.02 (2H, d, *J* = 5.4 Hz), 8.63 (2H, brs), 2H were vanished. Anal. Calcd. for $C_{21}H_{19}N_5OS•H_2O$: C, 61.90; H, 5.19; N, 17.19. Found: C, 61.74; H, 5.07; N, 16.97.

***N*-Methyl-3-(((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)amino)benzamide (30g).** This compound was prepared from 4-(3-(chloromethyl)-1*H*-1,2,4-triazol-5-yl)pyridine **23a** and 3-amino-*N*-methylbenzamide as described in the synthesis of **24c**, as white solids. mp 238–239 ºC. ^{1}H NMR (600 MHz, CD_3OD) δ 2.87 (3H, s), 4.59 (2H, s), 6.81 (1H, dd, *J* = 8.3, 2.4 Hz), 7.07 (1H, d, *J* = 7.3 Hz), 7.11 (1H, s), 7.19 (1H, t, *J* = 7.9 Hz), 7.99–8.05 (2H, m), 8.60-8.65 (2H, m), 3NH were vanished. ^{13}C NMR (151 MHz, CD_3OD) δ 28.62, 42.98, 114.28, 118.69, 118.91, 123.73, 132.08, 138.33, 142.04, 151.27, 152.52, 161.37, 161.86, 173.08. MS (ESI/APCI) *m/z* 309.2 $[M + H]^+$. HPLC purity 100.0%.

***N*-Phenyl-3-(((5-(4-pyridyl)-1*H*-1,2,4-triazol-3-yl)methyl)amino)benzamide (30h).** This compound was prepared from 3-(((5-(4-pyridyl)-1*H*-1,2,4-triazol-3-yl)methyl)amino)benzoic acid **34** and aniline **6a** as described in the synthesis of **30i**, as white solids. mp 161–167 °C. ^{1}H NMR (600 MHz, CD_3OD) δ 4.62 (2H, s), 6.86 (1H, dt, *J* = 8.0, 1.1 Hz), 7.09–7.15 (1H, m), 7.19–7.28 (3H, m), 7.33 (2H, dd, *J* = 8.4, 7.3 Hz), 7.63 (2H, d, *J* = 7.7 Hz), 7.99–8.04 (2H, m), 8.59-8.65 (2H, m), 3NH were vanished. ^{13}C NMR (151 MHz, CD_3OD) δ 42.96, 114.63, 118.97, 119.34, 123.74, 124.08, 127.32, 131.50, 132.15, 139.04, 141.56, 141.97, 151.33, 152.52, 161.35, 161.82, 171.20. Anal. Calcd. for $C_{21}H_{18}N_6O•H_2O$: C, 64.94; H, 5.19; N, 21.64. Found: C, 64.86; H, 5.27; N, 21.43. MS (ESI/APCI) *m/z* 371.2 $[M + H]^+$. HPLC purity 100.0%.

***N*-Benzyl-3-(((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)amino)benzamide (30i).** This compound was prepared from 4-(3-(chloromethyl)-1*H*-1,2,4-triazol-5-yl)pyridine **23a** and

3-amino-*N*-benzylbenzamide **29i** as described in the synthesis of **24c**, as white solids. mp 179–180 ºC. ^{1}H NMR (600 MHz, CD_3OD) δ 4.53 (2H, s), 4.59 (2H, s), 6.82 (1H, dd, *J* = 8.1, 1.5 Hz), 7.11–7.16 (2H, m), 7.18–7.23 (2H, m), 7.25–7.32 (4H, m), 7.98–8.02 (2H, m), 8.61 (2H, d, *J* = 5.9 Hz), 3NH were vanished. ^{13}C NMR (151 MHz, CD_3OD) δ 42.99, 46.19, 114.34, 118.86, 119.11, 123.73, 129.84, 130.16, 131.23, 132.12, 138.38, 141.97, 142.04, 151.28, 152.51, 161.39, 161.85, 172.48. Anal. Calcd. for $C_{22}H_{20}N_6O$•0.2H_2O: C, 68.10; H, 5.30; N, 21.66. Found: C, 67.98; H, 5.35; N, 21.37. MS (ESI/APCI) *m/z* 385.1 $[M + H]^+$. HPLC purity 99.6%.

***N*-(2-Phenylethyl)-3-(((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)amino)benzamide (30j).** This compound was prepared from 4-(3-(chloromethyl)-1*H*-1,2,4-triazol-5-yl)pyridine **23a** and 3-amino-*N*-(2-phenylethyl)benzamide **29j** as described in the synthesis of **24c**, as white solids. mp 151–154 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.81 (2H, t, *J* = 7.3 Hz), 3.42–3.48 (2H, m), 4.49 (2H, d, *J* = 5.9 Hz), 6.45 (1H, t, *J* = 5.3 Hz), 6.77 (1H, dd, *J* = 7.9, 2.0 Hz), 7.03 (1H, d, *J* = 7.3 Hz), 7.10 (1H, d, *J* = 1.8 Hz), 7.13–7.20 (2H, m), 7.21–7.23 (2H, m), 7.25–7.30 (2H, m), 7.87–7.95 (2H, m), 8.35 (1H, t, *J* = 5.7 Hz), 8.67 (2H, d, *J* = 5.9 Hz), 14.30 (1H, brs). ^{13}C NMR (151 MHz, DMSO-d_6) δ 35.04, 40.70, 111.11, 114.87, 115.01, 119.82, 125.91, 128.19, 128.51, 128.58, 135.47, 139.46, 148.01, 150.25, 166.54, 1 aliphatic and 3 aromatic carbons were not assigned. Because the aliphatic carbon was hidden by DMSO. Anal. Calcd. for $C_{23}H_{22}N_6O$•0.5H_2O: C, 67.80; H, 5.69; N, 20.63. Found: C, 67.88; H, 5.69; N, 20.44. MS (ESI/APCI) *m/z* 399.1 $[M + H]^+$. HPLC purity 99.4%.

***N*-(3-Phenylpropyl)-3-(((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)amino)benzamide (30k).**

This compound was prepared from 4-(3-(chloromethyl)-1*H*-1,2,4-triazol-5-yl)pyridine **23a** and 3-amino-*N*-(3-phenylpropyl)benzamide **29k** as described in the synthesis of **24c**, as white solids. mp 199–203 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.80 (2H, quintet, J = 7.4 Hz), 2.60 (2H, t, J = 7.7 Hz), 3.20–3.26 (2H, m), 4.49 (2H, d, J = 5.5 Hz), 6.44 (1H, brs), 6.77 (1H, dd, J = 8.1, 1.8 Hz), 7.05 (1H, d, J = 7.7 Hz), 7.12 (1H, s), 7.14–7.19 (2H, m), 7.19–7.24 (2H, m), 7.25–7.30 (2H, m), 7.83–7.95 (2H, m), 8.28 (1H, t, J = 5.5 Hz), 8.66 (2H, d, J = 4.8 Hz), 14.29 (1H, brs). ^{13}C NMR (151 MHz, DMSO-d_6) δ 30.82, 32.54, 38.69, 111.24, 114.77, 115.07, 119.81, 125.58, 128.15, 128.17, 128.56, 135.57, 141.66, 148.00, 150.24, 166.61, 1 aliphatic and 3 aromatic carbons were not assigned. Because the aliphatic carbon was hidden by DMSO. Anal. Calcd. for $C_{24}H_{24}N_6O$•0.2AcOEt: C, 69.25; H, 6.00; N, 19.54. Found: C, 69.16; H, 6.12; N, 19.55. MS (ESI/APCI) *m/z* 413.2 $[M + H]^+$. HPLC purity 98.6%.

***N*-Benzyl-*N*-methyl-3-(((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)amino)benzamide (30l).** This compound was prepared from 4-(3-(chloromethyl)-1*H*-1,2,4-triazol-5-yl)pyridine **23a** and 3-amino-*N*-benzyl-*N*-methylbenzamide **29l** as described in the synthesis of **24c**, as white foam. ^{1}H NMR (600 MHz, DMSO-d_6) syn:anti = ca. 8:7, δ 2.76 (1.6H, brs), 2.81 (1.4H, brs), 4.31–4.54 (3H, m), 4.63 (1H, brs), 6.47–6.65 (2H, m), 6.69 (2H, brs), 7.01–7.20 (2H, m), 7.24–7.40 (4H, m), 7.89 (2H, d, J = 5.5 Hz), 8.66 (2H, brs), 14.25 (1H, brs). ^{13}C NMR (151 MHz, DMSO-d_6) δ 32.21, 36.58, 49.53, 53.87, 110.09, 110.47, 113.05, 113.29, 114.70, 119.81, 126.61, 127.02, 127.38, 128.48, 128.86, 136.94, 137.33, 138.26, 147.93, 150.22, 156.69, 159.02, 170.67, 171.26, these chemical shift values

were not able to be assigned because this compound were syn and anti mixture at the amide bond. MS (ESI/APCI) *m/z* 399.1 $[M + H]^+$. HPLC purity 99.8%.

***N*-(3-(((3-(Pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)amino)phenyl)acetamide (30m).** This compound was prepared from 4-(3-(chloromethyl)-1*H*-1,2,4-triazol-5-yl)pyridine **23a** and *m*-aminoacetanilide as described in the synthesis of **24c**, as white foam. ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.19 (3H, s), 4.45 (1H, brs), 4.61 (2H, d, *J* = 4.4 Hz), 6.37 (1H, dd, *J* = 8.3, 2.0 Hz), 6.50 (1H, d, *J* = 8.1 Hz), 7.09 (1H, t, *J* = 8.1 Hz), 7.30 (1H, s), 7.92–7.97 (2H, m), 8.65–8.70 (2H, m), 2NH were not assigned. ^{13}C NMR (151 MHz, $CDCl_3$) δ 24.63, 40.44, 105.60, 110.32, 110.42, 120.49, 130.17, 138.39, 147.18, 150.31, 169.17, 3 aromatic carbons were not assigned. MS (ESI/APCI) *m/z* 309.2 $[M + H]^+$. HPLC purity 93.7%.

***N*-(2-Chloro-5-(((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)amino)phenyl)acetamide (30n).** This compound was prepared from 4-chloro-*N*-1-((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)benzene-1,3-diamine **41** and acetic acid as described in the synthesis of **30o** as white solids. mp 228–238 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.05 (3H, brs), 4.41 (2H, d, *J* = 5.5 Hz), 6.45 (1H, d, *J* = 7.3 Hz), 6.53 (1H, brs), 7.07 (1H, brs), 7.15 (1H, d, *J* = 8.8 Hz), 7.84–7.97 (2H, m), 8.67 (2H, d, *J* = 5.1 Hz), 9.23 (1H, s), 14.29 (1H, brs). ^{13}C NMR (151 MHz, DMSO-d_6) δ 23.31, 109.77, 110.09, 113.46, 119.83, 129.19, 135.09, 138.04, 147.27, 150.25, 156.88, 158.40, 168.23, 1 aliphatic carbon was not assigned. Because the aliphatic carbon was hidden by DMSO. MS (ESI/APCI) *m/z* 343.1 $[M + H]^+$. HPLC purity 100.0%.

General procedure for the preparation of *N*-(2-chloro-5-(((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)amino)phenyl)-2-phenylacetamide (30o). A mixture of 4-chloro-*N*-1-((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)benzene-1,3-diamine (**41**, 210 mg, 0.70 mmol), phenylacetic acid (238 mg), WSC•H_2O (402 mg) and HOBt•HCl (21 mg) in DMF (7.0 mL) was stirred at room temperature for 14 h. Potassium carbonate (145 mg), methanol (1.5 mL) and water (1.5 mL) were added. The mixture was stirred at room temperature for 2.5 h. Ethyl acetate was added to the reaction mixture and then the mixture was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting hexane and ethyl acetate, and by recrystallization from ethyl acetate and IPE to afford 184 mg of **30o** (0.44 mmol, 63%) as white solids. mp 199–201 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.69 (2H, s), 4.40 (2H, d, J = 5.1 Hz), 6.45 (1H, d, J = 7.0 Hz), 6.53 (1H, brs), 7.09 (1H, brs), 7.15 (1H, d, J = 8.8 Hz), 7.22–7.26 (1H, m), 7.29–7.41 (4H, m), 7.88–7.91 (2H, m), 8.67 (2H, d, J = 5.1 Hz), 9.36 (1H, s), 14.27 (1H, brs). ^{13}C NMR (151 MHz, DMSO-d_6) δ 42.61, 109.80, 109.88, 113.28, 119.83, 126.42, 128.18, 129.04, 129.21, 134.85, 135.81, 147.31, 150.24, 169.05, 1 aliphatic and 3 aromatic carbons were not assigned. Because the aliphatic carbon was hidden by DMSO. Anal. Calcd. for $C_{22}H_{19}ClN_6O$•0.1H_2O: C, 62.81; H, 4.60; N, 19.98. Found: C, 62.62; H, 4.51; N, 19.90. MS (ESI/APCI) *m/z* 419.0 $[M + H]^+$. HPLC purity 94.2%.

1-Benzyl-3-(3-(((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)amino)phenyl)urea (30p). This compound was prepared from 4-(3-(chloromethyl)-1*H*-1,2,4-triazol-5-yl)pyridine **23a** and *N*-(3-Aminophenyl)-*N*'-benzylurea **29p** as described in the synthesis of **24c**, as light red solids. mp 186–189 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.27 (2H, d, J = 5.9 Hz), 4.40 (2H, d, J = 5.9 Hz), 6.17–6.24 (2H, m), 6.49 (1H, t, J = 5.9 Hz), 6.67 (1H, dd, J = 8.1, 1.1 Hz), 6.75 (1H, t, J = 2.0 Hz), 6.93 (1H, t, J = 7.9 Hz), 7.20–7.25 (1H, m), 7.26–7.29 (2H, m), 7.30–7.35 (2H, m), 7.87–7.93 (2H, m), 8.30 (1H, s), 8.67 (2H, d, J = 5.9 Hz), 14.23 (1H, brs). ^{13}C NMR (151 MHz, DMSO-d_6) δ 39.76, 42.59, 102.12, 105.57, 106.73, 119.83, 126.57, 126.97, 128.16, 128.94, 137.96, 140.29, 140.98, 148.49, 150.22, 155.04, 157.71, 1 aromatic carbon was not assigned. Anal. Calcd. for $C_{22}H_{21}N_7O$•$0.4H_2O$: C, 64.98; H, 5.40; N, 24.11. Found: C, 65.28; H, 5.38; N, 23.74. MS (ESI/APCI) *m/z* 400.2 $[M + H]^+$. HPLC purity 98.8%.

***N*-Benzyl-2-(3-(((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)amino)phenyl)acetamide (30q).** This compound was prepared from 4-(3-(chloromethyl)-1*H*-1,2,4-triazol-5-yl)pyridine **23a** and 2-(3-aminophenyl)-*N*-benzylacetamide **29q** as described in the synthesis of **24c**, as off-white solids. mp 189–191 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.34 (2H, s), 4.24 (2H, d, J = 5.9 Hz), 4.43 (2H, d, J = 5.5 Hz), 6.25 (1H, brs), 6.49 (1H, d, J = 7.7 Hz), 6.53 (1H, d, J = 7.7 Hz), 6.60 (1H, s), 7.01 (1H, t, J = 7.7 Hz), 7.18–7.24 (3H, m), 7.27–7.32 (2H, m), 7.87–7.94 (2H, m), 8.42 (1H, t, J = 5.7 Hz), 8.67 (2H, d, J = 4.8 Hz), 14.26 (1H, brs). ^{13}C NMR (151 MHz, DMSO-d_6) δ 42.07, 42.60, 110.18, 113.37, 117.42, 119.83, 126.59, 127.06, 128.13, 128.64, 136.85, 138.26, 139.37, 148.00, 150.23,

157.01, 158.80, 170.05, 1 aliphatic carbon was not assigned. Because the aliphatic carbon was hidden by DMSO. Anal. Calcd. for $C_{23}H_{22}N_6O•0.2H_2O$: C, 68.71; H, 5.62; N, 20.90. Found: C, 68.60; H, 5.57; N, 20.67. MS (ESI/APCI) *m/z* 399.1 $[M + H]^+$. HPLC purity 96.3%.

General procedure for the alternative preparation of *N*-benzyl-3-(((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)amino)benzamide (30i). A mixture of 3-(((5-(4-pyridyl)-1*H*-1,2,4-triazol-3-yl)methyl)amino)benzoic acid (**34**, 1.00 g, 3.39 mmol), benzylamine (0.73 g), WSC•HCl (0.94 g) and HOBt•H_2O (52 mg) in DMF (15 mL) was stirred at room temperature for 14 h. Ethyl acetate was added to the reaction mixture and then the mixture was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by recrystallization from ethyl acetate and IPE to afford 1.04 g of **30i** (2.71 mmol, 80%) as white solids. The spectrum data of **30i** was described (*vide supra*).

Ethyl 3-((2-(2-(imino(4-pyridyl)methyl)hydrazino)-2-oxoethyl)(trifluoroacetyl)aminobenzoate (31). A mixture of *N*-(3-(ethoxycarbonyl)phenyl)-*N*-(trifluoroacetyl)glycine (**16**, 32.0 g, 100 mmol), pyridine-4- carboxyimidehydrazide (**22**, 14.3 g), WSC•HCl (28.8 g) and HOBt•H_2O (1.53 g) in DMF (300 mL) was stirred at room temperature for 15 h. Ethyl acetate was added to the reaction mixture and then the mixture was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by recrystallization from ethyl acetate to afford 37.3 g of **62** (85.3 mmol, 85%) as white solids. mp

174–178 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32 (3H, t, *J* = 7.2 Hz), 4.34 (2H, q, *J* = 7.2 Hz), 4.83 (2H, s), 6.72 (2H, s), 7.65 (1H, t, *J* = 8.0 Hz), 7.74 (2H, d, *J* = 5.1 Hz), 7.83 (1H, d, *J* = 9.3 Hz), 8.03 (1H, d, *J* = 8.7 Hz), 8.13 (1H, s), 8.66 (2H, d, *J* = 5.1 Hz), 10.31 (1H, s). Anal. Calcd. for $C_{19}H_{18}N_5O_4$: C, 52.18; H, 4.15; N, 16.01. Found: C, 52.14; H, 4.38; N, 16.04.

Ethyl 3-((2-(2-(imino(4-pyridyl)methyl)hydrazino)-2-oxoethyl)amino)benzoate (32). A solution of sodium ethoxide in ethanol (20%, 34.2 g) was added to a suspension of ethyl 3-((2-(2-(imino(4-pyridyl) methyl)hydrazino)-2-oxoethyl)(trifluoroacetyl)amino)benzoate (**31**, 22.0 g, 50.3 mmol) in ethanol (250 mL). The mixture was stirred at room temperature for 35 min. After the reaction mixture was treated with acetic acid (7.0 mL), the resulting mixture was concentrated under reduced pressure. Ethyl acetate was added to the residue and then the mixture was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford crude **32** (quantitative yield) as yellow oil. This compound was used without further purification. A part of crude product was recrystallized from ethyl acetate to afford **32** as pale yellow solids. mp 165–167 °C. ^{1}H NMR (300 MHz, DMSO-d_6) ratio: 63:37, δ 1.28 (1.88H, t, *J* = 7.2 Hz), 1.30 (1.12H, t, *J* = 7.2 Hz), 3.83 (0.75H, d, *J* = 6.0 Hz), 4.24 (1.25H, d, *J* = 5.7 Hz), 4.25 (1.25H, q, *J* = 7.0 Hz), 4.28 (0.75H, q, *J* = 6.9 Hz), 6.11 (0.63H, s), 6.25 (0.37H, s), 6.69 (2H, brs), 6.88 (1H, d, *J* = 9.0 Hz), 7.14–7.25 (3H, m), 7.73 (0.75H, d, *J* = 6.0 Hz), 7.79 (1.25H, dd, *J* = 4.5, 1.5 Hz), 8.62–8.65 (2H, m), 9.91 (0.37H, s), 10.15 (0.63H, s). Anal. Calcd. for $C_{17}H_{19}N_5O_3$•$0.25H_2O$: C, 59.03; H, 5.68; N, 20.25. Found: C, 58.98; H, 5.67; N, 19.85.

Ethyl 3-(((5-(4-pyridyl)-1*H*-1,2,4-triazol-3-yl)methyl)amino)benzoate (33). A mixture of ethyl 3-((2-(2-(imino(4-pyridyl)methyl)hydrazino)-2-oxoethyl)amino)benzoate **32** as above and acetic acid (10 mL) in ethanol (500 mL) was stirred at 90 ℃ for 5 h. The reaction mixture was concentrated under reduced pressure. Ethyl acetate was added to the residue and then the mixture was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting ethyl acetate and methanol, and by recrystallization from ethyl acetate to afford 9.98 g of **33** (30.9 mmol, 61%) as white solids. mp 143–146 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36 (3H, t, J = 7.1 Hz), 4.34 (2H, q, J = 7.1 Hz), 4.60 (1H, br), 4.65 (2H, s), 6.81 (1H, dd, J = 7.0, 2.6 Hz), 7.22–7.29 (1H, m), 7.34–7.36 (1H, m), 7.47 (1H, d, J = 7.8 Hz), 7.96 (2H, d, J = 6.2 Hz), 8.70 (2H, d, J = 6.2 Hz), 11.68 (1H, br).

General procedure of the preparation for 3-(((5-(4-pyridyl)-1*H*-1,2,4-triazol-3-yl)methyl)amino)benzoic acid (34). 4 N NaOH aqueous solution (55 mL) was added to a solution of ethyl 3-(((3-(4-pyridyl)-1*H*-1,2,4-triazol-5-yl) methyl)amino)benzoate (**33**, 14.3 g, 44.2 mmol) in ethanol (200 mL) and THF (200 mL). The mixture was stirred at room temperature for 11.5 h. The reaction mixture was neutralized with 12 N HCl aqueous solution and the precipitate was collected by filtration, followed by washing with acetone to afford 12.9g of **34** (43.7 mmol, 99%) as white solids. mp 231–238 °C (dec.). ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.51 (2H, d, J = 4.6 Hz), 6.61 (1H, br), 6.87 (1H, br), 7.18–7.23 (3H, m),

7.91 (2H, d, J = 6.0 Hz), 8.66 (2H, d, J = 6.0 Hz), CO_2H and 1,2,4-triazole-NH were not assigned. Anal. Calcd. for $C_{15}H_{13}N_5O_2 \cdot H_2O$: C, 57.50; H, 4.83; N, 22.35. Found: C, 57.27; H, 4.90; N, 21.94.

3-(2-Phenylethoxy)nitrobenzene (39c). A mixture of 3-nitrophenol (**37**, 7.00 g, 50.3 mmol) and potassium carbonate (31.3 g) in DMF (100 mL) was stirred at room temperature for 15 min. (2-Bromoethyl)benzene (37.2 g) was added. The mixture was stirred at room temperature for 17 h. Ethyl acetate was added to the reaction mixture and then the mixture was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting hexane and ethyl acetate to afford 10.2 g of **39c** (41.9 mmol, 83%) as pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.14 (2H, t, J = 7.1 Hz), 4.25 (2H, t, J = 6.9 Hz), 7.19–7.44 (7H, s), 7.72 (1H, t, J = 2.0 Hz), 7.81 (1H, ddd, J = 8.2, 2.0, 0.7 Hz).

General procedure for the preparation of *N*-benzyl-3-nitrobenzamide (39i). A mixture of 3-nitrobenzoic acid (**36**, 7.00 g, 41.9 mmol), benzylamine (4.49 g), WSC•HCl (12.0 g) and HOBt•H_2O (0.64 g) in DMF (50 mL) was stirred at room temperature for 12 h. Ethyl acetate was added to the reaction mixture and then the mixture was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by recrystallization from ethyl acetate to afford 8.69 g of **39i** (33.9 mmol, 81%) as white solids. The mother liquor was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting hexane and ethyl acetate to afford 1.22 g of

39i as white solids. Total: 9.11 g (38.7 mmol, 92% yield). mp 94–96 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.67 (2H, d, *J* = 5.7 Hz), 6.49 (1H, br), 7.30–7.38 (5H, m), 7.64 (1H, t, *J* = 8.0 Hz), 8.17 (1H, dt, *J* = 7.8, 1.5 Hz), 8.35 (1H, ddd, *J* = 8.3, 2.1, 1.1 Hz), 8.59 (1H, t, *J* = 2.0 Hz). Anal. Calcd. for $C_{14}H_{12}N_2O_3$: C, 65.62; H, 4.72; N, 10.93. Found: C, 65.59; H, 4.74; N, 10.87.

3-Nitro-*N*-(2-phenylethyl)benzamide (39j). This compound was prepared from 3-nitrobenzoic acid **36** and 2-phenylethylamine hydrochloride as described in the synthesis of **39i**, as pale yellow solids. mp 120–121 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.97 (2H, t, *J* = 6.8 Hz), 3.76 (2H, td, *J* = 6.9, 5.7 Hz), 6.20 (1H, br), 7.23–7.29 (3H, m), 7.31–7.37 (2H, m), 7.62 (1H, t, *J* = 7.8 Hz), 8.06 (1H, ddd, *J* = 7.7, 1.7, 1.0 Hz), 8.33 (1H, ddd, *J* = 8.1, 2.3, 1.1 Hz), 8.49 (1H, t, *J* = 1.7 Hz). Anal. Calcd. for $C_{15}H_{14}N_2O_3$: C, 66.66; H, 5.22; N, 10.36. Found: C, 66.41; H, 5.21; N, 10.43.

***N*-Benzyl-*N*-methyl-3-nitrobenzamide (39l).** This compound was prepared from 3-nitrobenzoic acid **36** and *N*-methylbenzylamine as described in the synthesis of **39i**, as pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) syn:anti = 1:1, δ 2.89 (1.5H, s), 3.09 (1.5H, s), 4.50 (1H, s), 4.78 (1H, s), 7.16 (1H, brs), 7.36–7.42 (4H, m), 7.61–7.65 (1H, m), 7.79 (1H, brs), 8.28 (1H, brs), 8.33 (1H, s).

***N*-Benzyl-*N*'-(3-nitrophenyl)urea (39p).** Benzylamine (5.9 mL) was added to a solution of 3-nitrophenylisocyanate (**38**, 8.10 g, 49.4 mmol) in THF (150 mL) at room temperature. The mixture was stirred at room temperature for 6.5 h. Hexane was added and then the mixture was stirred at room temperature. The precipitate was collected by filtration to afford 13.0 g of **39p** (47.9 mmol, 97%) as white solids. mp 190–191 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.31 (2H, d, *J* = 6.0 Hz),

6.83 (1H, t, *J* = 6.0 Hz), 7.21–7.35 (5H, m), 7.49 (1H, t, *J* = 8.1 Hz), 7.65 (1H, dd, *J* = 8.1, 1.8 Hz), 7.73 (1H, ddd, *J* = 8.1, 2.1, 0.9 Hz), 8.51 (1H, t, *J* = 2.3 Hz), 9.13 (1H, s). Anal. Calcd. for $C_{14}H_{13}N_3O_3$: C, 61.99; H, 4.83; N, 15.49. Found: C, 61.95; H, 4.93; N, 15.38.

4-Chloro-3-nitro-*N*-((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)aniline (40). This compound was prepared from 4-(3-(chloromethyl)-1*H*-1,2,4-triazol-5-yl)pyridine **23a** and 4-chloro-3-nitroaniline as described in the synthesis of **24c**, as yellow solids. mp 241–242 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 4.59 (2H, s), 6.88 (1H, dd, *J* = 8.7, 2.7 Hz), 7.67 (1H, d, *J* = 3.0 Hz), 7.33 (1H, d, *J* = 8.7 Hz), 8.02 (2H, dd, *J* = 4.7, 1.7 Hz), 8.64 (2H, dd, *J* = 4.7, 1.7 Hz), 2H were vanished. Anal. Calcd. for $C_{14}H_{11}ClN_6O_2$: C, 50.84; H, 3.35; N, 25.41. Found: C, 50.72; H, 3.46; N, 25.39.

4-Chloro-*N*-1-((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)benzene-1,3-diamine (41). This compound was prepared from 4-chloro-3-nitro-*N*-((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)aniline **40** as described in the synthesis of **29i**, as pale yellow solids. mp 192–195 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 4.49 (2H, s), 6.03 (1H, dd, *J* = 8.9, 2.3 Hz), 6.23 (1H, d, *J* = 2.1 Hz), 6.92 (1H, dd, *J* = 8.1, 1.2 Hz), 8.00–8.02 (2H, m), 8.62 (2H, dd, *J* = 6.0, 1.5 Hz). Anal. Calcd. for $C_{14}H_{13}ClN_6 \cdot 1/7H_2O$: C, 55.44; H, 4.41; N, 27.71. Found: C, 55.79; H, 4.43; N, 27.31.

General procedure for the preparation of 2-methyl-4-pyridinecarbaldehyde (44a). A solution of *n*-butyllithium in hexane (1.6 M, 60 mL) was added dropwise to a solution of 2,4-dimethylpyridine

(**42a**, 9.27 g, 86.5 mmol) in THF (100 mL) at –78 °C for 25 min under argon atmosphere. After the addition, the mixture was stirred at –78 °C for 35 min. Diethylamine (13.4 mL) was added at –78 °C for 25 min and then DMF (13.4 mL) was added at –78 °C for 25 min. The resulting mixture was stirred at –78 °C for 1.5 h. Saturated ammonium chloride aqueous solution was added and the mixture was extracted with chloroform. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The solution of the residue in DCM (100 mL) was added to a solution of sodium periodate (55.5 g) in water (200 mL) at 0 °C and the mixture was stirred at room temperature for 1 h. The mixture was treated with 8 N NaOH aqueous solution and the pH was adjusted to 9 – 10. The organic phase was separated, washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The solution of the residue in ethyl acetate was washed with water and brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to afford 9.64 g of **44a** (79.6 mmol, 92%) as orange oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.68 (3H, s), 7.52 (1H, d, J = 5.2 Hz), 7.57 (1H, s), 8.75 (1H, d, J = 5.2 Hz), 10.06 (1H, s).

3-Methyl-4-pyridinecarbaldehyde (44b). This compound was prepared from 3,4-dimethylpyridine **42b** as described in the synthesis of **44a**, as orange oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.59 (3H, s), 7.55 (1H, d, J = 5.2 Hz), 8.57 (1H, s), 8.65 (1H, d, J = 5.2 Hz), 10.27 (1H, s).

General procedure for the preparation of 2-methyl-4-pyridinecarbonitrile (45a). A solution of hydroxylamine hydrochloride (1.67 g) in 1 N NaOH aqueous solution (24 mL) was added to a

solution of 2-methyl-4-pyridinecarbaldehyde (**44a**, 2.42 g, 20.0 mmol) in acetonitrile (25 mL) and the mixture was stirred at room temperature for 30 min. The reaction mixture was concentrated under reduced pressure and then the mixture of the residue and acetic anhydride (25 mL) was stirred at reflux for 1 h. The reaction mixture was concentrated under reduced pressure and the residue was treated with water and 1 N NaOH aqueous solution at 0 °C. The mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting hexane and ethyl acetate to afford 1.54 g of **45a** (13.0 mmol, 65%) as pale yellow solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.64 (3H, s), 7.34 (1H, d, *J* = 5.2 Hz), 7.40 (1H, s), 8.69 (1H, d, *J* = 5.2 Hz).

3-Methyl-4-pyridinecarbonitrile (45b). This compound was prepared from 3-methyl-4-pyridinecarbaldehyde **44b** as described in the synthesis of **45a**, as pale yellow solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.55 (3H, s), 7.47 (1H, d, *J* = 5.2 Hz), 8.60 (1H, d, *J* = 5.2 Hz), 8.68 (1H, s).

4-(3-(Chloromethyl)-1*H*-1,2,4-triazol-5-yl)-2-methylpyridine (46a). This compound was prepared from 2-methyl-4-pyridinecarbonitrile **45a** as described in the synthesis of **23a**, as white solids. ^{1}H NMR (300 MHz, CD_3OD) δ 2.61 (3H, s), 4.79 (2H, s), 7.81 (1H, d, *J* = 7.2 Hz), 7.89 (1H, s), 8.53 (1H, d, *J* = 7.5 Hz), 1H was vanished.

4-(3-(Chloromethyl)-1*H*-1,2,4-triazol-5-yl)-3-methylpyridine (46b). This compound was prepared

from 3-methyl-4-pyridinecarbonitrile **45b** as described in the synthesis of **23a**, as white solids. ^{1}H NMR (300 MHz, CD_3OD) δ 2.62 (3H, s), 4.81 (2H, s), 7.83 (1H, d, *J* = 4.5 Hz), 8.46 (1H, d, *J* = 5.4 Hz), 8.52 (1H, s), 1H was vanished.

4-Chloro-*N*-((3-(2-methylpyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)aniline (47a). This compound was prepared from 4-(3-(chloromethyl)-1*H*-1,2,4-triazol-5-yl)-2-methylpyridine **46a** and 4-chloroaniline **1c** as described in the synthesis of **24c**, as white solids. mp 146–147 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.53 (3H, s), 4.44 (2H, brs), 6.49 (1H, brs), 6.64 (2H, d, *J* = 8.1 Hz), 7.11 (2H, d, *J* = 8.8 Hz), 7.70 (1H, dd, *J* = 5.0, 0.9 Hz), 7.78 (1H, s), 8.53 (1H, brs), 14.21 (1H, brs). ^{13}C NMR (151 MHz, DMSO-d_6) δ 24.00, 113.67, 117.06, 119.05, 119.86, 128.49, 138.56, 146.97, 149.55, 156.46, 158.58, 159.23, 1 aliphatic carbon was not assigned. Because the aliphatic carbon was hidden by DMSO. MS (ESI/APCI) *m/z* 300.1 $[M + H]^+$. HPLC purity 99.6%.

***N*-Benzyl-3-(((3-(3-methylpyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)amino)benzamide (47b).** This compound was prepared from 4-(3-(chloromethyl)-1*H*-1,2,4-triazol-5-yl)-3-methylpyridine **46b** and 3-amino-*N*-benzylbenzamide **29i** as described in the synthesis of **24c**, as white solids. mp 211–214 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.58 (3H, s), 4.44 (2H, d, *J* = 5.9 Hz), 4.51 (2H, d, *J* = 5.1 Hz), 6.45 (1H, brs), 6.81 (1H, dd, *J* = 7.9, 1.7 Hz), 7.12 (1H, d, *J* = 7.7 Hz), 7.17 (1H, d, *J* = 7.7 Hz), 7.19 (1H, s), 7.20–7.24 (1H, m), 7.27–7.32 (4H, m), 7.86 (1H, brs), 8.48 (1H, d, *J* = 5.1 Hz), 8.53 (1H, s), 8.84 (1H, t, *J* = 5.9 Hz), 14.24 (1H, brs). ^{13}C NMR (151 MHz, DMSO-d_6) δ 18.42, 42.39, 111.30, 115.08, 115.15, 121.79, 126.52, 126.99, 128.10, 128.63, 130.47, 135.19, 136.77,

139.72, 147.24, 148.10, 151.94, 155.91, 159.76, 166.61, 1 aliphatic carbon was not assigned. Because the aliphatic carbon was hidden by DMSO. Anal. Calcd. for $C_{23}H_{22}N_6O$•0.2AcOEt: C, 68.70; H, 5.72; N, 20.20. Found: C, 68.59; H, 5.82; N, 20.13. MS (ESI/APCI) *m/z* 399.1 $[M + H]^+$. HPLC purity 99.3%.

General procedure for the preparation of *N*-benzyl-3-(((3-(3-(hydroxymethyl)pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)amino)benzamide (47c). Sodium hydride (60% paraffin dispersion, 89 mg) was added to a suspension of (3-(*tert*-butyldimethylsilyloxy)methylpyridin-4-yl)carbonitrile (**53**, 830 mg, 3.34 mmol) and *N*-benzyl-3-((2-hydrazino-2-oxoethyl)amino)benzamide (**20g**, 665 mg) in ethanol (40 mL) and the mixture was stirred at 90 °C for 21.5 h. The reaction mixture was concentrated under reduced pressure and water was added to the residue. The mixture was neutralized with 1 N HCl aqueous solution and ethyl acetate was added. The mixture was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by recrystallization from ethanol to afford 749 mg of **47c** (1.81 mmol, 81%), as white solids. mp 194–195 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.06 (1H, t, J = 7.2 Hz), 4.44 (2H, d, J = 5.9 Hz), 4.52 (2H, d, J = 5.9 Hz), 4.88 (2H, s), 6.47 (1H, t, J = 5.9 Hz), 6.80 (1H, dd, J = 7.9, 1.7 Hz), 7.11–7.14 (1H, m), 7.15–7.19 (2H, m), 7.20–7.24 (1H, m), 7.28–7.32 (4H, m), 7.89 (1H, d, J = 5.1 Hz), 8.57 (1H, d, J = 5.1 Hz), 8.80 (1H, s), 8.84 (1H, t, J = 6.1 Hz), 1H was not assigned. ^{13}C NMR (151 MHz, DMSO-d_6) δ 42.40, 55.92, 60.07, 111.32, 115.03, 115.19, 121.38, 126.52, 126.99,

128.10, 128.67, 134.27, 135.04, 135.19, 139.72, 148.05, 148.29, 149.29, 156.52, 158.76, 166.60. Anal. Calcd. for $C_{23}H_{22}N_6O_2$•0.25H_2O: C, 65.94; H, 5.41; N, 20.06. Found: C, 65.86; H, 5.42; N, 19.76. MS (ESI/APCI) *m/z* 415.2 $[M + H]^+$. HPLC purity 95.7%.

2-(4-(5-(((4-Chlorophenyl)amino)methyl)-1*H*-1,2,4-triazol-3-yl)pyridin-3-yl)ethanol (47d). This compound was similarly prepared from 2-(4-cyanopyridin-3-yl)ethyl acetate **55** and 2-((4-chlorophenyl)amino)acetohydrazide **4c** as described in the synthesis of **47d**, as white foam. ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.21 (2H, brs), 3.60 (2H, t, *J* = 6.6 Hz), 4.44 (2H, brs), 4.63 (1H, brs), 6.47 (1H, brs), 6.66 (2H, d, *J* = 8.8 Hz), 7.11 (2H, d, *J* = 8.8 Hz), 7.81 (1H, brs), 8.48 (1H, d, *J* = 4.4 Hz), 8.53 (1H, brs), 14.25 (1H, brs). ^{13}C NMR (151 MHz, DMSO-d_6) δ 34.19, 61.09, 113.74, 119.75, 122.27, 128.46, 132.17, 146.99, 147.34, 152.51, 155.30, 159.61, 1 aliphatic carbon and 1 aromatic carbon were not assigned. Because the aliphatic carbon was hidden by DMSO. Exact Mass (FAB) $[M + H]^+$ for $C_{16}H_{17}ClN_5O$, Calcd: 330.1122. Found: 330.1122. MS (ESI/APCI) *m/z* 330.1 $[M + H]^+$. HPLC purity 92.8%.

(4-(5-(((3-(Benzylcarbamoyl)phenyl)amino)methyl)-1*H*-1,2,4-triazol-3-yl)pyridin-3-yl)methyl carbamate (47e). The mixture of *N*-benzyl-3-(((5-(3-(hydroxymethyl)pyridin-4-yl)-1*H*-1,2,4-triazol-3-yl)methyl)(trifluoroacetyl)amino)benzamide (**56**, 316 mg, 0.62 mmol) and trichloroacetyl isocyante (0.22 mL, 1.85 mmol) in acetonitrile (18 mL) was stirred at room temperature for 6 h. Potassium carbonate (513 mg), methanol (12 mL) and water (3.0 mL) were added. The mixture was stirred at room temperature for 4.5 h. The reaction mixture was concentrated

under reduced pressure. Ethyl acetate was added to the reaction mixture and then the mixture was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting ethyl acetate and methanol, and by recrystallization from ethyl acetate to afford 113 mg of **47e** (0.25 mmol, 40%) as white solids. mp 167–170 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.44 (2H, d, J = 5.9 Hz), 4.52 (2H, d, J = 5.9 Hz), 5.51 (2H, s), 6.46 (1H, t, J = 5.3 Hz), 6.81 (1H, dd, J = 8.1, 1.8 Hz + 2H, br), 7.12 (1H, d, J = 7.7 Hz), 7.17–7.20 (2H, m), 7.20–7.24 (1H, m), 7.26–7.32 (4H, m), 7.97 (1H, d, J = 4.4 Hz), 8.61 (1H, d, J = 5.1 Hz), 8.71 (1H, s), 8.84 (1H, t, J = 6.1 Hz), 14.36 (1H, brs). ^{13}C NMR (151 MHz, DMSO-d_6) δ 42.39, 61.76, 111.35, 115.03, 115.22, 121.58, 126.52, 126.99, 128.10, 128.69, 129.74, 135.18, 135.64, 139.71, 148.03, 148.91, 149.07, 156.34, 158.75, 166.59, 1 aliphatic carbon and 1 aromatic carbon were not assigned. Because the aliphatic carbon was hidden by DMSO. Anal. Calcd. for $C_{24}H_{23}N_7O_3$•2.5H_2O: C, 57.36; H, 5.62; N, 19.51. Found: C, 57.68; H, 5.44; N, 19.67. MS (ESI/APCI) *m/z* 458.2 $[M + H]^+$. HPLC purity 95.3%.

Dimethyl pyridine-3,4-dicarboxylate (49). 12 N HCl aqueous solution (100 mL) was added to a solution of pyridine-3,4-dicarboxylic acid (**48**, 50.0 g, 299 mmol) in methanol (300 mL). The mixture was stirred at reflux for 16 h. The reaction mixture was concentrated under reduced pressure and water was added. The mixture was treated with 1 N NaOH aqueous solution and the pH was adjusted to 10. The resulting mixture extracted with chloroform. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to afford 38.0

g of **21** (195 mmol, 65%) as yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.95 (6H, s), 7.51 (1H, dd, *J* = 5.2, 0.8 Hz), 8.84 (1H, d, *J* = 5.2 Hz), 9.07 (1H, d, *J* = 0.8 Hz).

General procedure for the preparation of pyridine-3,4-dimethanol (50). Sodium borohydride (6.80 g) was added to a solution of dimethyl pyridine-3,4-dicarboxylate (**49**, 20.0 g, 102 mmol) in ethanol (200 mL) at 0 °C. After the mixture was stirred at 0 °C for 15 min, calcium chloride (10.0 g) was added at 0 °C. After the addition, the mixture was allowed to warm to room temperature and then stirred for 16 h. The reaction mixture was concentrated under reduced pressure and water (50 mL) and conc. H_2SO_4 (8.80 g) were added. After the mixture was stirred at 90 °C for 20 min, the precipitate was removed by filtration. 12 N HCl aqueous solution (42.1 mL) was added to the filtrate and then the mixture was stirred at 90 °C for 30 min. The reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography on ion-exchanger resins (DOWEX 50WX8-100) afford 12.1 g of **22** (87.0 mmol, 85%) as white solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.52 (2H, s), 4.61 (2H, s), 5.26 (1H, br), 5.37 (1H, br), 7.43 (1H, d, *J* = 4.6 Hz), 8.45 (1H, s), 8.46 (1H, d, *J* = 4.6 Hz).

(3-(*tert*-Butyldimethylsilyloxy)methylpyridin-4-yl)methanol (51) and (4-(*tert*-butyldimethylsilyloxy) methylpyridin-3-yl)methanol (52). *tert*-Butyldimethylsilylchloride (5.28 g) was added to a solution of pyridine-3,4-dimethanol (**50**, 4.87 g, 35.0 mmol) and imidazole (2.76 g) in DMF (50 mL) at 0 °C. The mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure. Chloroform was added to the residue and then the

mixture was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting ethyl acetate to afford 0.95 g of **51** (3.75 mmol, 10%) as white solids and 2.01 g of **52** (7.93 mmol, 23%) as white solids. **(3-(*tert*-Butyldimethylsilyloxy)methylpyridin-4-yl)methanol (51)**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.13 (6H, s), 0.91 (9H, s), 3.27 (1H, t, J = 4.8 Hz), 4.73 (2H, d, J = 4.8 Hz), 4.78 (2H, s), 7.36 (1H, d, J = 5.2 Hz), 8.50 (1H, s), 8.56 (1H, d, J = 5.2 Hz).

(4-(*tert*-Butyldimethylsilyloxy)methylpyridin-3-yl)methanol (52): ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.13 (6H, s), 0.95 (9H, s), 3.62 (1H, br), 4.67 (2H, s), 4.84 (2H, s), 7.39 (1H, d, J = 5.2 Hz), 8.43 (1H, s), 8.49 (1H, d, J = 5.2 Hz).

(3-(*tert*-Butyldimethylsilyloxy)methylpyridin-4-yl)carbaldehyde. A solution of DMSO (1.36 mL) in DCM (5.0 mL) was added to a solution of oxalyl chloride (0.80 mL) in DCM (15 mL) and THF (15 mL) at –78 °C. After the addition, the mixture was stirred at –78 °C for 10 min and then a solution of (3-(*tert*-butyldimethylsilyloxy)methylpyridin-4-yl)methanol (**51**, 2.03 g, 8.0 mmol) in DCM (5.0 mL) was added at –78 °C. The resulting mixture was stirred at –78 °C for 30 min and then Et_3N (5.6 mL) was added. After the addition, the mixture was allowed to warm to room temperature and then stirred for 10 min. The reaction mixture was concentrated under reduced pressure. Ethyl acetate was added to the residue and then the mixture was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The

residue was purified by column chromatography on silica gel eluting ethyl acetate to afford 2.01 g of (3-(*tert*-butyldimethylsilyloxy)methylpyridin-4-yl)carbaldehyde (3.75 mmol, quantitative yield) as pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.13 (6H, s), 0.94 (9H, s), 5.12 (2H, s), 7.65 (1H, d, *J* = 5.2 Hz), 8.82 (1H, d, *J* = 5.2 Hz), 8.99 (1H, s), 10.30 (1H, s).

(3-(*tert*-Butyldimethylsilyloxy)methylpyridin-4-yl)carbonitrile (53). A solution of hydroxylamine hydrochloride (833 mg) in 1 N NaOH aqueous solution (12 mL) was added to a solution of (3-(*tert*-butyldimethylsilyloxy)methylpyridin-4-yl)carbaldehyde (2.51 g, 10.0 mmol) in acetonitrile (20 mL) and the mixture was stirred at room temperature for 30 min. The reaction mixture was concentrated under reduced pressure. Water was added to the residue and then the mixture was extracted with chloroform. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The mixture of the residue and carbonyldiimidazole (2.43 g) was stirred at reflux for 16 h. The reaction mixture was concentrated under reduced pressure. Water was added to the residue and then the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting hexane and ethyl acetate to afford 2.01 g of **53** (8.09 mmol, 81%) as pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.17 (6H, s), 0.96 (9H, s), 4.93 (2H, s), 7.48 (1H, d, *J* = 5.0 Hz), 8.70 (1H, d, *J* = 5.0 Hz), 8.92 (1H, s).

(4-((*tert*-Butyldimethylsilyloxy)methyl)pyridin-3-yl)acetonitrile (54). Tributylphosphine (3.7

mL) was added to a solution of (4-(*tert*-butyldimethylsilyloxy)methylpyridin-3-yl)methanol (**52**, 2.53 g, 10.0 mmol), acetone cyanohydrin (1.23 g) and 1,1'-(azodicarbonyl)dipiperidine (3.79 g) in THF (25 mL) 0 °C. The mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure. 1 N NaOH aqueous solution was added to the residue and then the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting hexane and ethyl acetate to afford 2.23 g of **54** (8.50 mmol, 85%) as pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.14 (6H, s), 0.95 (9H, s), 3.76 (2H, s), 4.78 (2H, s), 7.39 (1H, d, J = 5.2 Hz), 8.56 (1H, s), 8.60 (1H, d, J = 5.2 Hz).

Ethyl (4-hydroxymethylpyridin-3-yl)acetate. 12 N HCl aqueous solution (4.1 mL) was added to a solution of (4-((*tert*-butyldimethylsilyloxy)methyl)pyridin-3-yl)acetonitrile (**54**, 2.62 g, 10.0 mmol) in ethanol (25 mL). The mixture was stirred at reflux for 16 h. The reaction mixture was concentrated under reduced pressure. The residue was made alkaline with 1 N NaOH aqueous solution and then the solution was extracted with chloroform. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting ethyl acetate to afford 1.27 g of ethyl (4-hydroxymethylpyridin-3-yl)acetate (6.51 mmol, 65%) as yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (3H, t, J = 7.4 Hz), 3.73 (2H, s), 4.08 (2H, q, J = 7.4 Hz), 4.52 (2H, d, J = 5.4 Hz), 5.40 (1H, t, J = 5.4 Hz), 7.43 (1H, d, J = 5.2 Hz), 8.34 (1H, s), 8.45 (1H, d, J = 5.2 Hz).

(4-Hydroxymethylpyridin-3-yl)ethanol. This compound was prepared from ethyl (4-hydroxymethylpyridin- 3-yl)acetate as described in the synthesis of **50**, as yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.72 (2H, t, J = 7.0 Hz), 3.59 (2H, t, J = 7.0 Hz), 4.59 (2H, s), 7.39 (1H, d, J = 5.2 Hz), 8.32 (1H, s), 8.37 (1H, d, J = 5.2 Hz).

2-(4-Cyanopyridin-3-yl)ethyl acetate (55). Manganese(IV) oxide (12.5 g) was added to a solution of (4-hydroxymethylpyridin-3-yl)ethanol (2.01 g, 13.1 mmol) in chloroform (15 mL) and methanol (5.0 mL). The mixture was stirred at room temperature for 16 h. The reaction mixture was filtered and then washed with methanol. The filtrate was combined and then concentrated under reduced pressure. A solution of hydroxylamine hydrochloride (910 mg) in 1 N NaOH aqueous solution (13.1 mL) was added to a solution of the residue in acetonitrile (20 mL) and the mixture was stirred at room temperature for 30 min. The reaction mixture was concentrated under reduced pressure and then the mixture of the residue and acetic anhydride (15 mL) was stirred at reflux for 1 h. The reaction mixture was concentrated under reduced pressure and the residue was treated with water and 1 N NaOH aqueous solution at 0 °C. The mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting hexane and ethyl acetate to afford 1.89 g of **55** (9.94 mmol, 76%) as pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.05 (3H, s), 3.19 (2H, t, J = 6.6 Hz), 4.37 (2H, t, J = 6.6 Hz), 7.52 (1H, d, J = 5.2 Hz), 8.68 (1H, d, J = 5.2 Hz), 8.71 (1H, s).

_N_-Benzyl-3-(((5-(3-(hydroxymethyl)pyridin-4-yl)-1_H_-1,2,4-triazol-3-yl)methyl)(trifluoroacetyl) amino)benzamide (56). To a suspension of _N_-benzyl-3-(((3-(3-(hydroxymethyl)pyridin-4-yl)-1_H_-1,2,4-triazol-5-yl)methyl)amino)benzamide (**47c**, 304 mg, 0.73 mmol) in THF (17 mL) was added Et_3N (0.72 mL) and trifluoroacetic anhydride (0.61 mL) at 0 °C. The mixture was stirred at room temperature for 1 h. Methanol (4.0 mL) and water (2.0 mL) were added and then the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure. Ethyl acetate was added to the residue and then the mixture was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting ethyl acetate and methanol to afford 326 mg of **32** (0.64 mmol, 87%) as white foam. ^{1}H NMR (300 MHz, DMSO-d_6) ratio: 3:2, δ 4.43 (0.8H, s), 4.48 (1.2H, d, J = 6.0 Hz), 4.84–4.87 (2H, m), 5.11–5.16 (2H, m), 7.14–7.32 (6H, m), 7.58–7.66 (2H, m), 7.90–8.07 (2H, m), 8.55 (0.6H, s), 8.62 (0.4H, d, J = 4.5 Hz), 8.79 (0.4H, s), 8.81 (0.6H, s), 8.87 (0.4H, s), 9.13 (0.6H, t, J = 6.3 Hz).

2-(Acetylamino)-4-methylpyridine (58). A mixture of 2-amino-4-picoline (**57**, 48.7 g, 450 mmol) and acetic anhydride (130 mL) was stirred at 70 °C for 2 h. The reaction mixture was concentrated under reduced pressure. Water (100 mL) was added. The mixture was neutralized with 8N NaOH aqueous solution at 0 °C. The resulting mixture was extracted with ethyl acetate. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to afford 59.0 g

of **58** (393 mmol, 87%) as white solids. ^{1}H NMR (300 MHz, $CDC1_3$) δ 2.21 (3H, s), 2.38 (3H, s), 6.87 (1H, d, *J* = 4.8 Hz), 7.93 (1H , d, *J* = 5.7 Hz), 8.16 (1H, s), 10.30 (1H, br).

2-(Acetylamino)isonicotinic acid (59). Potassium permanganate (50.0 g) was carefully added to a solution of 2-(acetylamino)-4-methylpyridine (**58**, 20.0 g, 133 mmol) in water (200 mL) at 90 °C for 1 h. The mixture was stirred at 90 °C for 3 h. The reaction mixture was filtered with Celite and the filtrate was treated with conc. HCl aqueous solution to adjust the pH = 4. The resulting mixture was cooled in ice bath. The precipitate was collected and then dried over to afford 10.0 g of **59** (55.5 mmol, 21%) as white solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.12 (3H, s), 4.02 (1H, brs), 7.49 (1H, dd, *J* = 5.1, 1.5 Hz), 8.45 (1H, d, *J* = 5.1 Hz), 8.55 (1H, s), CO_2H was not assigned.

***N*'-(*tert*-Butoxycarbonyl)-2-(acetylamino)isonicotinic acid hydrazide (60).** Et_3N (20.0 mL) was added to a mixture of 2-(acetylamino)isonicotinic acid (**59**, 10.0 g, 55.5 mmol), *tert*-butylcarbazate (9.30 g), WSC•HCl (19.0 g) and HOBt•H_2O (15.5 g) in DMF (100 mL) at room temperature. The mixture was stirred at room temperature for 48 h. The reaction mixture was concentrated under reduced pressure. Ethyl acetate was added to the residue and then the mixture was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The solid residue was washed with diethyl ether to afford 7.90 g of **60** (26.8 mmol, 48%) as white solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.43 (9H, s), 2.12 (3H, s), 7.41 (1H, d, *J* = 4.8 Hz), 8.44 (2H, d, *J* = 5.1 Hz), 9.01 (1H, s), 10.42 (1H, s), 10.66 (1H, s).

2-(Acetylamino)isonicotinic acid hydrazide hydrochloride (61). A solution of 4 N HCl in ethyl

acetate (18 mL) was added to *N*'-(*tert*-butoxycarbonyl)-2-(acetylamino)isonicotinic acid hydrazide (**60**, 7.90 g, 26.8 mmol) at room temperature. The mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure to afford 7.00 g of **61** (quantitative yield) as white solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.13 (3H, s), 7.55–7.58 (1H, m), 8.46–8.50 (2H, m), 10.87 (1H, s).

General procedure for the preparation of *N*-methyl-(2-acetoxy)acetamide (63). Acetoxyacetyl chloride (**62**, 25.0 g, 183 mmol) was added to a solution of methylamine in THF (2.0 M, 100 mL) and Et_3N (20.2 g) in THF (200 mL) at 0 °C. The mixture was stirred at room temperature for 24 h. The reaction mixture was concentrated under reduced pressure. Diethyl ether was added to the residue and then the mixture was washed with water and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to afford 8.00 g of **63** (61.0 mmol, 31%) as crude light yellow oil. This product was used without further purification. ^{1}H NMR (300 MHz, $CDC1_3$) δ 2.17 (3H, s), 2.88 (3H, d, J = 7.2 Hz), 4.57 (2H, s), 6.00–6.43 (1H, br).

General procedure for the preparation of *N*-methyl-(2-acetoxy)thioacetamide (64). A mixture of *N*-methyl-(2-acetoxy)acetamide (**63**, 4.00 g, 30.5 mmol) and Lawesson's reagent (18.2 g) in THF (50 mL) was stirred at 70 °C for 4 h. The reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting hexane and ethyl acetate to afford 3.60 g of **64** (24.5 mmol, 79%) as white solids. ^{1}H NMR (300 MHz, $CDC1_3$) δ 2.19 (3H, s), 3.26 (3H, d, J = 4.8 Hz), 4.99 (2H, s), 8.04 (1H, br).

General procedure for the preparation of ((5-(2-(acetylamino)pyridin-4-yl)-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)acetate (65a). A solution of triethyloxonium tetrafluoroborate in DCM (1.0 M, 18.0 mL) was added to a solution of *N*-methyl-(2-acetoxy)thioacetamide (**64**, 2.20 g, 15.0 mmol) in DCM (20 mL) at 0 °C. The mixture was stirred at room temperature for 1.5 h. The reaction mixture was poured into iced water. The mixture was treated with saturated $NaHCO_3$ aqueous solution to adjust the pH = 9. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford oil product.

A solution of 2-(acetylamino)isonicotinic acid hydrazide hydrochloride (**61**, 2.70 g, 11.7 mmol) and Et_3N (1.7 mL) in 1-butanol (30 mL) was added to the above oil in 1-butanol (10 mL) at room temperature. The mixture was stirred at reflux for 20 h. The reaction mixture was concentrated under reduced pressure. MeOH (50 mL) was added and the mixture was filtered. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting ethyl acetate and methanol to afford 900 mg of **65a** (3.11 mmol, 27%) as white solids. ^{1}H NMR (300 MHz, $CDC1_3$) δ 2.16 (3H, s), 2.26 (3H, s), 3.82 (3H, s), 5.37 (2H, s), 7.51–7.56 (1H, m), 8.30 (1H, s), 8.43 (1H, d, J = 5.1 Hz), 8.48 (1H, s).

General procedure for the preparation of (4-methyl-5-(2-(methylamino)pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl)methanol (66b). A mixture of (5-(2-chloropyridin-4-yl)-4-methyl-4*H*-1,2,4-triazol-3-yl)methanol (**76**, 1.05 g, 4.67 mmol) and a

solution of methylamine in methanol (40%, 10 mL) was stirred at 150 ºC for 16 h in sealed tube. The reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting ethyl acetate and methanol and by recrystallization from methanol and ethyl acetate to afford 658 mg of **66b** (3.00 mmol, 64%) as white solids. mp 172 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.81 (3H, d, J = 4.9 Hz), 3.71 (3H, s), 4.65 (2H, d, J = 5.7 Hz), 5.60 (1H, t, J = 5.7 Hz), 6.72–6.80 (3H, m), 8.13 (1H, d, J = 5.3 Hz). Anal. Calcd. for $C_{10}H_{13}N_5O$: C, 54.78; H, 5.98; N, 31.94. Found: C, 54.68; H, 5.86; N, 31.96.

(5-(2-(Benzylamino)pyridin-4-yl)-4-methyl-4*H*-1,2,4-triazol-3-yl)methanol (66c). This compound was prepared from (5-(2-chloropyridin-4-yl)-4-methyl-4*H*-1,2,4-triazol-3-yl)methanol **76** and benzylamine as described in the synthesis of **66b**, as white solids. mp 198 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.67 (3H, s), 4.53 (2H, d, J = 5.8 Hz), 4.65 (2H, d, J = 5.7 Hz), 5.59 (1H, t, J = 5.7 Hz), 6.77–6.84 (2H, m), 7.19–7.26 (1H, m), 7.28–7.39 (5H, m), 8.11 (1H, d, J = 5.3 Hz). Anal. Calcd. for $C_{16}H_{17}N_5O$•0.1H_2O: C, 64.67; H, 5.83; N, 23.57. Found: C, 64.38; H, 5.75; N, 23.43.

(4-Methyl-5-(2-((2-phenylethyl)amino)pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl)methanol (66d). This compound was prepared from (5-(2-chloropyridin-4-yl)-4-methyl-4*H*-1,2,4-triazol-3-yl)methanol **76** and phenylethylamine as described in the synthesis of **66b**, as white solids. mp 135–138 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.86 (2H, t, J = 7.3 Hz), 3.52 (2H, dt, J = 7.5, 6.4 Hz), 3.71 (3H, s), 4.65 (2H, d, J = 5.7 Hz), 5.60 (1H, t, J = 5.7 Hz), 6.75–6.80 (2H, m), 6.89 (1H, t, J = 5.7 Hz), 7.17–7.23 (1H, m), 7.24–7.34 (4H, m), 8.14 (1H, dd, J = 5.1, 0.6 Hz). Anal. Calcd. for $C_{17}H_{19}N_5O$: C, 66.00; H,

6.19; N, 22.64. Found: C, 65.74; H, 6.22; N, 22.52.

(4-Methyl-5-(2-((3-phenylpropyl)amino)pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl)methanol (66e). This compound was prepared from (5-(2-chloropyridin-4-yl)-4-methyl-4*H*-1,2,4-triazol-3-yl)methanol **76** and phenylpropylamine as described in the synthesis of **66b**, as light yellow foam. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.85 (2H, quintet, J = 7.4 Hz), 2.67 (2H, t, J = 7.7 Hz), 3.24–3.31 (2H, m), 3.71 (3H, s), 4.65 (2H, d, J = 5.7 Hz), 5.60 (1H, t, J = 5.7 Hz), 6.73–6.78 (2H, m), 6.86 (1H, t, J = 5.4 Hz), 7.14–7.32 (5H, m), 8.10 (1H, d, J = 5.1 Hz).

(2-Acetylamino)-4-(5-(chloromethyl)-4-methyl-4*H*-1,2,4-triazol-3-yl)pyridine (67a). 1 N NaOH aqueous solution (4.0 mL) was added to a solution of ((5-(2-(acetylamino)pyridin-4-yl)-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)acetate (434 mg, 1.51 mmol) in methanol (2.0 mL) at room temperature. The mixture was stirred at room temperature for 10 min. The reaction mixture was neutralized with 1 N HCl aqueous solution. The resulting mixture was concentrated under reduced pressure. The residue (**66a**) was suspended with DCM (2.0 mL) and thionyl chloride (3.0 mL) was added at room temperature. The mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated under reduced pressure. The residue was dissolved with water (2.0 mL) and then treated with saturated $NaHCO_3$ aqueous solution. The solid residue was collected and dried to afford 290 mg of **67a** (1.09 mmol, 73%) as white solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.13 (3H, s), 3.77 (3H, s), 5.08 (2H, s), 7.43–7.49 (1H, m), 8.43 (1H, s), 8.48 (1H, d, J = 5.1 Hz), 10.73 (1H, s).

3-(((5-(2-Acetamidopyridin-4-yl)-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)amino)-*N*-(2-(trifluoro methyl)benzyl)benzamide (68a). This compound was prepared from 3-amino-*N*-(2-(trifluoromethyl)benzyl)benzamide **69a** as described in the synthesis of **24c**, as white solids. ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.13 (3H, s), 3.75 (3H, s), 4.55 (2H, d, J = 5.5 Hz), 4.65 (2H, d, J = 5.5 Hz), 6.48 (1H, t, J = 5.7 Hz), 6.95 (1H, dd, J = 8.1, 1.8 Hz), 7.16 (1H, d, J = 7.7 Hz), 7.20–7.25 (1H, m), 7.29 (1H, s), 7.42 (1H, dd, J = 5.1, 1.5 Hz), 7.47 (1H, t, J = 7.5 Hz), 7.52 (1H, d, J = 7.7 Hz), 7.64–7.68 (1H, m), 7.73 (1H, d, J = 7.7 Hz), 8.42 (1H, s), 8.47 (1H, d, J = 5.1 Hz), 8.94 (1H, t, J = 5.9 Hz), 10.71 (1H, s). ^{13}C NMR (151 MHz, DMSO-d_6) δ 23.85, 31.42, 37.93, 38.99, 111.52, 111.87, 115.01, 115.16, 117.71, 124.43 (q, J = 273.7 Hz), 125.59 (q, J = 5.9 Hz), 125.92 (q, J = 30.4 Hz), 127.09, 127.90, 128.79, 132.55, 134.89, 136.29, 137.74 (q, J = 1.1 Hz), 148.07, 148.60, 152.54, 152.55, 154.06, 167.05, 169.54. MS (ESI/APCI) *m/z* 524.2 $[M + H]^+$. HPLC purity 98.6%.

General procedure for the preparation of *N*-(2,6-difluorobenzyl)-3-(((4-methyl-5-(2-(methylamino)pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl)met hyl)amino)benzamide (68b). A mixture of 4-methyl-5-(2-(methylamino)pyridin-4-yl)-4*H*-1,2,4-triazole-3-carbaldehyde (**77b**, 245 mg, 1.13 mmol), *N*-2,6-difluorobenzyl-3-aminobenzamide (**69b**, 592 mg) and acetic acid (0.60 mL) in methanol (12 mL) was stirred at room temperature for overnight. The reaction mixture was concentrated under reduced pressure. The residue was dissolved with methanol (20 mL) and then sodium borohydride (128 mg) was added at 0 ºC. The resulting mixture was stirred at 0 ºC for 50 min

and at room temperature for 21 h. The reaction mixture was concentrated under reduced pressure. Ethyl acetate was added to the reaction mixture and then the mixture was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting ethyl acetate and methanol to afford 401 mg of **68b** (0.87 mmol, 77%) as light yellow foam. ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.81 (3H, d, J = 5.1 Hz), 3.69 (3H, s), 4.49 (4H, d, J = 5.5 Hz), 6.39 (1H, t, J = 5.7 Hz), 6.71–6.76 (3H, m), 6.89 (1H, dd, J = 8.1, 1.8 Hz), 7.04–7.09 (3H, m), 7.13–7.17 (1H, m), 7.18 (1H, t, J = 1.7 Hz), 7.34–7.41 (1H, m), 8.12 (1H, d, J = 5.1 Hz), 8.67 (1H, t, J = 5.3 Hz). ^{13}C NMR (151 MHz, DMSO-d_6) δ 27.81, 31.29 (t, J = 3.6 Hz), 31.29 (s), 37.92, 106.48, 109.86, 111.31 (dd, J = 21.0, 5.5 Hz), 111.54, 114.29 (t, J = 18.8 Hz), 114.74, 115.21, 128.57, 129.52 (t, J = 10.5 Hz), 134.96, 147.95, 148.32, 153.09, 153.59, 159.63, 161.08 (dd, J = 248.0, 8.6 Hz), 166.47, 1 aromatic carbon was not assigned. Anal. Calcd. for $C_{24}H_{23}F_2N_7O$•0.5H_2O•0.25AcOEt: C, 60.72; H, 5.30; N, 19.83. Found: C, 60.68; H, 5.24; N, 19.71. MS (ESI/APCI) *m/z* 464.2 $[M + H]^+$. HPLC purity 95.6%.

3-(((5-(2-(Benzylamino)pyridin-4-yl)-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)amino)-*N*-(2,6-difluorobenzyl)benzamide (68c). This compound was prepared from 5-(2-(benzylamino)pyridin-4-yl)-4-methyl-4*H*-1,2,4-triazole-3-carbaldehyde **77c** as described in the synthesis of **68b**, as light yellow foam. ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.65 (3H, s), 4.49 (4H, t, J = 4.6 Hz), 4.52 (2H, d, J = 6.2 Hz), 6.38 (1H, t, J = 5.5 Hz), 6.77 (1H, dd, J = 5.1, 1.5 Hz), 6.80 (1H, s), 6.88 (1H, dd, J = 8.1, 1.8 Hz), 7.04–7.10 (3H, m), 7.12–7.18 (2H, m), 7.20–7.24 (1H, m),

7.30–7.39 (6H, m), 8.10 (1H, d, J = 5.1 Hz), 8.67 (1H, t, J = 5.3 Hz). ^{13}C NMR (151 MHz, DMSO-d_6) δ 31.27, 31.29 (t, J = 4.4 Hz), 37.92, 44.08, 106.92, 110.32, 111.31 (dd, J = 21.0, 5.0 Hz), 111.54, 114.29 (t, J = 18.8 Hz), 114.74, 115.21, 126.49, 127.13, 128.13, 128.57, 129.52 (t, J = 10.2 Hz), 134.96, 135.09, 140.15, 147.95, 148.28, 152.98, 153.63, 158.85, 161.08 (dd, J = 8.3, 247.7, 8.3 Hz), 166.46. Anal. Calcd. for $C_{30}H_{27}F_2N_7O$•1/3H_2O: C, 66.04; H, 5.11; N, 17.97. Found: C, 65.84; H, 5.19; N, 17.67. MS (ESI/APCI) *m/z* 540.2 $[M + H]^+$. HPLC purity 96.6%.

***N*-(2,6-Difluorobenzyl)-3-(((4-methyl-5-(2-((2-phenylethyl)amino)pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)benzamide (68d).** This compound was prepared from 4-methyl-5-(2-((2-phenylethyl)amino)pyridin-4-yl)-4*H*-1,2,4-triazole-3-carbaldehyde **77d** as described in the synthesis of **68b**, as white foam. ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.85 (2H, t, J = 7.3 Hz), 3.50–3.55 (2H, m), 3.69 (3H, s), 4.49 (4H, d, J = 5.5 Hz), 6.39 (1H, t, J = 5.5 Hz), 6.75 (1H, dd, J = 5.5, 1.5 Hz), 6.77 (1H, s), 6.86 (1H, t, J = 5.7 Hz), 6.89 (1H, dd, J = 8.1, 1.8 Hz), 7.04–7.10 (3H, m), 7.14–7.17 (1H, m), 7.17–7.21 (2H, m), 7.25–7.27 (2H, m), 7.28–7.31 (2H, m), 7.35–7.41 (1H, m), 8.13 (1H, d, J = 5.1 Hz), 8.67 (1H, t, J = 5.3 Hz). ^{13}C NMR (151 MHz, DMSO-d_6) δ 31.29 (t, J = 3.9 Hz), 31.31, 35.00, 37.93, 42.31, 106.99, 109.98, 111.31 (dd, J = 20.5, 5.0 Hz), 111.54, 114.30 (t, J = 19.1 Hz), 114.74, 115.21, 125.87, 128.17, 128.57, 129.52 (t, J = 10.2 Hz), 134.96, 135.00, 139.78, 147.95, 148.34, 153.05, 153.60, 158.92, 161.08 (dd, J = 247.7, 8.3 Hz), 166.47. Anal. Calcd. for $C_{31}H_{29}F_2N_7O$•H_2O: C, 65.14; H, 5.47; N, 17.15. Found: C, 65.43; H, 5.37; N, 16.99. MS (ESI/APCI) *m/z* 554.2 $[M + H]^+$. HPLC purity 97.9%.

***N*-(2,6-Difluorobenzyl)-3-(((4-methyl-5-(2-((3-phenylpropyl)amino)pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)benzamide (68e).** This compound was prepared from 4-methyl-5-(2-((3-phenylpropyl)amino)pyridin-4-yl)-4*H*-1,2,4-triazole-3-carbaldehyde **77e** as described in the synthesis of **68b**, as white solids. mp 190 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.85 (2H, quintet, J = 7.3 Hz), 2.67 (2H, t, J = 7.7 Hz), 3.26–3.30 (2H, m), 3.69 (3H, s), 4.49 (4H, d, J = 5.5 Hz), 6.39 (1H, t, J = 5.5 Hz), 6.73 (1H, dd, J = 5.3, 1.3 Hz), 6.75 (1H, s), 6.84 (1H, t, J = 5.5 Hz), 6.89 (1H, dd, J = 7.9, 2.0 Hz), 7.04–7.10 (3H, m), 7.14–7.19 (3H, m), 7.21–7.24 (2H, m), 7.26–7.30 (2H, m), 7.35–7.41 (1H, m), 8.09 (1H, d, J = 5.1 Hz), 8.67 (1H, t, J = 5.3 Hz). ^{13}C NMR (151 MHz, DMSO-d_6) δ 30.63, 31.29 (t, J = 3.3 Hz), 31.31, 32.59, 37.93, 40.23, 106.85, 109.81, 111.31 (dd, J = 21.0, 5.5 Hz), 111.54, 114.30 (t, J = 19.1 Hz), 114.74, 115.21, 125.58, 128.16, 128.18, 128.57, 129.52 (t, J = 10.5 Hz), 134.96, 141.76, 147.95, 148.28, 153.08, 153.59, 159.09, 161.08 (dd, J = 247.7, 8.3 Hz), 166.46, 1 aromatic carbon was not assigned. Anal. Calcd. for $C_{32}H_{31}F_2N_7O$•0.5H_2O: C, 66.65; H, 5.59; N, 17.00. Found: C, 66.55; H, 5.45; N, 17.00. MS (ESI/APCI) *m/z* 568.3 $[M + H]^+$. HPLC purity 99.8%.

3-Amino-*N*-(2-(trifluoromethyl)benzyl)benzamide (69a). This compound was prepared from 3-aminobenzoic acid **35a** and 2-trifluoromethylbenzylamine as described in the synthesis of **29i**, as white solids. mp 153–155 ºC. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.79 (2H, brs), 4.81 (2H, d, J = 6.0 Hz), 6.45 (1H, s), 6.80 (1H, ddd, J = 8.0, 2.6, 0.7 Hz), 7.05 (1H, d, J = 7.8 Hz), 7.14 (1H, t, J = 2.0 Hz), 7.19 (1H, t, J = 7.7 Hz), 7.39 (1H, t, J = 7.5 Hz), 7.53 (1H, t, J = 7.5 Hz), 7.64 (1H, d, J = 8.4 Hz),

7.67 (1H, d, J = 9.0 Hz). Anal. Calcd. for $C_{15}H_{13}F_3N_2O$: C, 61.22; H, 4.45; N, 9.52. Found: C, 61.06; H, 4.36; N, 9.60.

***N*-2,6-Difluorobenzyl-3-aminobenzamide (69b).** This compound was prepared from 3-aminobenzoic acid **35a** and 2,6-difluorobenzylamine as described in the synthesis of **29i**, as white solids. ^{1}H NMR (300 MHz, CDC1$_3$) δ 3.78 (2H, br), 4.72 (2H, d, J = 5.7 Hz), 6.40 (1H, br), 6.75–6.78 (1H, m), 6.87–6.92 (2H, m), 7.01–7.04 (1H, m), 7.10–7.27 (3H, m).

2-Chloroisonicotinohydrazide (71). A mixture of methyl 2-chloronicotinate (**70**, 48.7 g, 284 mmol) and hydrazine hydrate (21.3 g) in ethanol (300 mL) was stirred at 90 ºC for 4 h. The reaction mixture was cooled to room temperature. The solid residue was rinsed with ethyl acetate and IPE, and dried to afford 42.2 g of **71** (246 mmol, 87%) as light yellow solids. mp 165 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.67 (2H, s), 7.75 (1H, dd, J = 5.2, 1.4 Hz), 7.82 (1H, s), 8.55 (1H, d, J = 5.1 Hz), 10.19 (1H, s). Anal. Calcd. for $C_6H_6ClN_3O$: C, 42.00; H, 3.52; N, 24.49. Found: C, 41.66; H, 3.66; N, 24.43.

Ethyl 2-(methylamino)-2-oxoacetate (73). This compound was prepared from ethyl chloroglyoxylate **72** as described in the synthesis of **63**, as brown oil. This product was used without further purification.

Ethyl 2-(methylamino)-2-thioxoacetate (74). This compound was prepared from ethyl 2-(methylamino)-2-oxoacetate **73** as described in the synthesis of **64**, as brown oil. This product was used without further purification.

Ethyl 5-(2-chloropyridin-4-yl)-4-methyl-4*H*-1,2,4-triazole-3-carboxylate (75). This compound was prepared from 2-chloroisonicotinohydrazide **71** and ethyl 2-(methylamino)-2-thioxoacetate **74** as described in the synthesis of **65a**, as light yellow solids. mp 165 ºC. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.49 (3H, t, *J* = 7.2 Hz), 4.06 (3H, s), 4.53 (2H, q, *J* = 7.2 Hz), 7.56 (1H, dd, *J* = 5.1, 1.5 Hz), 7.69 (1H, dd, *J* = 1.4, 0.7 Hz), 8.61 (1H, dd, *J* = 5.1, 0.8 Hz). Anal. Calcd. for $C_{11}H_{11}ClN_4O_2$: C, 49.54; H, 4.16; N, 21.01. Found: C, 49.34; H, 4.06; N, 21.13.

General procedure for the preparation of (5-(2-chloropyridin-4-yl)-4-methyl-4*H*-1,2,4-triazol-3-yl)methanol (76). To a suspension of ethyl 5-(2-chloropyridin-4-yl)-4-methyl-4*H*-1,2,4-triazole-3-carboxylate (**75**, 13.3 g, 49.9 mmol) in ethanol (500 mL) were added sodium borohydride (2.26 g) and calcium chloride (3.32 g) at 0 ºC. The mixture was stirred at 0 ºC for 12.5 h. Conc. H_2SO_4 (2.93 g) was added to the reaction mixture at 0 ºC and then the resulting mixture was stirred at room temperature for 12 h. The reaction mixture was filtered with Celite and then washed with ethanol. The filtrate was concentrated under reduced pressure. The solid residue was washed with ethanol and ethyl acetate to afford 6.37 g of **76** as white solids. 2nd crop was 1.30 g. Total: 7.67 g (34.1 mmol, 68%). mp 178–179 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.80 (3H, s), 4.68 (2H, d, *J* = 5.8 Hz), 5.69 (1H, t, *J* = 5.7 Hz), 7.80 (1H, dd, *J* = 5.3, 1.5 Hz), 7.88 (1H, d, *J* = 0.8 Hz), 8.60 (1H, d, *J* = 4.5 Hz).

General procedure for the preparation of 4-methyl-5-(2-(methylamino)pyridin-4-yl)-4*H*-1,2,4-triazole-3-carbaldehyde (77b). A mixture of

(4-methyl-5-(2-(methylamino)pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl)methanol (**66b**, 500 mg, 2.28 mmol) and manganese(IV) oxide (2.30 g) in methanol (20 mL) was stirred at room temperature for 1 h. The reaction mixture was filtered with Celite and washed with methanol. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting ethyl acetate and methanol and by recrystallization from methanol and ethyl acetate to afford 487 mg of **77b** (2.24 mmol, 98%) as white solids. mp 179–182 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.82 (3H, d, J = 4.9 Hz), 3.92 (3H, s), 6.79–6.83 (2H, m), 6.88 (1H, q, J = 4.4 Hz), 8.18 (1H, dd, J = 5.2, 0.7 Hz), 10.05 (1H, s). Anal. Calcd. for $C_{10}H_{11}N_5O$•0.1H_2O: C, 54.84; H, 5.15; N, 31.97. Found: C, 54.56; H, 5.08; N, 31.57.

5-(2-(Benzylamino)pyridin-4-yl)-4-methyl-4*H*-1,2,4-triazole-3-carbaldehyde (77c). This compound was prepared from (5-(2-(benzylamino)pyridin-4-yl)-4-methyl-4*H*-1,2,4-triazol-3-yl)methanol **66c** as described in the synthesis of **77b**, as white solids. mp 154–155 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.88 (3H, s), 4.54 (2H, d, J = 5.8 Hz), 6.84 (1H, dd, J = 5.3, 1.3 Hz), 6.88 (1H, s), 7.19–7.27 (1H, m), 7.29–7.38 (4H, m), 7.47 (1H, t, J = 5.9 Hz), 8.16 (1H, d, J = 5.3 Hz), 10.04 (1H, s). Anal. Calcd. for $C_{16}H_{15}N_5O$•0.1H_2O: C, 65.12; H, 5.19; N, 23.73. Found: C, 64.94; H, 5.06; N, 23.55.

4-Methyl-5-(2-((2-phenylethyl)amino)pyridin-4-yl)-4*H*-1,2,4-triazole-3-carbaldehyde (77d). This compound was prepared from (4-methyl-5-(2-((2-phenylethyl)amino)pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl)methanol **66d** as described

in the synthesis of **77b**, as light yellow solids. mp 131 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.86 (2H, t, J = 7.3 Hz), 3.49–3.57 (2H, m), 3.92 (3H, s), 6.82 (1H, dd, J = 5.2, 1.4 Hz), 6.84 (1H, s), 7.01 (1H, t, J = 5.5 Hz), 7.16–7.24 (1H, m), 7.24–7.34 (4H, m), 8.19 (1H, d, J = 5.3 Hz), 10.05 (1H, s).

4-Methyl-5-(2-((3-phenylpropyl)amino)pyridin-4-yl)-4*H*-1,2,4-triazole-3-carbaldehyde (77e). This compound was prepared from (4-methyl-5-(2-((3-phenylpropyl)amino)pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl)methanol **66e** as described in the synthesis of **77b**, as light yellow solids. mp 88–89 ºC. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.99 (2H, quintet, J = 7.4 Hz), 2.75 (2H, t, J = 7.5 Hz), 3.32–3.40 (2H, m), 4.00 (3H, s), 4.79 (1H, t, J = 5.5 Hz), 6.65 (1H, s), 6.75 (1H, dd, J = 5.2, 1.4 Hz), 7.15–7.23 (3H, m), 7.24–7.33 (2H, m), 8.26 (1H, d, J = 5.3 Hz), 10.17 (1H, s). Anal. Calcd. for $C_{18}H_{19}N_5O$: C, 67.27; H, 5.96; N, 21.79. Found: C, 67.17; H, 5.92; N, 21.89.

General procedure for the preparation of ethyl 2,4-dioxo-4-(4-pyridyl)butanoate. A solution of lithium bis(trimethylsilyl)amide in THF (1.1 M, 200 mL) was added dropwise to a solution of 4-acetylpyridine (**78a**, 26.7 g, 220 mmol) and diethyl oxalate (32.2 g, 220 mmol) in THF (440 mL) at –70 °C for 20 min under nitrogen atmosphere. After the addition, the mixture was allowed to warm to room temperature and then stirred for 3 h. After the resulting mixture was cooled to –60 °C, 6 N HCl aqueous solution (160 mL) was added at –40 ~ –60 °C. After the addition, the mixture was allowed to warm to room temperature and then stirred for 30 min. The reaction mixture was concentrated under reduced pressure. The pH of the residue was adjusted to 4 with 1 N NaOH

aqueous solution. The precipitate was collected by filtration, followed by washing with water and diethyl ether to afford 64.0 g of ethyl 2,4-dioxo-4-(4-pyridyl)butanoate (crude). This compound was used without further purification.

General procedure for the preparation of 5-(4-pyridyl)-1*H*-pyrazole-3-carboxylic acid hydrochloride (79a). After the above ethyl 2,4-dioxo-4-(4-pyridyl)butanoate (32.0 g) was treated with 5.5 N HCl aqueous solution (140 mL), hydrazine hydrate (4.5 mL) was added at 0 °C. The mixture was stirred at 85 °C for 90 min. The pH of the resulting mixture was adjusted to 4 with 8 N NaOH aqueous solution. The precipitate was collected by filtration, followed by washing with isopropanol and diethyl ether to afford 13.4 g of **79a** (70.8 mmol, 64%) as pale yellow solids. mp >300 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.80 (1H, s), 8.42 (2H, d, J = 6.9 Hz), 8.90 (2H, dd, J = 5.6, 1.4 Hz). Anal. Calcd. for $C_9H_7N_3O_2$•HCl: C, 47.91; H, 3.57; N, 18.62; Cl, 15.71. Found: C, 47.55; H, 3.59; N, 18.42, Cl; 15.58.

Ethyl 4-methyl-5-(4-pyridyl)-1*H*-pyrazole-3-carboxylate (79b). This compound was similarly prepared from 4-propionylpyridine **78b** as described in the synthesis of **79a**, as white solids. mp 154–155 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 (3H, t, J = 7.1 Hz), 2.52 (3H, s), 4.44 (2H, q, J = 7.1 Hz), 7.61 (2H, dd, J = 4.7, 1.4 Hz), 8.70 (2H, dd, J = 4.7, 1.7 Hz), 11.08 (1H, br). Anal. Calcd. for $C_{12}H_{13}N_3O_2$•0.1H_2O: C, 61.84; H, 5.71; N, 18.03. Found: C, 61.76; H, 5.81; N, 18.19.

***N*-Methoxy-*N*-methyl-5-(4-pyridyl)-1*H*-pyrazole-3-carboxamide.** The mixture of 5-(4-pyridyl)-1*H*- pyrazole-3-carboxylic acid hydrochloride (**79a**, 2.32 g, 10.3 mmol),

N-methoxy-*N*-methylamine hydrochloride (1.20 g), Et_3N (3.6 mL) WSC•HCl (2.37 g) and HOBt•H_2O (160 mg) in DMF (50 mL) was stirred at room temperature for 60 h. Water was added to the reaction mixture and then the mixture was extracted with chloroform. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by recrystallization from ethyl acetate to afford 2.20 g of *N*-methoxy-*N*-methyl-5-(4-pyridyl)-1*H*-pyrazole-3-carboxamide as white solids. The mother liquor was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting ethyl acetate and methanol to afford 0.11 g of the above compound. Total: 2.31 g (9.95 mmol, 97% yield). mp 193–197 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.43 (3H, s), 3.85 (3H, s), 7.20 (1H, s), 7.74 (2H, d, J = 6.0 Hz), 8.67 (2H, d, J = 6.0 Hz), 11.55 (1H, br). Anal. Calcd. for $C_{11}H_{12}N_4O_2$: C, 56.89; H, 5.21; N, 24.12. Found: C, 56.71; H, 5.20; N, 23.99.

5-(4-Pyridyl)-1*H*-pyrazole-3-carbaldehyde. Lithium aluminum hydride (87 mg) was added to a solution of *N*-methoxy-*N*-methyl-3-(4-pyridyl)-1*H*-pyrazole-5-carboxamide (535 mg, 2.30 mmol) in THF (35 mL) at –30 °C. The resulting mixture was allowed to warm to 0 °C and then stirred at 0 °C for 1 h. The reaction mixture was carefully treated with water (10 mL) at –30 °C and then brine was added. The mixture was extracted with THF and then chloroform. The combined organic layer was dried over anhydrous Na_2SO_4, filtered through a pad of Celite and concentrated under reduced pressure to afford 585 mg of 5-(4-pyridyl)-1*H*-pyrazole-3-carbaldehyde (quantitative yield) as pale yellow solids. mp 223–235 °C (dec.). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.58 (1H, s), 7.85 (2H, dd, J

= 4.5, 1.5 Hz), 8.67 (2H, d, *J* = 5.4 Hz), 9.94 (1H, s), NH was not assigned. Anal. Calcd. for $C_9H_7N_3O$•0.1H_2O: C, 61.78; H, 4.15; N, 24.02. Found: C, 61.87; H, 4.17; N, 23.90.

General procedure for the preparation of (5-(4-pyridyl)-1*H*-pyrazol-3-yl)methanol (80a). Sodium borohydride (131 mg) was added to a suspension of the above 3-(4-pyridyl)-1*H*-pyrazole-5-carbaldehyde in methanol (45 mL) at 0 °C. The resulting mixture was allowed to warm to room temperature and then stirred for 7 h. Acetone (2.0 mL) was added to the reaction mixture. The mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting ethyl acetate and methanol and by recrystallization from methanol and ethyl acetate to afford 309 mg of **80a** (1.76 mmol, 77%) as white solids. mp 205–207 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.53 (2H, d, *J* = 5.7 Hz), 5.37 (1H, s), 6.76 (1H, s), 7.74 (2H, d, *J* = 6.0 Hz), 8.57 (2H, s), 13.08 (1H, brs). Anal. Calcd. for $C_9H_9N_3O$•0.25H_2O: C, 60.16; H, 5.33; N, 23.38. Found: C, 59.95; H, 5.25; N, 23.65.

General procedure for the preparation of (4-methyl-5-(4-pyridyl)-1*H*-pyrazol-3-yl)methanol (80b). Lithium aluminum hydride (96 mg) was added to a suspension of ethyl 4-methyl-5-(4-pyridyl)-1*H*-pyrazole-3-carboxylate (**79b**, 586 mg, 2.53 mmol) in THF (20 mL) at –40 °C. The resulting mixture was stirred at between –30 ~ –40 °C for 1 h. After the reaction mixture was treated with sodium sulfate decahydrate (816 mg) at –20 °C, the resulting mixture was allowed to warm to room temperature and then stirred at room temperature for overnight. The resulting mixture was filtered through a pad of Celite. The filtrate was concentrated under reduced pressure.

The residue was purified by column chromatography on silica gel eluting chloroform and methanol to afford 64 mg of 4-methyl-5-(4-pyridyl)-1*H*-pyrazole-3-carbaldehyde (0.34 mmol, 13%) as white solids and 84 mg of **80b** (0.44 mmol, 17%) as pale yellow solids. 4-Methyl-5-(4-pyridyl)-1*H*-pyrazole-3-carbaldehyde: mp 213–215 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.47 (3H, s), 7.64 (2H, dd, J = 4.7, 1.4 Hz), 8.68 (2H, d, J = 4.8 Hz), 9.99 (1H, s), NH was not assigned. Anal. Calcd. for $C_{10}H_9N_3O$•0.2H_2O: C, 62.95; H, 4.97; N, 22.02. Found: C, 63.08; H, 4.84; N, 21.68. **80b**: mp 179–182 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.21 (3H, s), 4.48 (2H, d, J = 3.6 Hz), 5.21 (1H, brs), 7.64 (2H, s), 8.59 (2H, s), 12.97 (1H, br). Anal. Calcd. for $C_{10}H_{11}N_3O$: C, 63.48; H, 5.86; N, 22.21. Found: C, 63.34; H, 5.91; N, 22.28.

General procedure for the preparation of 4-(3-(chloromethyl)-1*H*-pyrazol-5-yl)pyridine hydrochloride. Thionyl chloride (3.0 mL) was added to (5-(4-pyridyl)-1*H*-pyrazol-3-yl)methanol (**80a**, 273 mg, 1.56 mmol) at 0 °C. The resulting mixture was stirred at room temperature for 30 min. The reaction mixture was concentrated under reduced pressure to afford 345 mg of 4-(3-(chloromethyl)-1*H*-pyrazol-5-yl)pyridine hydrochloride (1.50 mmol, 96%) as white solids. mp 269–273 °C (dec.). ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.89 (2H, s), 7.33 (1H, s), 8.39 (2H, d, J = 6.9 Hz), 8.90 (2H, d, J = 6.6 Hz).

4-(3-(Chloromethyl)-4-methyl-1*H*-pyrazol-5-yl)pyridine hydrochloride. This compound was prepared from (4-methyl-5-(4-pyridyl)-1*H*-pyrazol-3-yl)methanol **80b** as described in the synthesis of 4-(3-(chloromethyl)-1*H*-pyrazol-5-yl)pyridine hydrochloride, as pale yellow solids. ^{1}H NMR (300

MHz, DMSO-d_6) δ 2.34 (3H, s), 4.88 (2H, s), 8.20 (2H, d, J = 5.1 Hz), 8.87 (2H, d, J = 6.0 Hz).

***N*-Benzyl-3-(((3-(pyridin-4-yl)-1*H*-pyrazol-5-yl)methyl)amino)benzamide (81a).** This compound was prepared from 4-(3-(chloromethyl)-1*H*-pyrazol-5-yl)pyridine hydrochloride and 3-amino-*N*-benzylbenzamide **29i** as described in the synthesis of **24c**, as white solids. mp 172–174 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.34 (1H, brs), 4.50 (2H, d, J = 4.0 Hz), 4.63 (2H, d, J = 5.9 Hz), 6.36 (1H, brs), 6.63 (1H, s), 6.77 (1H, dd, J = 8.1, 1.8 Hz), 7.05 (1H, d, J = 8.1 Hz), 7.20–7.24 (2H, m), 7.27–7.32 (2H, m), 7.33–7.36 (4H, m), 7.60 (2H, d, J = 5.9 Hz), 8.62–8.65 (2H, m). ^{13}C NMR (151 MHz, $CDCl_3$) δ 40.46, 44.18, 112.25, 116.07, 116.24, 119.85, 127.67, 127.89, 128.81, 129.59, 135.68, 138.09, 150.36, 6 aromatic carbons were not assigned. Anal. Calcd. for $C_{23}H_{21}N_5O$•$0.2H_2O$: C, 71.37; H, 5.57; N, 18.09. Found: C, 71.32; H, 5.58; N, 17.87. MS (ESI/APCI) *m/z* 384.1 $[M + H]^+$. HPLC purity 98.8%.

***N*-Benzyl-3-(((4-methyl-3-(pyridin-4-yl)-1*H*-pyrazol-5-yl)methyl)amino)benzamide (81b).** This compound was prepared from 4-(3-(chloromethyl)-4-methyl-1*H*-pyrazol-5-yl)pyridine hydrochloride and 3-amino-*N*-benzylbenzamide **29i** as described in the synthesis of **24c**, as white solids. mp 185–188 °C. ^{1}H NMR (600 MHz, DMSO-d_6) (2:1) δ 2.24 (3H, s), 4.28 (2H, brs), 4.45 (2H, d, J = 5.9 Hz), 6.15 (1H, brs), 6.82 (1H, brs), 7.09 (1H, brs), 7.17 (2H, d, J = 11.0 Hz), 7.21–7.25 (1H, m), 7.28–7.37 (4H, m), 7.52–7.72 (2H, m), 8.59 (2H, brs), 8.83 (1H, brs), 13.02 (2/3H, brs), 13.12 (1/3H, brs). ^{13}C NMR (151 MHz, DMSO-d_6) δ 8.96, 36.98, 42.40, 110.91, 111.28, 114.99, 120.46, 120.90, 126.52, 127.02, 128.11, 128.55, 135.15, 139.40, 139.77, 146.15, 148.30, 149.73, 150.07, 166.72.

Anal. Calcd. for $C_{24}H_{23}N_5O$: C, 72.52; H, 5.83; N, 17.62. Found: C, 72.41; H, 5.94; N, 17.57. MS (ESI/APCI) *m/z* 398.1 $[M + H]^+$. HPLC purity 99.6%.

General procedure for the preparation of (4-(4-pyridyl)-1-trityl-1*H*-imidazol-2-yl)methanol. Sodium borohydride (60 mg) was added to a solution of 4-(4-pyridyl)-1-trityl-1*H*-imidazole-2-carbaldehyde (**85**, 662 mg, 1.59 mmol) in methanol (30 mL) at 0 °C. The mixture was stirred at room temperature for 20 min. Acetone (2.0 mL) was added and then the mixture was stirred at room temperature for 30 min. The reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting ethyl acetate and methanol, and by recrystallization from ethyl acetate and IPE to afford 522 mg of (4-(4-pyridyl)-1-trityl-1*H*-imidazol-2-yl)methanol (1.25 mmol, 78%) as white solids. mp 146–148 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.92 (1H, br), 3.67 (2H, s), 7.15–7.18 (6H, m), 7.27 (1H, s), 7.36–7.40 (9H, m), 7.57 (2H, dd, *J* = 4.8, 1.8 Hz), 8.53 (2H, dd, *J* = 4.7, 1.7 Hz).

4-(2-(Chloromethyl)-1-trityl-1*H*-imidazol-4-yl)pyridine hydrochloride. This compound was prepared from (4-(4-pyridyl)-1-trityl-1*H*-imidazol-2-yl)methanol as described in the synthesis of 4-(3-(chloromethyl)-1*H*-pyrazol-5-yl)pyridine hydrochloride, as white solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.85 (2H, s), 7.13–7.43 (15H, m) 8.30 (2H, d, *J* = 6.9 Hz), 8.47 (1H, s), 8.80 (2H, d, *J* = 6.6 Hz).

***N*-Benzyl-3-(((4-(pyridin-4-yl)-1*H*-imidazol-2-yl)methyl)amino)benzamide (81c).** This compound was prepared from 4-(2-(chloromethyl)-1-trityl-1*H*-imidazol-4-yl)pyridine hydrochloride and

3-amino-*N*-benzylbenzamide **29i** as described in the synthesis of **24c**, as white solids. mp 105–110 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.35 (2H, d, J = 5.5 Hz), 4.44 (2H, d, J = 5.9 Hz), 6.31 (1H, t, J = 5.5 Hz), 6.80 (1H, d, J = 8.1 Hz), 7.10 (1H, d, J = 7.7 Hz), 7.14–7.17 (1H, m), 7.19 (1H, s), 7.20–7.25 (1H, m), 7.27–7.33 (4H, m), 7.70 (2H, d, J = 5.5 Hz), 7.81 (1H, d, J = 1.1 Hz), 8.47 (1H, d, J = 5.1 Hz + 1H, br), 8.83 (1H, t, J = 5.9 Hz), 12.26 (1H, brs). ^{13}C NMR (151 MHz, DMSO-d_6) δ 41.04, 42.39, 111.29, 114.91, 114.99, 115.86, 118.51, 126.51, 126.99, 128.10, 128.59, 135.13, 137.17, 139.74, 141.79, 147.30, 148.33, 149.64, 166.65. Anal. Calcd. for $C_{23}H_{21}N_5O$•MeOH•0.2H_2O: C, 68.78; H, 6.11; N, 16.71. Found: C, 68.54; H, 6.10; N, 16.93. MS (ESI/APCI) *m/z* 384.1 $[M + H]^+$. HPLC purity 95.8%.

***N*-Benzyl-3-(((5-(pyridin-4-yl)-1,2-oxazol-3-yl)methyl)amino)benzamide (81d).** This compound was prepared from 4-(3-(chloromethyl)isoxazol-5-yl)pyridine **86** as described in the synthesis of **24c**, as light yellow solids. mp 170–171 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.45 (4H, d, J = 5.9 Hz), 6.48 (1H, t, J = 6.2 Hz), 6.77–6.83 (1H, m), 7.09–7.13 (1H, m), 7.16–7.24 (4H, m), 7.28–7.33 (4H, m), 7.79–7.83 (2H, m), 8.70–8.76 (2H, m), 8.85 (1H, t, J = 6.1 Hz). ^{13}C NMR (151 MHz, DMSO-d_6) δ 38.45, 42.39, 102.55, 111.32, 114.93, 115.11, 119.27, 126.53, 127.00, 128.10, 128.71, 133.22, 135.23, 139.73, 147.98, 150.64, 163.96, 166.45, 166.60. Anal. Calcd. for $C_{23}H_{20}N_4O_2$: C, 71.86; H, 5.24; N, 14.57. Found: C, 71.55; H, 5.43; N, 14.33. MS (ESI/APCI) *m/z* 385.1 $[M + H]^+$. HPLC purity 100.0%.

***N*-Benzyl-3-(((3-(pyridin-4-yl)-1,2-oxazol-5-yl)methyl)amino)benzamide (81e).** This compound

was prepared from 4-(5-(chloromethyl)isoxazol-3-yl)pyridine **87** as described in the synthesis of **24c**, as white solids. mp 168–169 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.40 (1H, brs), 4.60 (2H, d, *J* = 5.1 Hz), 4.63 (2H, d, *J* = 5.5 Hz), 6.35 (1H, brs), 6.53 (1H, s), 6.78 (1H, dd, *J* = 8.1, 2.2 Hz), 7.06 (1H, d, *J* = 7.7 Hz), 7.20–7.25 (2H, m), 7.28–7.32 (1H, m), 7.32–7.40 (4H, m), 7.57–7.67 (2H, m), 8.63–8.81 (2H, m). ^{13}C NMR (151 MHz, $CDCl_3$) δ 40.18, 44.17, 100.12, 112.29, 115.94, 116.21, 120.96, 127.67, 127.90, 128.81, 129.63, 135.79, 136.28, 138.12, 146.97, 150.62, 160.69, 167.38, 171.77. Exact Mass (FAB) $[M + H]^+$ for $C_{23}H_{21}N_4O_2$, Calcd: 385.1665. Found: 385.1668. MS (ESI/APCI) *m/z* 385.1 $[M + H]^+$. HPLC purity 98.2%.

2-(4-Pyridyl)-1*H*-imidazole-4-carbaldehyde. A solution of diisobutylalminum hydride in toluene (1.5 M, 3.7 mL) was added to a suspension of 2-(4-pyridyl)-1*H*-imidazole-4-carbonitrile (**90**, 628 mg, 3.69 mmol) in THF (20 mL) at –78 °C under argon atmosphere. The resulting mixture was stirred at –78 °C for 30 min and at 0 °C for 1 h. After ethanol (2.0 mL) was added to the reaction mixture at 0 °C, the mixture was stirred at 0 °C for 1 h. The resulting mixture was filtered through a pad of Celite and then was washing with ethyl acetate and methanol (10:1). The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting ethyl acetate and methanol to afford 266 mg of 2-(4-pyridyl)-1*H*-imidazole-4-carbaldehyde (1.54mmol, 42%) as pale yellow solids. ^{1}H NMR (300 MHz, CD_3OD) δ 7.99 (2H, dd, *J* = 4.7, 1.7 Hz), 8.09 (1H, s), 8.68 (2H, dd, *J* = 4.7, 1.7 Hz), 9.84 (1H, s), NH was vanished.

(2-(4-Pyridyl)-1*H*-imidazol-4-yl)methanol. These compounds were prepared from

2-(4-pyridyl)-1*H*-imidazole-4-carbaldehyde as described in the synthesis of (4-(4-pyridyl)-1-trityl-1*H*-imidazol-2-yl)methanol, as white solids. mp 178–183 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 4.63 (2H, s), 7.21 (1H, br), 7.87 (2H, dd, *J* = 4.7, 1.7 Hz), 8.60 (2H, dd, *J* = 4.7, 1.7 Hz), 2H were vanished. Anal. Calcd. for $C_9H_9N_3O$•$0.1H_2O$: C, 61.08; H, 5.24; N, 23.74. Found: C, 61.09; H, 5.25; N, 23.57.

4-(4-(Chloromethyl)-1*H*-imidazol-2-yl)pyridine hydrochloride. This compound was prepared from (2-(4-pyridyl)-1*H*-imidazol-4-yl)methanol as described in the synthesis of 4-(3-(chloromethyl)-1*H*-pyrazol-5-yl)pyridine hydrochloride, as pale yellow solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.82 (2H, s), 7.72 (1H, s), 8.47 (2H, d, *J* = 6.9 Hz), 8.93 (2H, d, *J* = 6.9 Hz), NH was not assigned.

***N*-Benzyl-3-(((2-(4-pyridyl)-1*H*-imidazol-4-yl)methyl)amino)benzamide (81f).** This compound was prepared from 4-(4-(chloromethyl)-1*H*-imidazol-2-yl)pyridine hydrochloride as described in the synthesis of **24c**, as pale yellow solids. mp 178–180 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.25 (2H, brs), 4.45 (2H, d, *J* = 6.2 Hz), 6.14 (1H, brs), 6.81 (1H, dd, *J* = 8.1, 1.8 Hz), 7.07 (1H, brs), 7.15 (1H, t, *J* = 7.7 Hz), 7.18 (1H, s), 7.17–7.19 (1H, m), 7.21–7.24 (1H, m), 7.28–7.34 (4H, m), 7.81–7.86 (2H, m), 8.58–8.63 (2H, m), 8.83 (1H, t, *J* = 5.9 Hz), 12.78 (1H, brs). ^{13}C NMR (151 MHz, DMSO-d_6) δ 41.07, 42.38, 110.89, 114.36, 115.05, 116.29, 118.51, 126.51, 127.00, 128.10, 128.53, 135.08, 137.18, 139.78, 141.29, 142.53, 148.58, 150.08, 166.73. Anal. Calcd. for $C_{23}H_{21}N_5O$: C, 72.04; H, 5.52; N, 18.26. Found: C, 71.88; H, 5.54; N, 18.24. MS (ESI/APCI) *m/z* 384.2 $[M + H]^+$.

HPLC purity 99.6%.

***N*-Benzyl-3-(((4-(pyridin-4-yl)-1*H*-pyrrol-2-yl)methyl)amino)benzamide (81g).** A mixture of 4-(4-pyridyl)-1*H*-pyrrole-2-carbaldehyde (**93**, 57 mg, 0.33 mmol) and 3-amino-*N*-benzylbenzamide (**29i**, 91 mg) in methanol (5.0 mL) and acetic acid (0.25 mL) was stirred at room temperature for 30 min. After sodium triacetoxyborohydride (211 mg) was added, the mixture was stirred at room temperature for 15 h. The reaction mixture was concentrated under reduced pressure. Ethyl acetate was added to the residue and the mixture was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by recrystallization from methanol and ethyl acetate to afford 27 mg of **81g** (0.071 mmol, 21%) as pale yellow solids. mp 184–186 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.22 (2H, d, J = 5.1 Hz), 4.45 (2H, d, J = 5.9 Hz), 6.07 (1H, t, J = 4.8 Hz), 6.50 (1H, brs), 6.80 (1H, d, J = 7.3 Hz), 7.08 (1H, d, J = 7.3 Hz), 7.15 (1H, t, J = 7.7 Hz), 7.18 (1H, brs), 7.21–7.26 (1H, m), 7.27–7.33 (4H, m), 7.40 (1H, brs), 7.44 (2H, d, J = 5.1 Hz), 8.38 (2H, d, J = 4.8 Hz), 8.83 (1H, t, J = 5.5 Hz), 11.16 (1H, brs). ^{13}C NMR (151 MHz, DMSO-d_6) δ 40.11, 42.39, 103.89, 111.02, 114.63, 115.13, 116.80, 118.66, 120.39, 126.52, 127.01, 128.10, 128.51, 131.62, 135.07, 139.78, 143.14, 148.53, 149.58, 166.70. MS (ESI/APCI) *m/z* 383.2 $[M + H]^+$. HPLC purity 72.3%.

***N*-Benzyl-3-(((1-(pyridin-4-yl)-1*H*-pyrrol-3-yl)methyl)amino)benzamide (81h).** This compound was prepared from 1-(4-pyridyl)-1*H*-pyrrole-3-carbaldehyde **96** as described in the synthesis of **81g**, as white solids. mp 117–122 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.15 (2H, d, J = 5.9 Hz), 4.45

(2H, d, J = 6.2 Hz), 6.05 (1H, t, J = 5.7 Hz), 6.36 (1H, dd, J = 2.9, 1.8 Hz), 6.80 (1H, dd, J = 8.1, 1.8 Hz), 7.06 (1H, d, J = 7.7 Hz), 7.11–7.16 (1H, m), 7.17 (1H, t, J = 1.7 Hz), 7.20–7.25 (1H, m), 7.28–7.33 (4H, m), 7.54–7.61 (4H, m), 8.54 (2H, d, J = 6.2 Hz), 8.83 (1H, t, J = 6.1 Hz). ^{13}C NMR (151 MHz, DMSO-d_6) δ 39.82, 42.39, 110.97, 112.10, 112.23, 114.37, 115.02, 116.21, 118.70, 125.49, 126.51, 127.01, 128.09, 128.50, 135.07, 139.79, 145.33, 148.73, 150.97, 166.77. Anal. Calcd. for $C_{24}H_{22}N_4O$•0.1H_2O: C, 75.02; H, 5.82; N, 14.58. Found: C, 74.96; H, 5.83; N, 14.57. MS (ESI/APCI) *m/z* 383.1 $[M + H]^+$. HPLC purity 99.4%.

(5-(4-Pyridyl)-1,3,4-oxadiazol-2-yl)methanol. This compound was prepared from ethyl 5-(4-pyridyl)-1,3,4- oxadiazole-2-carboxylate **98** as described in the synthesis of **76**, as white solids. mp 150–152 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.76 (2H, d, J = 6.3 Hz), 6.02 (1H, t, J = 6.3 Hz), 7.94 (2H, dd, J = 4.4, 1.7 Hz), 8.85 (2H, dd, J = 4.4, 1.7 Hz). Anal. Calcd. for $C_8H_7N_3O_2$: C, 54.24; H, 3.98; N, 23.72. Found: C, 54.08; H, 3.94; N, 23.67.

4-(2-(Chloromethyl)-1,3,4-oxadiazol-5-yl)pyridine hydrochloride. This compound was prepared from (5-(4-pyridyl)-1,3,4-oxadiazol-2-yl)methanol as described in the synthesis of 4-(3-(chloromethyl)-1*H*-pyrazol-5-yl)pyridine hydrochloride, as white solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.20 (2H, s), 8.08 (2H, dd, J = 4.7, 1.7 Hz), 8.92 (2H, dd, J = 4.7, 1.7 Hz).

***N*-Benzyl-3-(((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)methyl)amino)benzamide (81i).** This compound was prepared from 4-(2-(chloromethyl)-1,3,4-oxadiazol-5-yl)pyridine hydrochloride as described in the synthesis of **24c**, as white solids. mp 168–170 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ

4.45 (2H, d, J = 5.9 Hz), 4.71 (2H, d, J = 6.2 Hz), 6.64 (1H, t, J = 6.2 Hz), 6.87 (1H, dd, J = 8.1, 1.5 Hz), 7.13–7.17 (1H, m), 7.18–7.24 (2H, m), 7.24–7.26 (1H, m), 7.28–7.34 (4H, m), 7.84–7.91 (2H, m), 8.80–8.84 (2H, m), 8.86 (1H, t, J = 5.9 Hz). ^{13}C NMR (151 MHz, DMSO-d_6) δ 37.82, 42.40, 111.35, 115.06, 115.59, 119.96, 126.53, 126.99, 128.10, 128.77, 130.24, 135.25, 139.69, 147.51, 150.92, 162.59, 166.02, 166.53. Anal. Calcd. for $C_{22}H_{19}N_5O_2$•0.1H_2O: C, 68.24; H, 5.00; N, 18.09. Found: C, 68.00; H, 5.03; N, 17.73. MS (ESI/APCI) *m/z* 386.1 $[M + H]^+$. HPLC purity 98.3%.

***N*-Benzyl-3-(((3-(pyridin-4-yl)-1,2,4-oxadiazol-5-yl)methyl)amino)benzamide (81j).** This compound was prepared from 4-(5-(chloromethyl)-1,2,4-oxadiazol-3-yl)pyridine **99** as described in the synthesis of **24c**, as pale yellow solids. mp 147–149 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.44 (2H, d, J = 6.2 Hz), 4.81 (2H, d, J = 6.6 Hz), 6.71 (1H, t, J = 6.4 Hz), 6.84 (1H, dd, J = 7.9, 1.7 Hz), 7.13–7.17 (1H, m), 7.18–7.24 (3H, m), 7.26–7.32 (4H, m), 7.89–7.93 (2H, m), 8.77–8.82 (2H, m), 8.86 (1H, t, J = 6.1 Hz). ^{13}C NMR (151 MHz, DMSO-d_6) δ 39.05, 42.38, 111.18, 114.99, 115.68, 120.82, 126.54, 126.98, 128.10, 128.84, 133.23, 135.30, 139.68, 147.44, 150.82, 166.23, 166.49, 179.67. Anal. Calcd. for $C_{22}H_{19}N_5O$•H_2O: C, 68.20; H, 5.46; N, 18.08. Found: C, 68.58; H, 5.44; N, 18.24. MS (ESI/APCI) *m/z* 386.1 $[M + H]^+$. HPLC purity 96.8%.

***N*-Benzyl-3-(((4-(pyridin-4-yl)-1,3-thiazol-2-yl)methyl)amino)benzamide (81k).** This compound was prepared from 2-chloromethyl-4-(4-pyridyl)thiazole hydrochloride **101** as described in the synthesis of **24c**, as white solids. mp 152–154 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.43 (2H, d, J = 5.9 Hz), 4.70 (2H, d, J = 6.2 Hz), 6.77–6.81 (1H, m), 6.86 (1H, t, J = 6.2 Hz), 7.12–7.15 (1H, m),

7.16–7.23 (3H, m), 7.26–7.31 (4H, m), 7.89–7.93 (2H, m), 8.33 (1H, s), 8.60–8.67 (2H, m), 8.86 (1H, t, J = 6.1 Hz). ^{13}C NMR (151 MHz, DMSO-d_6) δ 42.38, 45.02, 111.33, 115.08, 115.52, 118.14, 120.04, 126.52, 126.99, 128.09, 128.80, 135.25, 139.68, 140.71, 147.70, 150.20, 151.61, 166.50, 173.60. MS (ESI/APCI) *m/z* 401.1 $[M + H]^+$. HPLC purity 99.6%.

***N*-Benzyl-3-(((2-(pyridin-4-yl)-1,3-thiazol-4-yl)methyl)amino)benzamide (81l).** The mixture of 4-(4-(chloromethyl)thiazol-2-yl)pyridine (**103**, 439 mg, 2.34mmol), *N*-benzyl-3-((trifluoroacetyl)amino) benzamide (**104**, 718 mg, 2.23 mmol) prepared from 3-amino-*N*-benzylbenzamide **29i** with trifluoroacetic acid anhydride (93% yield) and potassium carbonate (462 mg) in DMF (10 mL) was stirred at 80 °C for 7 h. Potassium carbonate (308 mg) and water (5.0 mL) were added. The mixture was stirred at room temperature for 2 h. Ethyl acetate was added to the reaction mixture and then the mixture was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting hexane and ethyl acetate, and by recrystallization from ethyl acetate to afford 429 mg of **81l** (1.07 mmol, 48%) as pale yellow solids. mp 136–140 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.44 (2H, d, J = 5.9 Hz), 4.51 (2H, d, J = 5.9 Hz), 6.47 (1H, t, J = 6.1 Hz), 6.81 (1H, dd, J = 8.1, 1.8 Hz), 7.09 (1H, d, J = 7.7 Hz), 7.14–7.18 (1H, m), 7.19–7.24 (2H, m), 7.27–7.33 (4H, m), 7.62 (1H, s), 7.85–7.89 (2H, m), 8.68–8.72 (2H, m), 8.84 (1H, t, J = 6.1 Hz). ^{13}C NMR (151 MHz, DMSO-d_6) δ 42.38, 43.25, 111.05, 114.78, 115.02, 117.70, 119.81, 126.52, 126.99, 128.10, 128.65, 135.16, 139.45, 139.76, 148.24, 150.64, 156.88,

164.24, 166.66. Anal. Calcd. for $C_{23}H_{20}N_4OS•0.1H_2O$: C, 68.67; H, 5.06; N, 13.93. Found: C, 68.37; H, 5.06; N, 13.67. MS (ESI/APCI) *m/z* 401.1 $[M + H]^+$. HPLC purity 96.1%.

***N*-Benzyl-3-(((4-phenyl-3-(pyridin-4-yl)-1*H*-pyrazol-5-yl)methyl)amino)benzamide (81m).** 4-Phenyl-5-(4-pyridyl)-1*H*-pyrazole-3-carbaldehyde was prepared from *N*-methoxy-*N*-methyl-4-phenyl-5-(4-pyridyl)-1*H*-pyrazole-3-carboxamide (**107**, 123 mg, 0.40 mmol) as described in the synthesis of 4-methyl-5-(4-pyridyl)-1*H*-pyrazole-3-carbaldehyde, as a crude compound. A mixture of the above 4-phenyl-5-(4-pyridyl)-1*H*-pyrazole-3-carbaldehyde and 3-amino-*N*-benzylbenzamide **29i** (226 mg, 1.00 mmol) in methanol (20 mL) and acetic acid (1.0 mL) was stirred at room temperature for 12.5 h. After sodium triacetoxyborohydride (254 mg), 1,2-dichloroethane (15 mL) and acetic acid (1.0 mL) were added, the mixture was stirred at room temperature for 8 h. The reaction mixture was concentrated under reduced pressure. Ethyl acetate was added to the residue and then the mixture was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified column chromatography on silica gel eluting hexane and ethyl acetate to afford 13 mg of **81m** (0.028 mmol, 7%) as white solids. mp 200–207 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.37 (2H, s + 1H, br), 4.61 (2H, d, *J* = 5.5 Hz), 6.41 (1H, d, *J* = 4.8 Hz), 6.65 (1H, dd, *J* = 7.7, 2.2 Hz), 7.02 (1H, d, *J* = 7.7 Hz), 7.11–7.18 (2H, m), 7.20–7.24 (2H, m), 7.27–7.30 (3H, m), 7.31–7.35 (4H, m), 7.36–7.44 (3H, m), 8.47 (2H, brs), NH was not assigned. ^{13}C NMR (151 MHz, $CDCl_3$) δ 39.44, 44.16, 112.15, 115.95, 116.30, 119.02, 121.70, 127.65, 127.70, 127.86, 128.79, 128.98, 129.48,

129.91, 132.07, 135.45, 138.09, 139.50, 147.78, 149.94, 167.76, 2 aromatic carbons were not assigned. MS (ESI/APCI) *m/z* 460.2 $[M + H]^+$. HPLC purity 99.8%.

4-(1-Trityl-1*H*-imidazol-4-yl)pyridine (84). A mixture of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaboran-2-yl)pyridine **82** (2.04 g, 9.95 mmol), 4-iodo-1-trityl-1*H*-imidazole **83** (4.34 g, 9.95 mmol), potassium phosphate (16.9 g) in DMF (150 mL) was stirred at room temperature for 30 min under argon atmosphere. After tetrakis(triphenylphosphine)palladium (1.15 g) was added, the resulting mixture was stirred at 110 °C for 19 h under argon atmosphere. The reaction mixture was concentrated under reduced pressure. Ethyl acetate was added to the residue and the mixture was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting hexane and ethyl acetate, and by recrystallization from ethyl acetate and IPE to afford 3.19 g of **84** (8.23 mmol, 83%) as white solids. mp 215–220 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16–7.21 (6H, m), 7.30 (1H, d, *J* = 1.2 Hz), 7.35–7.39 (9H, m), 7.54 (1H, d, *J* = 1.5 Hz), 7.59 (2H, dd, *J* = 4.5, 1.5 Hz), 8.54 (2H, dd, *J* = 4.7, 1.4 Hz). Anal. Calcd. for $C_{28}H_{21}N_3O$•$0.25H_2O$: C, 82.73; H, 5.53; N, 10.72. Found: C, 82.87; H, 5.55; N, 10.32.

General procedure for the preparation of 4-(4-pyridyl)-1-trityl-1*H*-imidazole-2-carbaldehyde (85). A solution of *n*-butyllithium in hexane (1.6 M, 0.85 mL) was added dropwise to a solution of 4-(1-trityl-1*H*-imidazol-4-yl)pyridine (**84**, 500 mg, 1.29 mmol) in THF (15 mL) at –78 °C for 5 min

under argon atmosphere. After the addition, the mixture was stirred at –78 °C for 30 min and then DMF (0.15 mL) was added at –78 °C. The reaction mixture was stirred at –78 °C for 15 min and allowed to warm up to room temperature for 80 min. Water was added and the resulting mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting hexane and ethyl acetate, and by recrystallization from ethyl acetate to afford 261 mg of **85** (0.63 mmol, 49%) as white solids. mp 109–113 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 (6H, br), 7.35–7.40 (9H, m), 7.42 (1H, s), 7.65 (2H, dd, *J* = 4.4, 1.7 Hz), 8.60 (2H, dd, *J* = 4.8, 1.5 Hz), 9.23 (1H, s). Anal. Calcd. for $C_{28}H_{21}N_3O$•AcOEt: C, 76.32; H, 5.80; N, 8.34. Found: C, 76.31; H, 5.69; N, 8.31.

Ethyl 5-(4-pyridyl)-3-isoxazolecarboxylate and **ethyl 3-(4-pyridyl)-5-isoxazolecarboxylate.** These compounds were similarly prepared from hydroxylamine hydrochloride as described in the synthesis of **79b**, as crude inseparable products.

(5-(4-Pyridyl)isoxazol-3-yl)methanol and **(3-(4-pyridyl)isoxazol-5-yl)methanol.** These compounds were prepared from a mixture of ethyl 5-(4-pyridyl)-3-isoxazolecarboxylate and ethyl 3-(4-pyridyl)-5-isoxazolecarboxylate as described in the synthesis of **80b**, as inseparable products. **(5-(4-Pyridyl)isoxazol-3-yl)methanol**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.58 (2H, d, *J* = 6.0 Hz), 5.61 (1H, t, *J* = 5.9 Hz), 7.30 (1H, s), 7.84 (2H, dd, *J* = 4.2, 1.8 Hz), 8.74 (2H, dd, *J* = 4.5, 1.5 Hz). **(3-(Pyridin-4-yl)isoxazol-5-yl)methanol**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.64 (2H, d, *J* = 6.0 Hz),

5.77 (1H, t, J = 6.0 Hz), 7.08 (1H, s), 7.84–7.86 (2H, m), 8.71–8.73 (2H, m).

4-(3-(Chloromethyl)isoxazol-5-yl)pyridine (86) and **4-(5-(chloromethyl)isoxazol-3-yl)pyridine (87).** Thionyl chloride (1.8 mL) was added to a mixture of (5-(4-pyridyl)isoxazol-3-yl)methanol and (3-(4-pyridyl)isoxazol- 5-yl)methanol (206 mg, 1.17 mmol) at 0 °C. The resulting mixture was stirred at room temperature for 1.5 h. After ethyl acetate was added to the reaction mixture, the mixture was washed with saturated $NaHCO_3$ aqueous solution and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting hexane and ethyl acetate to afford 160 mg of **86** (0.82 mmol, 70%) as white solids and 38.8 mg of **87** (0.20 mmol, 17%) as white solids. **4-(3-(Chloromethyl)isoxazol-5-yl)pyridine (86)**: mp 102–104 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.66 (2H, s), 6.82 (1H, s), 7.64 (2H, dd, J = 4.5, 1.5 Hz), 8.76 (2H, dd, J = 4.4, 1.7 Hz). **4-(5-(Chloromethyl)isoxazol-3-yl)pyridine (87)**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.69 (2H, s), 6.70 (1H, s), 7.68 (2H, dd, J = 4.4, 1.7 Hz), 8.74 (2H, d, J = 4.8 Hz).

4-(4-(Trifluoromethyl)-1*H*-imidazol-2-yl)pyridine (89). To a solution of sodium acetate (21.9 g) in water (50 mL) was added 3,3-dibromo-1,1,1-trifluoroacetone (36.1 g, 134 mmol). The mixture was stirred at 110 °C for 30 min. After cooling to room temperature, a solution of isonicotinealdehyde (11.5 g) in 25% aqueous ammonia solution (13 mL) and methanol (50 mL) was added. The mixture was stirred at room temperature for 36 h. The reaction mixture was concentrated under reduced pressure. After ethyl acetate was added to the residue, the mixture was washed with water and brine.

The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting hexane and ethyl acetate, and by recrystallization from ethyl acetate to afford 7.04 g of **89** (33.0 mmol, 31%) as light yellow solids. ^{1}H NMR (300 MHz, CD_3OD) δ 7.78 (1H, s), 7.92 (2H, dd, *J* = 4.4, 1.7 Hz), 8.65 (2H, dd, *J* = 4.7, 1.7 Hz), NH was vanished.

2-(4-Pyridyl)-1*H*-imidazole-4-carbonitrile (90). The mixture of 4-(4-(trifluoromethyl)-1*H*-imidazol-2-yl) pyridine (**89**, 1.14 g, 5.35 mmol), 5% ammonia aqueous solution (110 mL) and methanol (55 mL) was stirred at 60 °C for 15 h. The reaction mixture was concentrated under reduced pressure. The precipitate was collected to afford 765 mg of **90** (4.50 mmol, 84%) as white solids. ^{1}H NMR (300 MHz, CD_3OD) δ 7.91 (2H, dd, *J* = 4.7, 1.7 Hz), 8.07 (1H, s), 8.67 (2H, dd, *J* = 4.7, 1.7 Hz), NH was vanished.

4-(4-Pyridyl)-1*H*-pyrrole-2-carbaldehyde (93). 4-(1*H*-Pyrrol-3-yl)pyridine **92** was derived from 4-vinylpyridine **91** and TOSMIC (tosylmethyl isocyanide) in 49% yield. To a solution of 4-(1*H*-pyrrol-3-yl) pyridine (**92**, 1.45 g, 10.0 mmol) in acetonitrile (30 mL) was slowly added chlroromethylenedimethyliminium chloride (3.20 g, 25.0 mmol). The mixture was stirred at reflux for 5 h. After the reaction mixture was cooled to room temperature, water and saturated $NaHCO_3$ aqueous solution were added. The mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by recrystallization from methanol and IPE to afford 800 mg of **93** (4.65

mmol, 46%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (1H, s), 7.42 (2H, d, *J* = 5.0 Hz), 7.53 (1H, brs), 8.59 (2H, d, *J* = 5.2 Hz), 9.62 (1H, s + 1H, br).

1-(4-Pyridyl)-1*H*-pyrrole-3-carbaldehyde (96). A mixture of 4-aminopyridine (**94**, 2.12 g, 22.5 mmol) and 2,5-dimethoxy-3-tetrahydrofurancarboxaldehyde (**95**, 4.50 g, 28.1 mmol) in acetic acid (25 mL) was stirred at 90 °C for 2 h. The reaction mixture was concentrated under reduced pressure and the pH of the residue was adjusted to 11 with 1 N NaOH aqueous solution. Brine was added and then the mixture was extracted with chloroform. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting hexane and ethyl acetate, and by recrystallization from ethyl acetate and IPE to afford 0.86 g of **96** (4.99 mmol, 22%) as pale yellow solids. mp 91–93 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.87 (1H, dd, *J* = 6.2, 1.7 Hz), 7.23–7.24 (1H, m), 7.37 (2H, dd, *J* = 4.5, 1.5 Hz), 7.81 (1H, t, *J* = 1.8 Hz), 8.72 (2H, dd, *J* = 4.7, 1.7 Hz), 9.90 (1H, s). Anal. Calcd. for $C_{10}H_8N_2O$: C, 69.76; H, 4.68; N, 16.27. Found: C, 69.70; H, 4.67; N, 16.20.

4-(1*H*-Tetrazol-5-yl)pyridine (97). The mixture of 4-cyanopyridine (**21**, 8.00 g, 76.8 mmol), sodium azide (6.49 g) and ammonium chloride (5.34 g) in DMF (45 mL) was stirred at 150 °C for 60 h. The cooled reaction mixture was poured into water (200 mL) and then the solution was acidified with 12 N HCl aqueous solution to adjust the pH = 2. After the solution was stored at refrigerator for 12 h, the precipitate was collected by filtration, followed by washing with ethanol and water (1:1) to afford 3.84 g of **97** (26.1 mmol, 34%) as brown solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.03 (2H, dd, *J* =

4.4, 1.7 Hz), 8.84 (2H, dd, J = 4.5, 1.5 Hz), NH was not assigned. Anal. Calcd. for $C_6H_5N_5$: C, 48.98; H, 3.43; N, 47.60. Found: C, 48.78; H, 3.71; N, 47.53.

Ethyl 5-(4-pyridyl)-1,3,4-oxadiazole-2-carboxylate (98). To a suspension of 4-(1*H*-tetrazol-5-yl)pyridine (**97**, 2.09 g, 14.2 mmol) in toluene (140 mL) was added ethyl chlorooxoacetate (1.9 mL) and 2,4,6-collidine (2.3 mL). The mixture was stirred at 120 °C for 1 h. Ethyl acetate was added to the reaction mixture and the mixture was washed with saturated $NaHCO_3$ aqueous solution and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting hexane and ethyl acetate, and by recrystallization from ethyl acetate to afford 828 mg of **98** (3.78 mmol, 27%) as white solids. mp 87–88 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 (3H, t, J = 7.2 Hz), 4.58 (2H, q, J = 7.1 Hz), 8.02 (2H, dd, J = 4.4, 1.7 Hz), 8.88 (2H, dd, J = 4.4, 1.7 Hz). Anal. Calcd. for $C_{10}H_9N_3O_3$: C, 54.79; H, 4.14; N, 19.17. Found: C, 54.92; H, 4.03; N, 19.02.

***N*-Hydroxy-4-pyridinecarboximidamide**. A mixture of 4-cyanopyridine (**21**, 7.91 g, 76.0 mmol) and aqueous hydroxylamine (50%, 20 mL) in ethanol (300 mL) was stirred at 90 °C for 70 min. The reaction mixture was concentrated under reduced pressure. The precipitate was collected by filtration, followed by washing with diethyl ether to afford 10.2 g of *N*-hydroxy-4-pyridinecarboximidamide (74.4 mmol, 98%) as white solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.03 (2H, s), 7.64 (2H, dd, J = 4.5, 1.8 Hz), 8.58 (2H, dd, J = 4.4, 1.7 Hz), 10.06 (1H, s).

4-(5-(Chloromethyl)-1,2,4-oxadiazol-3-yl)pyridine (99). A mixture of *N*-hydroxy-4-

pyridinecarboximidamide (6.50 g, 47.4 mmol) and chloroacetic anhydride (12.2 g) in toluene (50 mL) was stirred at 120 °C for 12 h. Ethyl acetate was added to the reaction mixture and then the mixture was washed with saturated $NaHCO_3$ aqueous solution, water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting hexane and ethyl acetate afford 1.35 g of **99** (6.90 mmol, 15%) as light yellow solids. mp 65 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.78 (2H, s), 7.60 (2H, dd, *J* = 4.5, 1.8 Hz), 8.81 (2H, dd, *J* = 4.5, 1.8 Hz). Anal. Calcd. for $C_8H_6ClN_3O$: C, 49.12; H, 3.09; N, 21.48. Found: C, 49.07; H, 3.11; N, 21.58.

4-Bromoacetylpyridine hydrobromide. To a solution of 4-acetylpyridine (**78a**, 25.7 g, 212 mmol) in acetic acid (200 mL) was added hydrobromic acid aqueous solution (48%, 26.4 mL) and then a solution of bromine (33.9 g) in acetic acid (20 mL). The mixture was stirred at 90 °C for 20 min. After the reaction mixture was cooled to room temperature, the precipitate was collected by filtration, followed by washing with ethanol and then diethyl ether to afford 48.2 g of 4-bromoacetylpyridine hydrobromide (172 mmol, 81%) as white solids. ^{1}H NMR (300 MHz, D_2O) δ 3.79 (2H, s), 8.23 (2H, d, *J* = 6.4 Hz), 8.84 (2H, d, *J* = 6.4 Hz).

Ethyl 4-(4-pyridyl)thiazole-2-carboxylate (100). A mixture of 4-bromoacetylpyridine hydrobromide (8.14 g, 29.0 mmol) and ethyl thiooxamate (3.86 g) in ethanol (120 mL) was stirred at reflux for 16 h. The reaction mixture was concentrated under reduced pressure and water was added to the residue. The pH of the mixture was adjusted to 10 with 8 N NaOH aqueous solution at 0 °C.

The mixture was extracted with chloroform. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to afford 4.86 g of **100** (20.7 mmol, 72%) as brown solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.48 (3H, t, *J* = 6.8 Hz), 4.53 (2H, q, *J* = 6.8 Hz), 7.84 (2H, dd, *J* = 4.4, 1.6 Hz), 7.97 (1H, s), 8.70 (2H, dd, *J* = 4.4, 1.6 Hz).

4-(4-Pyridyl)thiazole-2-methanol. A solution of diisobutylalminum hydride in toluene (1.5 M, 50 mL) was added to a suspension of ethyl 4-(4-pyridyl)thiazole-2-carboxylate (**100**, 5.86g, 25.0 mmol) in toluene (50 mL) at 0 °C. The resulting mixture was stirred at 0 ℃ for 20 min. After the reaction mixture was treated with sodium sulfate decahydrate (36.3 g), the resulting mixture was stirred for 3 h. The resulting mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting ethyl acetate and methanol to afford 2.72 g of 4-(4-pyridyl)thiazole-2-methanol (14.1 mmol, 57%) as white solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.82 (2H, d, *J* = 5.8 Hz), 6.20 (1H, t, *J* = 5.8 Hz), 7.89 (2H, dd, *J* = 4.6, 1.8 Hz), 8.40 (1H, s), 8.62 (2H, dd, *J* = 4.6, 1.8 Hz).

2-Chloromethyl-4-(4-pyridyl)thiazole hydrochloride (101). Thionyl chloride (15 mL) was added to 4-(4-pyridyl)thiazole-2-methanol (1.54 g, 8.0 mmol) at 0 °C. The resulting mixture was stirred at room temperature for 1 h. The precipitate was collected by filtration, followed by washing with diethyl ether and IPE to afford 1.82 g of **101** (7.36 mmol, 92%) as yellow solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.25 (2H, s), 8.50 (2H, d, *J* = 7.0 Hz), 8.96 (2H, d, *J* = 7.0 Hz), 9.07 (1H, s).

4-(4-(Chloromethyl)thiazol-2-yl)pyridine (103). A mixture of isonicotinethioamide (**102**, 2.50 g, 18.1 mmol) and 1,3-dichloroacetone (2.53 g) in ethanol (30 mL) was stirred at 90 °C for 8 h. Ethyl acetate was added to the reaction mixture and then the mixture was washed with saturated $NaHCO_3$ aqueous solution and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting hexane and ethyl acetate to afford 1.34 g of **103** (6.36 mmol, 35%) as pale yellow solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.77 (2H, s), 7.45 (1H, s), 7.81 (2H, dd, J = 4.7, 1.7 Hz), 8.72 (2H, dd, J = 4.4, 1.7 Hz).

4-Iodo-*N*-methoxy-*N*-methyl-5-(4-pyridyl)-1*H*-pyrazole-3-carboxamide (106). A mixture of *N*-methoxy-*N*-methyl-5-(4-pyridyl)-1*H*-pyrazole-3-carboxamide (**105**, 540 mg, 2.33 mmol) and iodine (1.77 g) and ceric ammonium nitrate (1.27 g) in acetonitrile (10 mL) was stirred at 95 °C for 23 h. After the resulting mixture was treated with 5% sodium hydrogen sulfite aqueous solution, the mixture was concentrated under reduced pressure. Ethyl acetate and saturated $NaHCO_3$ aqueous solution were added to the residue. The precipitate was filtered and then filtrate was separated. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting ethyl acetate and methanol to afford 684 mg of **106** (1.91 mmol, 82%) as pale yellow foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.43 (3H, s), 3.77 (3H, s), 7.80 (2H, dd, J = 4.7, 1.7 Hz), 8.73 (2H, dd, J = 4.4, 1.7 Hz).

***N*-Methoxy-*N*-methyl-4-phenyl-5-(4-pyridyl)-1*H*-pyrazole-3-carboxamide (107).** A mixture of 4-iodo-*N*-methoxy-*N*-methyl-5-(4-pyridyl)-1*H*-pyrazole-3-carboxamide (**106**, 358 mg, 1.00 mmol), phenylboronic acid (244 mg) and potassium carbonate (332 mg) in toluene (20 mL), ethanol (2.0 mL) and water (2.0 mL) was stirred at room temperature for 30 min under argon atmosphere. After tetrakis(triphenylphosphine)palladium (116 mg) was added, the resulting mixture was stirred at 100 °C for 11.5 h under argon atmosphere. Ethyl acetate was added to the reaction mixture and then the mixture was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting ethyl acetate and methanol to afford 63.6 mg of **107** (0.21 mmol, 21%) as white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.21 (3H, s), 3.60 (3H, s), 7.26–7.39 (7H, m), 8.51 (2H, dd, J = 4.4, 1.7 Hz), 11.82 (1H, br).

2-Isonicotinoyl-*N*-methylhydrazinecarbothioamide. A solution of methyl isothiocyanate (8.60 g) in ethanol (50 mL) was added to a suspension of isonicotinic hydrazide (**25**, 13.7 g, 99.9 mmol) in ethanol (250 mL). The mixture was stirred at 90 °C for 4 h. After the reaction mixture was cooled to room temperature, the precipitate was collected by filtration to afford 20.9 g of 2-isonicotinoyl-*N*-methylhydrazinecarbothioamide (99.4 mmol, 99%) as white solids. mp 213–215 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.88 (3H, d, J = 4.2 Hz), 7.81 (2H, dd, J = 4.5, 1.5 Hz), 8.13 (1H, q, J = 3.6 Hz), 8.76 (2H, dd, J = 4.4, 1.7 Hz), 9.43 (1H, s), 10.64 (1H, s). Anal. Calcd. for $C_8H_{10}N_4OS$: C, 45.70; H, 4.79; N, 26.65. Found: C, 45.69; H, 4.97; N, 26.92.

4-Methyl-5-(4-pyridyl)-4*H*-1,2,4-triazole-3-thiol (108). A mixture of 2-isonicotinoyl-*N*-methylhydrazinecarbothioamide (10.2 g, 48.5 mmol) and 1 M aqueous sodium bicarbonate solution (485 mL) was stirred at 120 °C for 22 h. After the hot reaction mixture was filtered, the filtrate was neutralized with 12 N HCl aqueous solution. The precipitate was collected by filtration to afford 9.24 g of **108** (48.1 mmol, 99%) as white solids. mp 282–287 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.60 (3H, s), 7.77 (2H, dd, J = 4.4, 1.7 Hz), 8.79 (2H, dd, J = 4.5, 1.8 Hz), SH were not assigned. Anal. Calcd. for $C_8H_8N_4S$•$0.1H_2O$: C, 49.52; H, 4.26; N, 28.87. Found: C, 49.27; H, 4.13; N, 28.81.

4-(4-Methyl-4*H*-1,2,4-triazol-3-yl)pyridine (109). A mixture of 4-methyl-5-(4-pyridyl)-4*H*-1,2,4-triazole- 3-thiol (**108**, 13.8 g, 71.8 mmol) and 17% nitric acid aqueous solution (380 mL) was stirred at 120 °C for 30 min. The reaction mixture was cooled to 0 °C and then the pH was adjusted to 14 with pellets of potassium hydroxide. The resulting mixture was extracted with chloroform. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by recrystallization from THF and IPE to afford 10.3 g of **109** (64.3 mmol, 90%) as white solids. mp 169–171 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.86 (3H, s), 7.66 (2H, dd, J = 4.4, 1.7 Hz), 8.26 (1H, s), 8.81 (2H, dd, J = 4.4, 1.7 Hz). Anal. Calcd. for $C_8H_8N_4$: C, 59.99; H, 5.03; N, 34.98. Found: C, 59.78; H, 5.05; N, 34.82.

4-Methyl-5-(4-pyridyl)-4*H*-1,2,4-triazole-3-carbaldehyde (110). This compound was prepared from 4-(4-methyl-4*H*-1,2,4-triazol-3-yl)pyridine **109** as described in the synthesis of **85**, as white

solid. mp 115–116 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.08 (3H, s), 7.66 (2H, dd, *J* = 4.4, 1.7 Hz), 8.87 (2H, dd, *J* = 4.5, 1.5 Hz), 10.20 (1H, s).

General procedure for the preparation of ethyl *N*-methylaminooxoacetate. A solution of methylamine in THF (2.0 M, 200 mL) was added to a solution of ethyl chlorooxoacetate (49.2 g, 360 mmol) and pyridine (37.0 g) in dichloromethane (500 mL) at 0 °C for 30 min. After the addition, the mixture was allowed to warm to room temperature and then stirred for 24 h. The precipitate was filtered and washed with THF. The filtrate was combined and then concentrated under reduced pressure. The pH of the residue was adjusted to 1 with 1 N HCl aqueous solution. The mixture was extracted with chloroform. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to afford 47.3 g of ethyl *N*-methylaminooxoacetate (361 mmol, quantitative yield) as brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (3H, t, *J* = 7.2 Hz), 2.94 (3H, d, *J* = 5.1 Hz), 4.35 (2H, q, *J* = 7.2 Hz), 7.12 (1H, s).

General procedure for the preparation of ethyl *N*-methylaminothiooxoacetate. A mixture of the above ethyl *N*-methylaminooxoacetate (all amount) and Lawesson's reagent (218 g) in toluene (450 mL) was stirred at 90 °C for 2 h. The reaction mixture was cooled to room temperature. The precipitate was filtered and washed with toluene. The filtrate was combined and then concentrated under reduced pressure. The residue was purified by distillation to afford 31.7 g of ethyl *N*-methylaminothiooxoacetate (215 mmol, 60%) as brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (3H, t, *J* = 7.2 Hz), 3.24 (3H, d, *J* = 5.1 Hz), 4.38 (2H, q, *J* = 7.2 Hz), 9.00 (1H, s).

General procedure for the preparation of ethyl 4-methyl-5-(4-pyridyl)-4*H*-1,2,4-triazole-3-carboxylate (111). Meerwein's reagent in DCM (1.0 M, 168 mL) was added to a solution of ethyl *N*-methylaminothiooxoacetate (16.5 g, 112 mmol) in DCM (150 mL). The mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with DCM and then washed with water, saturated $NaHCO_3$ aqueous solution and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford ethyl (*S*-ethylthio-*N*-methylimino)acetate as brown oil. This compound was used without further purification. A mixture of ethyl (*S*-ethylthio-*N*-methylimino)acetate and isonicotinic hydrazide (**25**, 9.75 g, 71.1 mmol) in toluene (200 mL) was stirred at 130 °C for 16 h with Dean-Stark removal of toluene/water azeotrope. The reaction mixture was concentrated under reduced pressure and then chloroform was added. The mixture was washed with water and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting ethyl acetate and methanol to afford 9.90 g of **111** (42.6 mmol, 60%) as yellow solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.49 (3H, t, *J* = 7.2 Hz), 4.04 (3H, s), 4.53 (2H, q, *J* = 7.1 Hz), 7.62 (2H, dd, *J* = 4.4, 1.4 Hz), 8.84 (2H, dd, *J* = 4.4, 1.4 Hz).

(4-Methyl-5-(4-pyridyl)-4*H*-1,2,4-triazol-3-yl)methanol (112). This compound was prepared from ethyl 4-methyl-5-(4-pyridyl)-4*H*-1,2,4-triazole-3-carboxylate **111** as described in the synthesis of **76**, as white solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.78 (3H, s), 4.69 (2H, d, *J* = 4.4 Hz), 5.65 (1H, s), 7.76 (2H, dd, *J* = 4.6, 1.8 Hz), 8.76 (2H, dd, *J* = 4.6, 1.6 Hz).

The alternative preparation of 4-methyl-5-(4-pyridyl)-4*H*-1,2,4-triazole-3-carbaldehyde (110). Manganese(IV) oxide (10.0 g) was added to a solution of (4-methyl-5-(4-pyridyl)-4*H*-1,2,4-triazol-3-yl)methanol **112** (2.00 g, 10.5 mmol) in methanol (100 mL) and acetone (50 mL). The mixture was stirred at room temperature for 5 h. The reaction mixture was filtered through a pad of Celite and then the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting ethyl acetate and methanol to afford 1.89 g of **110** (10.0 mmol, 96%) as white solid.

General procedure for the preparation of ethyl 3-(((4-methyl-5-(4-pyridyl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)benzoate (113). A mixture of 4-methyl-5-(4-pyridyl)-4*H*-1,2,4-triazole-3-carbaldehyde (**110**, 4.47 g, 23.8 mmol) and ethyl 3-aminobenzoate (7.85 g) in ethanol (350 mL) and acetic acid (17.5 mL) was stirred at room temperature for 19 h. The reaction mixture was concentrated under reduced pressure and the residue was dissolved with ethanol (200 mL). Sodium borohydride (2.70 g) was added to the solution at 0 °C. After the addition, the mixture was allowed to warm to room temperature and then stirred for 16 h. The reaction mixture was concentrated under reduced pressure and ethyl acetate was added to the residue. The mixture was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting ethyl acetate and methanol, and by recrystallization from ethyl acetate and IPE to afford 5.42 g of **113** (16.1 mmol, 68%) as white solids. mp 115–116 °C. ^{1}H NMR

(300 MHz, $CDCl_3$) δ 1.40 (3H, t, *J* = 7.2 Hz), 3.79 (3H, s), 4.37 (2H, q, *J* = 7.1 Hz), 4.53 (1H, t, *J* = 5.1 Hz), 4.60 (2H, d, *J* = 4.8 Hz), 7.00 (1H, dd, *J* = 8.0, 2.9 Hz), 7.30 (1H, t, *J* = 8.0 Hz), 7.43 (1H, t, *J* = 2.1 Hz), 7.50 (1H, d, *J* = 7.5 Hz), 7.61 (2H, dd, *J* = 4.4, 1.7 Hz), 8.80 (2H, dd, *J* = 4.5, 1.5 Hz). Anal. Calcd. for $C_{18}H_{19}N_5O_2$: C, 64.08; H, 5.68; N, 20.76. Found: C, 63.71; H, 5.88; N, 20.64.

3-(((4-Methyl-5-(4-pyridyl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)benzoic acid (114). This compound was prepared from ethyl 3-(((4-methyl-5-(4-pyridyl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)benzoate **113** as described in the synthesis of **34**, as white solids. mp 239–242 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.77 (3H, s), 4.54 (2H, d, *J* = 5.4 Hz), 6.56 (1H, t, *J* = 5.4 Hz), 6.99 (1H, dt, *J* = 7.2, 2.2 Hz), 7.17–7.24 (2H, m), 7.35 (1H, s), 7.74 (2H, dd, *J* = 4.7, 1.7 Hz), 8.75 (2H, dd, *J* = 4.5, 1.8 Hz), CO_2H was not assigned. Anal. Calcd. for $C_{16}H_{15}N_5O_2$•$0.8H_2O$: C, 59.36; H, 5.17; N, 21.63. Found: C, 59.13; H, 4.80; N, 21.63.

General procedure for the preparation of 3-(((4-methyl-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)-*N*-(2-(trifluoromethyl)benzyl)benzamide (115h). A mixture of 3-(((4-methyl-5-(4-pyridyl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)benzoic acid (**114**, 155 mg, 0.50 mmol), 2-(trifluoromethyl)benzylamine (175 mg), WSC•HCl (144 mg) and HOBt•H_2O (7.7 mg) in DMF (2.0 mL) was stirred at room temperature for 18 h. Ethyl acetate was added to the reaction mixture and then the mixture was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by

column chromatography on silica gel eluting ethyl acetate and methanol to afford 225 mg of **115h** (0.48 mmol, 96%) as white foam. ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.78 (3H, s), 4.56 (2H, d, J = 5.5 Hz), 4.64 (2H, d, J = 5.5 Hz), 6.50 (1H, t, J = 5.5 Hz), 6.95 (1H, dd, J = 8.1, 1.5 Hz), 7.16 (1H, d, J = 7.3 Hz), 7.20–7.25 (1H, m), 7.29 (1H, s), 7.47 (1H, t, J = 7.5 Hz), 7.52 (1H, d, J = 7.7 Hz), 7.63–7.69 (1H, m), 7.70–7.78 (3H, m), 8.76 (2H, d, J = 5.9 Hz), 8.94 (1H, t, J = 5.9 Hz). ^{13}C NMR (151 MHz, DMSO-d_6) δ 22.71, 31.43, 37.90, 111.57, 115.00, 115.13, 122.30, 124.43 (q, J = 274.0 Hz), 125.59 (q, J = 5.5 Hz), 125.92 (q, J = 31.0 Hz), 127.09, 127.90, 128.78, 132.54, 134.51, 134.89, 137.74 (q, J = 1.7 Hz), 148.07, 150.19, 152.32, 154.14, 167.03. Anal. Calcd. for $C_{24}H_{21}F_3N_6O$•1.2H_2O: C, 59.06; H, 4.83; N, 17.22. Found: C, 59.16; H, 4.84; N, 16.84. MS (ESI/APCI) *m/z* 467.1 $[M + H]^+$. HPLC purity 99.0%.

***N*-(4-Methoxybenzyl)-3-(((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)amino)benzamide (115a).** This compound was prepared from 3-(((5-(4-pyridyl)-1*H*-1,2,4-triazol-3-yl)methyl)amino)benzoic acid **34** and 4-methoxybenzylamine as described in the synthesis of **115h**, as white solids. mp 201–204 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.71 (3H, s), 4.36 (2H, d, J = 6.2 Hz), 4.49 (2H, d, J = 6.2 Hz), 6.46 (1H, t, J = 6.1 Hz), 6.77–6.81 (1H, m), 6.84–6.87 (2H, m), 7.09 (1H, d, J = 7.7 Hz), 7.15–7.18 (2H, m), 7.21 (2H, d, J = 8.8 Hz), 7.88–7.93 (2H, m), 8.67 (2H, d, J = 5.9 Hz), 8.77 (1H, t, J = 6.1 Hz), NH was not assigned. ^{13}C NMR (151 MHz, DMSO-d_6) δ 41.84, 54.93, 111.22, 113.51, 114.98, 115.11, 119.81, 128.35, 128.62, 131.69, 135.27, 137.63, 148.04, 150.25, 157.72, 158.00, 166.47, 1 aliphatic and 1 aromatic

carbons were not assigned. Because the aliphatic carbon was hidden by DMSO. Anal. Calcd. for $C_{23}H_{22}N_6O_2$•1/3H_2O: C, 65.70; H, 5.43; N, 19.99. Found: C, 65.84; H, 5.52; N, 19.66. MS (ESI/APCI) *m/z* 415.1 $[M + H]^+$. HPLC purity 99.0%.

***N*-(3-Methoxybenzyl)-3-(((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)amino)benzamide (115b).** This compound was prepared from 3-(((5-(4-pyridyl)-1*H*-1,2,4-triazol-3-yl)methyl)amino)benzoic acid **34** and 3-methoxybenzylamine as described in the synthesis of **115h**, as white solids. mp 128–130 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.71 (3H, s), 4.41 (2H, d, *J* = 5.9 Hz), 4.49 (2H, d, *J* = 5.9 Hz), 6.47 (1H, t, *J* = 5.9 Hz), 6.78–6.81 (2H, m), 6.84–6.87 (2H, m), 7.11 (1H, d, *J* = 8.1 Hz), 7.15–7.19 (2H, m), 7.21 (1H, t, *J* = 8.1 Hz), 7.88–7.94 (2H, m), 8.67 (2H, d, *J* = 5.9 Hz), 8.82 (1H, t, *J* = 6.1 Hz), 14.25 (1H, brs). ^{13}C NMR (151 MHz, DMSO-d_6) δ 42.34, 54.83, 111.24, 111.87, 112.72, 115.00, 115.11, 119.15, 119.81, 128.67, 129.17, 135.19, 137.66, 141.34, 148.07, 150.24, 157.74, 159.15, 166.62, 1 aliphatic and 1 aromatic carbon were not assigned. Because the aliphatic carbon was hidden by DMSO. Anal. Calcd. for $C_{23}H_{22}N_6O_2$•2H_2O: C, 61.32; H, 5.82; N, 18.66. Found: C, 61.51; H, 5.66; N, 18.43. MS (ESI/APCI) *m/z* 415.1 $[M + H]^+$. HPLC purity 98.8%.

***N*-(2-Methoxybenzyl)-3-(((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)amino)benzamide (115c).** This compound was prepared from 3-(((5-(4-pyridyl)-1*H*-1,2,4-triazol-3-yl)methyl)amino)benzoic acid **34** and 2-methoxybenzylamine as described in the synthesis of **115h**, as white solids. mp 138–140 °C. ^{1}H NMR (600 MHz,

DMSO-d_6) δ 3.81 (3H, s), 4.41 (2H, d, J = 5.9 Hz), 4.50 (2H, d, J = 5.9 Hz), 6.47 (1H, t, J = 5.9 Hz), 6.80 (1H, dd, J = 7.5, 1.7 Hz), 6.84–6.89 (1H, m), 6.97 (1H, d, J = 8.1 Hz), 7.13 (2H, d, J = 7.7 Hz), 7.16–7.24 (3H, m), 7.89–7.93 (2H, m), 8.64 (1H, t, J = 6.1 Hz), 8.66–8.71 (2H, m), 14.25 (1H, brs). ^{13}C NMR (151 MHz, DMSO-d_6) δ 37.38, 55.20, 110.29, 111.25, 114.99, 115.16, 119.81, 119.94, 126.93, 127.66, 128.66, 135.25, 137.58, 148.06, 150.25, 156.39, 157.66, 166.73, 1 aliphatic and 2 aromatic carbons were not assigned. Because the aliphatic carbon was hidden by DMSO. Anal. Calcd. for $C_{23}H_{22}N_6O_2$•$2H_2O$: C, 61.32; H, 5.82; N, 18.66. Found: C, 61.48; H, 5.98; N, 18.63. MS (ESI/APCI) *m/z* 415.1 $[M + H]^+$. HPLC purity 99.4%.

***N*-(2-Chlorobenzyl)-3-(((3-(pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl)methyl)amino)benzamide (115d).** This compound was prepared from 3-(((5-(4-pyridyl)-1*H*-1,2,4-triazol-3-yl)methyl)amino)benzoic acid **34** and 2-chlorobenzylamine as described in the synthesis of **115h**, as white solids. mp 162–164 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.50 (4H, d, J = 5.9 Hz), 6.50 (1H, t, J = 5.9 Hz), 6.82 (1H, dd, J = 8.1, 1.5 Hz), 7.13–7.16 (1H, m), 7.18–7.22 (2H, m), 7.27–7.33 (3H, m), 7.41–7.47 (1H, m), 7.87–7.94 (2H, m), 8.67 (2H, d, J = 5.9 Hz), 8.85 (1H, t, J = 5.9 Hz), 14.22 (1H, brs). ^{13}C NMR (151 MHz, DMSO-d_6) δ 40.33, 111.30, 115.13, 115.16, 119.82, 126.98, 128.26, 128.33, 128.72, 128.96, 131.75, 134.91, 136.41, 137.59, 148.10, 150.25, 157.95, 166.84, 1 aliphatic and 1 aromatic carbons were not assigned. Because the aliphatic carbon was hidden by DMSO. Anal. Calcd. for $C_{22}H_{19}ClN_6O$•$2.5H_2O$: C, 56.96; H, 5.21; N, 18.12. Found: C, 57.27; H, 4.91; N, 17.76. MS (ESI/APCI) *m/z* 419.0 $[M + H]^+$. HPLC purity 98.5%.

***N*-Benzyl-3-(((4-methyl-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)benzamide (115e).** This compound was prepared from 4-methyl-5-(4-pyridyl)-4*H*-1,2,4-triazole-3-carbaldehyde **110** and 3-amino-*N*-benzylbenzamide **29i** as described in the synthesis of **113**, as white foam. ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.77 (3H, s), 4.46 (2H, d, J = 6.2 Hz), 4.54 (2H, d, J = 5.9 Hz), 6.45 (1H, t, J = 5.7 Hz), 6.92 (1H, dd, J = 8.1, 1.5 Hz), 7.11 (1H, d, J = 7.7 Hz), 7.17–7.21 (1H, m), 7.21–7.25 (1H, m), 7.25–7.27 (1H, m), 7.29–7.35 (4H, m), 7.71–7.76 (2H, m), 8.72–8.78 (2H, m), 8.85 (1H, t, J = 6.1 Hz). ^{13}C NMR (151 MHz, DMSO-d_6) δ 31.44, 37.92, 42.43, 111.56, 114.79, 115.11, 122.31, 126.55, 127.04, 128.14, 128.69, 134.52, 135.29, 139.74, 148.02, 150.20, 152.33, 154.16, 166.70. Exact Mass (FAB) $[M + H]^+$ for $C_{23}H_{23}N_6O$, Calcd: 399.1921. Found: 399.1933. MS (ESI/APCI) *m/z* 399.1 $[M + H]^+$. HPLC purity 99.5%.

3-(((4-Methyl-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)-*N*-(4-(trifluoromethyl)benzyl)benzamide (115f). This compound was prepared from 3-(((4-methyl-5-(4-pyridyl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)benzoic acid **114** and 4-(trifluoromethyl)benzylamine as described in the synthesis of **115h**, as white foam. ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.77 (3H, s), 4.53 (2H, d, J = 6.2 Hz), 4.55 (2H, d, J = 5.9 Hz), 6.47 (1H, t, J = 5.7 Hz), 6.93 (1H, dd, J = 8.1, 1.8 Hz), 7.12 (1H, d, J = 7.7 Hz), 7.18–7.23 (1H, m), 7.27 (1H, t, J = 1.8 Hz), 7.53 (2H, d, J = 8.1 Hz), 7.69 (2H, d, J = 8.1 Hz), 7.72–7.76 (2H, m), 8.76 (2H, d, J = 5.1 Hz), 8.96 (1H, t, J = 6.1 Hz). ^{13}C NMR (151 MHz, DMSO-d_6) δ 31.43, 37.90, 42.15, 111.52, 114.95, 115.09, 122.29, 124.17 (q, J = 275.3 Hz), 125.04 (q, J = 3.9 Hz), 127.28 (q, J = 31.5 Hz), 127.70,

128.73, 134.50, 135.01, 144.66 (d, J = 1.1 Hz), 148.05, 150.19, 152.32, 154.14, 166.87. Anal. Calcd. for $C_{24}H_{21}F_3N_6O$•1.5H_2O: C, 58.41; H, 4.90; N, 17.03. Found: C, 58.75; H, 4.69; N, 16.82. MS (ESI/APCI) *m/z* 467.1 $[M + H]^+$. HPLC purity 97.1%.

3-(((4-Methyl-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)-*N*-(3-(trifluoromethyl)benzyl)benzamide (115g). This compound was prepared from 3-(((4-methyl-5-(4-pyridyl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)benzoic acid **114** and 3-(trifluoromethyl)benzylamine as described in the synthesis of **115h**, as white foam. ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.77 (3H, s), 4.54 (4H, t, J = 5.9 Hz), 6.48 (1H, t, J = 5.7 Hz), 6.94 (1H, dd, J = 7.9, 2.0 Hz), 7.11 (1H, d, J = 7.7 Hz), 7.18–7.23 (1H, m), 7.26 (1H, s), 7.56–7.64 (3H, m), 7.65 (1H, s), 7.71–7.77 (2H, m), 8.75 (2H, d, J = 6.2 Hz), 8.96 (1H, t, J = 6.1 Hz). ^{13}C NMR (151 MHz, DMSO-d_6) δ 31.42, 37.89, 42.10, 111.56, 114.89, 115.05, 122.30, 123.35 (q, J = 3.9 Hz), 123.57 (q, J = 3.7 Hz), 124.18 (q, J = 272.8 Hz), 125.98, 128.76, 128.87 (q, J = 31.2 Hz), 131.26, 134.51, 134.98, 141.27, 148.07, 150.19, 152.32, 154.13, 166.86. Anal. Calcd. for $C_{24}H_{21}F_3N_6O$•1.5H_2O: C, 58.41; H, 4.90; N, 17.03. Found: C, 58.62; H, 4.78; N, 16.91. MS (ESI/APCI) *m/z* 467.1 $[M + H]^+$. HPLC purity 98.9%.

***N*-(2-Methoxybenzyl)-3-(((4-methyl-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)benzamide (115i).** This compound was prepared from 3-(((4-methyl-5-(4-pyridyl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)benzoic acid **114** and 2-methoxybenzylamine as described in the synthesis of **115h**, as white solids. mp 109–111 ºC. ^{1}H

NMR (600 MHz, DMSO-d_6) δ 3.77 (3H, s), 3.83 (3H, s), 4.42 (2H, d, J = 5.9 Hz), 4.55 (2H, d, J = 5.5 Hz), 6.45 (1H, t, J = 5.7 Hz), 6.88–6.91 (1H, m), 6.93 (1H, dd, J = 8.1, 1.5 Hz), 6.98 (1H, d, J = 7.7 Hz), 7.13 (1H, d, J = 7.7 Hz), 7.17 (1H, d, J = 7.3 Hz), 7.18–7.24 (2H, m), 7.26–7.29 (1H, m), 7.71–7.77 (2H, m), 8.65 (1H, t, J = 5.9 Hz), 8.73–8.79 (2H, m). ^{13}C NMR (151 MHz, DMSO-d_6) δ 31.42, 37.39, 37.90, 55.21, 110.28, 111.52, 114.76, 115.13, 119.98, 122.29, 126.94, 126.99, 127.67, 128.68, 134.50, 135.32, 147.99, 150.18, 152.31, 154.15, 156.40, 166.82. Anal. Calcd. for $C_{24}H_{24}N_6O_2$•H_2O: C, 64.56; H, 5.87; N, 18.82. Found: C, 64.18; H, 5.84; N, 18.63. MS (ESI/APCI) *m/z* 429.1 $[M + H]^+$. HPLC purity 98.5%.

***N*-(2-Chlorobenzyl)-3-(((4-methyl-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)benzamide (115j).** This compound was prepared from 3-(((4-methyl-5-(4-pyridyl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)benzoic acid **114** and 2-chlorobenzylamine as described in the synthesis of **115h**, as white solids. mp 165–170 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.78 (3H, s), 4.52 (2H, d, J = 5.9 Hz), 4.55 (2H, d, J = 5.5 Hz), 6.48 (1H, t, J = 5.7 Hz), 6.94 (1H, dd, J = 8.1, 1.8 Hz), 7.15 (1H, d, J = 7.7 Hz), 7.19–7.23 (1H, m), 7.27–7.36 (4H, m), 7.45 (1H, dd, J = 7.9, 0.9 Hz), 7.72–7.76 (2H, m), 8.73–8.78 (2H, m), 8.87 (1H, t, J = 5.9 Hz). ^{13}C NMR (151 MHz, DMSO-d_6) δ 31.42, 37.89, 40.33, 111.57, 114.91, 115.13, 122.29, 127.03, 128.31, 128.34, 128.74, 128.95, 131.75, 134.50, 134.99, 136.41, 148.04, 150.19, 152.31, 154.14, 166.93. Anal. Calcd. for $C_{23}H_{21}ClN_6O$•$0.5H_2O$: C, 62.51; H, 5.02; N, 19.02. Found: C, 62.54; H, 4.92; N, 19.04. MS (ESI/APCI) *m/z* 433.1 $[M + H]^+$. HPLC purity 98.8%.

Ethyl 2-(ethylamino)-2-oxoacetate. This compound was prepared from ethyl chlorooxoacetate and ethylamine as described in the synthesis of ethyl *N*-methylaminooxoacetate, as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.21 (3H, t, *J* = 7.2 Hz), 1.39 (3H, t, *J* = 7.2 Hz), 3.33–3.46 (2H, m), 4.35 (2H, dd, *J* = 7.2 Hz), 7.12 (1H, br).

Ethyl 2-(ethylamino)-2-thioxoacetate. This compound was prepared from ethyl 2-(ethylamino)-2-oxoacetate as described in the synthesis of ethyl *N*-methylaminothiooxoacetate, as red oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27–1.48 (6H, m), 3.66–3.76 (2H, m), 4.33–4.43 (2H, m), 8.89 (1H, br).

Ethyl 4-ethyl-5-(4-pyridyl)-4*H*-1,2,4-triazole-3-carboxylate (116a). This compound was prepared from ethyl 2-(ethylamino)-2-thioxoacetate as described in the synthesis of **111**, as light yellow solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (3H, t, *J* = 7.2 Hz), 1.42 (3H, t, *J* = 7.2 Hz), 4.41–4.58 (4H, m), 7.59 (2H, dd, *J* = 4.4, 1.4 Hz), 8.83 (2H, dd, *J* = 4.4, 1.4 Hz).

Ethyl 2-(propylamino)-2-oxoacetate. This compound was prepared from ethyl chlorooxoacetate and *n*-propylamine as described in the synthesis of ethyl *N*-methylaminooxoacetate, as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 (3H, t, *J* = 7.2 Hz), 1.41 (3H, t, *J* = 7.2 Hz), 1.54–1.66 (2H, m), 3.32 (2H, q, *J* = 7.2 Hz), 4.35 (2H, q, *J* = 7.2 Hz), 7.14 (1H, br).

Ethyl 2-(propylamino)-2-thioxoacetate. This compound was prepared from ethyl 2-(propylamino)-2-oxoacetate as described in the synthesis of ethyl *N*-methylaminothiooxoacetate, as red oil. ^{1}H NMR (300 MHz, $CDCl_3$) ratio: 9:1, δ 1.01 (3H, t, *J* = 7.2 Hz), 1.41 (2.7H, t, *J* = 7.2

Hz), 1.54 (0.3H, t, *J* = 7.2 Hz), 1.69–1.81 (2H, m), 3.61–3.68 (2H, m), 4.38 (1.8H, q, *J* = 7.2 Hz), 4.68 (0.2H, q, *J* = 7.2 Hz), 8.93 (1H, br).

Ethyl 4-propyl-5-(4-pyridyl)-4*H*-1,2,4-triazole-3-carboxylate (116b). This compound was prepared from ethyl 2-(propylamino)-2-thioxoacetate as described in the synthesis of **111**, as light yellow solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (3H, t, *J* = 7.2 Hz), 1.49 (3H, t, *J* = 7.2 Hz), 1.68–1.89 (2H, m), 4.34–4.44 (2H, m), 4.53 (2H, q, *J* = 7.2 Hz), 7.57 (2H, dd, *J* = 4.4, 1.4 Hz), 8.83 (2H, dd, *J* = 4.4, 1.4 Hz).

Ethyl 2-(isopropylamino)-2-oxoacetate. This compound was prepared from ethyl chlorooxoacetate and isopropylamine as described in the synthesis of ethyl *N*-methylaminooxoacetate, as light yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (6H, d, *J* = 6.6 Hz), 1.39 (3H, t, *J* = 7.2 Hz), 4.03–4.18 (1H, m), 4.34 (2H, q, *J* = 7.2 Hz), 6.97 (1H, br).

Ethyl 2-(isopropylamino)-2-thioxoacetate. This compound was prepared from ethyl 2-(isopropylamino)-2-oxoacetate as described in the synthesis of ethyl *N*-methylaminothiooxoacetate, as red oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32 (6H, d, *J* = 6.6 Hz), 1.41 (3H, t, *J* = 7.2 Hz), 4.37 (2H, q, *J* = 7.2 Hz), 4.46–4.74 (1H, m), 8.72 (1H, br).

Ethyl 4-isopropyl-5-(4-pyridyl)-4*H*-1,2,4-triazole-3-carboxylate (116c). This compound was prepared from ethyl 2-(isopropylamino)-2-thioxoacetate as described in the synthesis of **111**, as light yellow solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.48 (3H, t, *J* = 7.2 Hz), 1.58 (6H, d, *J* = 6.8 Hz), 4.53 (2H, q, *J* = 7.2 Hz), 4.76–4.97 (1H, m), 7.50 (2H, dd, *J* = 4.4, 1.4 Hz), 8.82 (2H, dd, *J* = 4.4, 1.4 Hz).

Ethyl 2-(benzylamino)-2-oxoacetate. This compound was prepared from ethyl chlorooxoacetate and benzylamine as described in the synthesis of ethyl *N*-methylaminooxoacetate, as light yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (3H, t, *J* = 7.2 Hz), 4.34 (2H, dd, *J* = 7.2 Hz), 4.52 (2H, d, *J* = 6.2 Hz), 7.28–7.38 (5H, m), 7.41 (1H, br).

Ethyl 2-(benzylamino)-2-thioxoacetate. This compound was prepared from ethyl 2-(benzylamino)-2-oxoacetate as described in the synthesis of ethyl *N*-methylaminothiooxoacetate, as orange oil. ^{1}H NMR ($CDCl_3$) δ 1.40 (3H, t, *J* = 7.2 Hz), 4.37 (2H, dd, *J* = 7.2 Hz), 4.83 (2H, d, *J* = 5.4 Hz), 7.29–7.45 (5H, m), 9.06 (1H, br).

Ethyl 4-benzyl-5-(4-pyridyl)-4*H*-1,2,4-triazole-3-carboxylate (116d). This compound was prepared from ethyl 2-(benzylamino)-2-thioxoacetate as described in the synthesis of **111**, as white solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (3H, t, *J* = 7.0 Hz), 4.45 (2H, q, *J* = 7.0 Hz), 5.69 (2H, s), 6.88–7.01 (2H, m), 7.28–7.42 (3H, m), 7.47 (2H, dd, *J* = 4.4, 1.4 Hz), 8.73 (2H, dd, *J* = 4.4, 1.4 Hz).

Ethyl 2-((2-methoxyethyl)amino)-2-oxoacetate. This compound was prepared from ethyl chlorooxoacetate and (2-methoxyethyl)amine as described in the synthesis of ethyl *N*-methylaminooxoacetate, as yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (3H, t, *J* = 7.2 Hz), 3.37 (3H, s), 3.50–3.60 (4H, m), 4.35 (2H, q, *J* = 7.2 Hz), 7.43 (1H, brs).

Ethyl 2-((2-methoxyethyl)amino)-2-thioxoacetate. This compound was prepared from ethyl 2-((2-methoxyethyl)amino)-2-oxoacetate as described in the synthesis of ethyl *N*-methylaminothiooxoacetate, as orange oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (3H, t, *J* = 7.2 Hz),

3.40 (3H, s), 3.63 (2H, t, *J* = 6.9 Hz), 3.87 (2H, q, *J* = 7.2 Hz), 4.38 (2H, q, *J* = 7.2 Hz), 9.23 (1H, br).

Ethyl 4-(2-methoxyethyl)-5-(4-pyridyl)-4*H*-1,2,4-triazole-3-carboxylate (116e). This compound was prepared from ethyl 2-((2-methoxyethyl)amino)-2-thioxoacetate as described in the synthesis of **111**, as light yellow solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.49 (3H, t, *J* = 6.9 Hz), 3.24 (3H, s), 3.73 (2H, t, *J* = 4.8 Hz), 4.40–4.62 (4H, m), 7.74–7.78 (2H, m), 8.78–8.79 (2H, m).

Ethyl 2-((2-benzyloxyethyl)amino)-2-oxoacetate. This compound was prepared from ethyl chlorooxoacetate and (2-benzyloxyethyl)amine as described in the synthesis of ethyl *N*-methylaminooxoacetate, as yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (3H, t, *J* = 6.9 Hz), 3.51–3.62 (4H, m), 4.35 (2H, q, *J* = 6.9 Hz), 4.53 (2H, s), 7.23–7.38 (5H, m), 7.44 (1H, br).

Ethyl 2-((2-benzyloxyethyl)amino)-2-thioxoacetate. This compound was prepared from ethyl 2-((2-benzyloxyethyl)amino)-2-oxoacetate as described in the synthesis of ethyl *N*-methylaminothiooxoacetate, as red oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (3H, t, *J* = 7.0 Hz), 3.72 (2H, t, *J* = 5.2 Hz), 3.89 (2H, q, *J* = 5.7 Hz), 4.38 (2H, q, *J* = 7.4 Hz), 4.56 (2H, s), 7.30–7.42 (5H, m), 9.18 (1H, br).

Ethyl 4-(2-benzyloxyethyl)-5-(4-pyridyl)-4*H*-1,2,4-triazole-3-carboxylate (116f). This compound was prepared from ethyl 2-((2-benzyloxyethyl)amino)-2-thioxoacetate as described in the synthesis of **111**, as white solids. ^{1}H NMR ($CDCl_3$) δ 1.45 (3H, t, *J* = 7.2 Hz), 3.78 (2H, t, *J* = 4.8 Hz), 4.39 (2H, s), 4.46 (2H, q, *J* = 7.2 Hz), 4.58 (2H, t, *J* = 4.8 Hz), 7.05–7.09 (2H, m), 7.28–7.32 (3H, m),

7.63–7.68 (2H, m), 8.69–8.74 (2H, m).

Ethyl 2-((2,2,2-trifluoroethyl)amino)-2-oxoacetate. This compound was prepared from ethyl chlorooxoacetate and (2,2,2-trifluoroethyl)amine as described in the synthesis of ethyl *N*-methylaminooxoacetate, as light yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (3H, t, *J* = 7.2 Hz), 4.02-4.03 (2H, m), 4.38 (2H, q, *J* = 7.2 Hz), 7.21 (1H, br).

Ethyl 2-((2,2,2-trifluoroethyl)amino)-2-thioxoacetate. This compound was prepared from ethyl 2-((2,2,2-trifluoroethyl)amino)-2-oxoacetate as described in the synthesis of ethyl *N*-methylaminothiooxoacetate, as red oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (3H, t, *J* = 7.2 Hz), 4.35–4.52 (4H, m), 8.90 (1H, br).

Ethyl 4-(2,2,2-trifluoroethyl)-5-(4-pyridyl)-4*H*-1,2,4-triazole-3-carboxylate (116g). This compound was prepared from ethyl 2-((2,2,2-trifluoroethyl)amino)-2-thioxoacetate as described in the synthesis of **111**, as white solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.49 (3H, t, *J* = 7.8 Hz), 4.54 (2H, q, *J* = 10.8 Hz), 5.20 (2H, q, *J* = 12.0 Hz), 7.53–7.54 (2H, m), 8.87–8.88 (2H, m).

(4-Ethyl-5-(4-pyridyl)-4*H*-1,2,4-triazol-3-yl)methanol (117a). This compound was prepared from ethyl 4-ethyl-5-(4-pyridyl)-4*H*-1,2,4-triazole-3-carboxylate **116a** as described in the synthesis of **76**, as light yellow solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.24 (3H, t, *J* = 7.0 Hz), 4.23 (2H, q, *J* = 7.0 Hz), 4.65 (2H, d, *J* = 5.8 Hz), 5.70 (1H, t, *J* = 5.8 Hz), 7.72 (2H, dd, *J* = 4.4, 1.4 Hz), 8.77 (2H, dd, *J* = 4.4, 1.4 Hz).

(4-Propyl-5-(4-pyridyl)-4*H*-1,2,4-triazol-3-yl)methanol (117b). This compound was prepared from

ethyl 4-propyl-5-(4-pyridyl)-4*H*-1,2,4-triazole-3-carboxylate **116b** as described in the synthesis of **76**, as light yellow solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.74 (3H, t, J = 7.2 Hz), 1.48–1.79 (2H, m), 4.12–4.21 (2H, m), 4.70 (2H, s), 5.70 (1H, br), 7.72 (2H, dd, J = 4.4, 1.4 Hz), 8.77 (2H, dd, J = 4.4, 1.4 Hz).

(4-Isopropyl-5-(4-pyridyl)-4*H*-1,2,4-triazol-3-yl)methanol (117c). This compound was prepared from ethyl 4-isopropyl-5-(4-pyridyl)-4*H*-1,2,4-triazole-3-carboxylate **116c** as described in the synthesis of **76**, as light yellow solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.42 (6H, d, J = 7.0 Hz), 4.47–4.69 (1H, m), 4.74 (2H, s), 5.72 (1H, br), 7.59 (2H, dd, J = 4.4, 1.6 Hz), 8.77 (2H, dd, J = 4.4, 1.6 Hz).

(4-Benzyl-5-(4-pyridyl)-4*H*-1,2,4-triazol-3-yl)methanol (117d). This compound was prepared from ethyl 4-benzyl-5-(4-pyridyl)-4*H*-1,2,4-triazole-3-carboxylate **116d** as described in the synthesis of **76**, as white solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.65 (2H, d, J = 5.8 Hz), 5.52 (2H, s), 5.77 (1H, t, J = 5.8 Hz), 6.92–7.03 (2H, m), 7.22–7.35 (3H, m), 7.57 (2H, dd, J = 4.4, 1.4 Hz), 8.64 (2H, dd, J = 4.4, 1.4 Hz).

(4-Methoxyethyl-5-(4-pyridyl)-4*H*-1,2,4-triazol-3-yl)methanol (117e). This compound was prepared from ethyl 4-(2-methoxyethyl)-5-(4-pyridyl)-4*H*-1,2,4-triazole-3-carboxylate **116e** as described in the synthesis of **76**, as white solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.08 (3H, s), 3.56 (2H, t, J = 4.8 Hz), 4.37 (2H, t, J = 4.8 Hz), 4.71 (2H, d, J = 4.5 Hz), 5.68 (1H, t, J = 4.5 Hz), 7.74–7.78 (2H, m), 8.74–8.89 (2H, m).

(4-Benzyloxyethyl-5-(4-pyridyl)-4*H*-1,2,4-triazol-3-yl)methanol (117f). This compound was prepared from ethyl 4-(2-benzyloxyethyl)-5-(4-pyridyl)-4*H*-1,2,4-triazole-3-carboxylate **116f** as described in the synthesis of **76**, as white solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.65 (2H, t, J = 5.4 Hz), 4.32 (2H, s), 4.44 (2H, t, J = 5.4 Hz), 4.70 (2H, d, J = 5.7 Hz), 5.68 (1H, t, J = 5.4 Hz), 6.98–7.08 (2H, m), 7.22–7.33 (3H, m), 7.66–7.72 (2H, m), 8.64–8.70 (2H, m).

(4-(2,2,2-Trifluoroethyl)-5-(4-pyridyl)-4*H*-1,2,4-triazol-3-yl)methanol (117g). This compound was prepared from ethyl 4-(2,2,2-trifluoroethyl)-5-(4-pyridyl)-4*H*-1,2,4-triazole-3-carboxylate **116g** as described in the synthesis of **76**, as white solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.77 (2H, s), 5.30 (2H, q, J = 8.8 Hz), 5.93 (1H, br), 7.76 (2H, dd, J = 4.4, 1.8 Hz), 8.79 (2H, dd, J = 4.4, 1.8 Hz).

General procedure for the preparation of *N*-benzyl-3-(((4-ethyl-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)benzamide (119a). Thionyl chloride (3.67 mL) was added to (4-ethyl-5-(4-pyridyl)-4*H*-1,2,4-triazol-3-yl)methanol (**117a**, 1.02 g, 4.99 mmol) at 0 ºC. The mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure. After toluene was added, the resulting mixture was concentrated under reduced pressure. The residue was dissolved with DMF (10 mL) and 3-amino-*N*-benzylbenzamide **29i** (3.39 g) was added at room temperature. The mixture was stirred at 90 ºC for 2 h. The reaction mixture was concentrated under reduced pressure. Water was added and 8 N NaOH aqueous solution was added to adjust the pH = 9. The resulting mixture was extracted with chloroform. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered and

concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting ethyl acetate, methanol and Et_3N to afford 930 mg of **119a** (2.25 mmol, 45%) as light yellow foam. ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.20 (3H, t, J = 7.2 Hz), 4.23 (2H, q, J = 7.1 Hz), 4.45 (2H, d, J = 6.2 Hz), 4.56 (2H, d, J = 5.9 Hz), 6.47 (1H, t, J = 5.7 Hz), 6.92 (1H, dd, J = 7.9, 1.7 Hz), 7.11 (1H, d, J = 7.7 Hz), 7.17–7.21 (1H, m), 7.21–7.25 (1H, m), 7.25 (1H, t, J = 1.8 Hz), 7.30–7.34 (4H, m), 7.69–7.73 (2H, m), 8.73–8.77 (2H, m), 8.85 (1H, t, J = 6.1 Hz). ^{13}C NMR (151 MHz, DMSO-d_6) δ 15.32, 37.94, 38.91, 42.40, 111.48, 114.79, 115.09, 122.24, 126.53, 127.02, 128.12, 128.70, 134.90, 135.28, 139.72, 147.96, 150.32, 151.77, 153.59, 166.68. MS (ESI/APCI) *m/z* 413.2 $[M + H]^+$. HPLC purity 99.1%.

***N*-Benzyl-3-(((4-propyl-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)benzamide (119b).** This compound was prepared from (4-propyl-5-(4-pyridyl)-4*H*-1,2,4-triazol-3-yl)methanol **117b** and 3-amino-*N*-benzylbenzamide **29i** as described in the synthesis of **119a**, as light yellow foam. ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.72 (3H, t, J = 7.5 Hz), 1.54 (2H, dq, J = 15.1, 7.4 Hz), 4.13–4.19 (2H, m), 4.45 (2H, d, J = 6.2 Hz), 4.56 (2H, d, J = 5.9 Hz), 6.47 (1H, t, J = 5.7 Hz), 6.92 (1H, dd, J = 8.1, 1.8 Hz), 7.11 (1H, d, J = 7.7 Hz), 7.17–7.21 (1H, m), 7.21–7.25 (1H, m), 7.25 (1H, t, J = 1.8 Hz), 7.30–7.34 (4H, m), 7.68–7.72 (2H, m), 8.73–8.77 (2H, m), 8.85 (1H, t, J = 6.1 Hz). ^{13}C NMR (151 MHz, DMSO-d_6) δ 10.54, 22.90, 38.00, 42.40, 45.08, 111.47, 114.76, 115.08, 122.25, 126.54, 127.02, 128.12, 128.70, 135.11, 135.28, 139.72, 147.99, 150.32, 151.93, 153.81, 166.68. MS (ESI/APCI) *m/z* 427.2 $[M + H]^+$. HPLC purity 97.5%.

***N*-Benzyl-3-(((4-isopropyl-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)benzamide (119c).** This compound was prepared from (4-isopropyl-5-(4-pyridyl)-4*H*-1,2,4-triazol-3-yl)methanol **117c** and 3-amino-*N*-benzylbenzamide **29i** as described in the synthesis of **119a**, as light yellow foam. ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.39 (6H, d, J = 7.0 Hz), 4.46 (2H, d, J = 6.2 Hz), 4.54–4.62 (3H, m), 6.43 (1H, t, J = 5.5 Hz), 6.94 (1H, dd, J = 8.1, 1.8 Hz), 7.13 (1H, d, J = 8.1 Hz), 7.18-7.21 (1H, m), 7.22–7.25 (1H, m), 7.25 (1H, t, J = 1.8 Hz), 7.30–7.34 (4H, m), 7.55–7.59 (2H, m), 8.74–8.78 (2H, m), 8.86 (1H, t, J = 6.1 Hz). ^{13}C NMR (151 MHz, DMSO-d_6) δ 21.84, 38.98, 42.41, 48.34, 111.43, 115.00, 115.10, 124.00, 126.53, 127.02, 128.12, 128.68, 135.26, 136.43, 139.73, 148.07, 149.98, 152.29, 152.82, 166.71. MS (ESI/APCI) *m/z* 427.2 $[M + H]^+$. HPLC purity 93.9%.

***N*-Benzyl-3-(((4-benzyl-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)benzamide (119d).** This compound was prepared from (4-benzyl-5-(4-pyridyl)-4*H*-1,2,4-triazol-3-yl)methanol **117d** and 3-amino-*N*-benzylbenzamide **29i** as described in the synthesis of **119a**, as light yellow foam. ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.45 (2H, d, J = 5.9 Hz), 4.48 (2H, d, J = 5.9 Hz), 5.54 (2H, s), 6.43 (1H, t, J = 5.9 Hz), 6.83 (1H, dd, J = 8.1, 1.8 Hz), 6.93 (2H, d, J = 7.0 Hz), 7.10–7.13 (1H, m), 7.15–7.19 (2H, m), 7.20–7.27 (4H, m), 7.30–7.35 (4H, m), 7.53–7.59 (2H, m), 8.60–8.67 (2H, m), 8.83 (1H, t, J = 6.1 Hz). ^{13}C NMR (151 MHz, DMSO-d_6) δ 38.07, 42.40, 46.74, 111.68, 114.77, 115.16, 122.17, 125.96, 126.53, 127.01, 127.60, 128.11, 128.63, 128.68, 134.50, 135.23, 135.46, 139.73, 147.81, 150.10, 152.41, 154.15, 166.64. MS (ESI/APCI) *m/z* 475.2 $[M + H]^+$. HPLC purity

97.9%.

***N*-Benzyl-3-(((4-(2-methoxyethyl)-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)benzamide (119e).** This compound was prepared from (4-methoxyethyl-5-(4-pyridyl)-4*H*-1,2,4-triazol-3-yl)methanol **117e** and 3-amino-*N*-benzylbenzamide **29i** as described in the synthesis of **119a**, as light yellow foam. ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.08 (3H, s), 3.52 (2H, t, J = 5.1 Hz), 4.38 (2H, t, J = 5.1 Hz), 4.45 (2H, d, J = 5.9 Hz), 4.57 (2H, d, J = 5.5 Hz), 6.43 (1H, t, J = 5.7 Hz), 6.91 (1H, dd, J = 7.9, 2.0 Hz), 7.11 (1H, d, J = 7.7 Hz), 7.17–7.21 (1H, m), 7.21–7.26 (2H, m), 7.29–7.34 (4H, m), 7.73 (2H, d, J = 5.9 Hz), 8.74 (2H, d, J = 5.5 Hz), 8.84 (1H, t, J = 6.1 Hz). ^{13}C NMR (151 MHz, DMSO-d_6) δ 38.08, 42.40, 43.60, 58.11, 70.18, 111.58, 114.90, 115.11, 122.73, 126.53, 127.01, 128.12, 128.67, 135.11, 135.27, 139.72, 147.97, 150.13, 152.55, 154.00, 166.68. MS (ESI/APCI) *m/z* 443.2 $[M + H]^+$. HPLC purity 90.6%.

***N*-Benzyl-3-(((4-benzyloxy-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)benzamide (119f').** This compound was prepared from (4-benzyloxyethyl-5-(4-pyridyl)-4*H*-1,2,4-triazol-3-yl)methanol **117f** and 3-amino-*N*-(2-(trifluoromethyl)benzyl)benzamide **69a** as described in the synthesis of **119a**, as light yellow foam. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.60 (2H, t, J = 5.1 Hz), 4.32 (2H, s), 4.45 (2H, t, J = 4.8 Hz), 4.57 (2H, d, J = 5.4 Hz), 4.63 (2H, d, J = 5.4 Hz), 6.47 (1H, t, J = 5.7 Hz), 6.90 (1H, d, J = 7.1 Hz), 7.00–7.11 (2H, m), 7.12–7.33 (6H, m), 7.43–7.58 (2H, m), 7.60–7.75 (4H, m), 8.67–8.75

(2H, m), 8.93 (1H, t, *J* = 5.7 Hz).

3-(((4-(2-Hydroxyethyl)-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)-*N*-(2-(trifluoromethyl)benzyl)benzamide (119f). A mixture of *N*-benzyl-3-(((4-benzyloxy-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)benzamide (**119f'**, 200 mg, 0.34 mmol), 10% palladium – on activated carbon (100 mg) and ammonium formate (214 mg) in methanol (15 mL) and water (2.0 mL) was stirred at 80 ºC for 3 days. The reaction mixture was filtered. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting ethyl acetate and methanol to afford 45 mg of **119f** (0.091 mmol, 27%) as light yellow foam. ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.60 (2H, q, *J* = 5.1 Hz), 4.28 (2H, t, *J* = 5.3 Hz), 4.58 (2H, d, *J* = 5.5 Hz), 4.64 (2H, d, *J* = 5.5 Hz), 5.13 (1H, t, *J* = 5.1 Hz), 6.46 (1H, t, *J* = 5.7 Hz), 6.94 (1H, dd, *J* = 8.1, 1.8 Hz), 7.16 (1H, d, *J* = 7.7 Hz), 7.20–7.24 (1H, m), 7.28 (1H, t, *J* = 1.8 Hz), 7.47 (1H, t, *J* = 7.7 Hz), 7.52 (1H, d, *J* = 8.1 Hz), 7.65–7.68 (1H, m), 7.73 (1H, d, *J* = 7.7 Hz), 7.75–7.79 (2H, m), 8.72–8.76 (2H, m), 8.93 (1H, t, *J* = 5.9 Hz). ^{13}C NMR (151 MHz, DMSO-d_6) δ 38.16, 46.11, 59.47, 111.58, 115.12, 115.13, 122.69, 124.42 (q, *J* = 274.2 Hz), 125.57 (q, *J* = 6.0 Hz), 126.01 (q, *J* = 30.2 Hz), 127.09, 127.89, 128.76, 132.54, 134.85, 135.18, 137.73, 148.08, 150.12, 152.45, 154.11, 167.02, 1 aliphatic carbon was not assigned. MS (ESI/APCI) *m/z* 497.1 $[M + H]^+$. HPLC purity 83.8%.

3-Aminoisonicotinic acid (121). Bromine (33.7 g) was added to a solution of 1*H*-pyrrolo[3,4-*c*]pyridine-1,3(2*H*)-dione (**120**, 30.0 g, 203 mmol) in 10% NaOH aqueous solution

(500 mL) at 0 ºC for 15 min. The mixture was stirred at 85 ºC for 1 h. The reaction mixture was cooled to room temperature and then treated with acetic acid to adjust the pH = 6. The resulting mixture was cooled to 0 ºC and then collected. The solids residue was washed with water and dried to afford 17.4 g of **121** (126 mmol, 62%) as light brown solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.46 (1H, d, J = 5.1 Hz), 7.72 (1H, d, J = 5.1 Hz), 8.19 (1 H, s), CO_2H and NH_2 were not assigned.

3-Hydroxyisonicotinic acid (122). Conc. H_2SO_4 (10 mL) was added to a suspension of 3-aminoisonicotinic acid (**121**, 13.8 g, 100 mmol) in water (200 mL) at room temperature. The mixture was heated to 60 ºC to dissolve. After the solution was cooled to 8 ºC, an aqueous solution of sodium nitrite (7.59 g, 60 mL) was added at 8 ºC for 20 min. The resulting mixture was stirred at 85 ºC for 30 min. After acetic acid (10 mL) was added, 28% ammonium aqueous solution (25 mL) was added at 0 ºC. The resulting mixture was stirred at 0 ºC for a while. The solid residue was collected and dried to afford 10.8 g of **122** (77.6 mmol, 78%) as light brown solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.81 (1H, d, J = 5.3 Hz), 8.08 (1H, d, J = 5.3 Hz), 8.36 (1H, s), CO_2H and OH were not assigned.

Ethyl 3-hydroxyisonicotinate (123). A suspension of 3-hydroxyisonicotinic acid (**122**, 9.73 g, 69.9 mmol) and conc. H_2SO_4 (20 mL) in ethanol (300 mL)was stirred at 90 ºC for 21 h. The reaction mixture was concentrated under reduced pressure. The residue was dissolved with water and neutralized with sodium carbonate aqueous solution. The resulting mixture was extracted with chloroform (three times). The organic layer was dried over anhydrous Na_2SO_4, filtered and

concentrated under reduced pressure to afford 10.1 g of **123** (60.4 mmol, 86%) as light brown solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 (3H, t, *J* = 7.2 Hz), 4.46 (2H, q, *J* = 7.2 Hz), 7.62 (1H, dd, *J* = 5.1, 0.6 Hz), 8.22 (1H, d, *J* = 5.1 Hz), 8.50 (1H, s), 10.35 (1H, s).

Ethyl 3-(2-(((benzyloxy)carbonyl)amino)ethoxy)isonicotinate (124). Diisopropyl azodicarboxylate (8.33 g) was added to a solution of ethyl 3-hydroxyisonicotinate (**123**, 5.74 g, 34.3 mmol), 2-(carbobenzoxyamino)-1-ethanol (8.04 g) and triphenylphosphine (10.8 g) in THF (200 mL) at 0 ºC for 10 min. The mixture was stirred at room temperature for 22 h. The reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting hexane and ethyl acetate to afford crude **124** including diisopropyl hydrazine-1,2-dicarboxylate as colorless oil. This product was used withouth further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37 (3H, t, *J* = 7.2 Hz), 3.67 (2H, q, *J* = 5.3 Hz), 4.26 (2H, t, *J* = 4.9 Hz), 4.38 (2H, q, *J* = 7.2 Hz), 5.12 (2H, s), 5.70 (1H, s), 7.29–7.38 (5H, m), 7.61 (1H, d, *J* = 4.7 Hz), 8.36 (1H, d, *J* = 4.9 Hz), 8.43 (1H, s).

3,4-Dihydropyrido[4,3-*f*][1,4]oxazepin-5(2*H*)-one (125). 10% Palladium – on activated carbon (1.18 g) was added to a solution of crude ethyl 3-(2-(((benzyloxy)carbonyl)amino)ethoxy)isonicotinate (**124**, all amount) in ethanol (160 mL) at room temperature. The mixture was stirred at room temperature for 12 h under hydrogen (balloon). The reaction mixture was filtered with Celite and washed with ethanol. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting

ethyl acetate and methanol and by recrystallization from ethyl acetate and IPE to afford 3.30 g of **125** (20.1 mmol, 59%) as white solids. mp 176 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.60 (2H, q, *J* = 4.5 Hz), 4.50 (2H, t, *J* = 4.2 Hz), 7.04 (1H, s), 7.89 (1H, d, *J* = 5.3 Hz), 8.35 (1H, d, *J* = 5.1 Hz), 8.45 (1H, s). Anal. Calcd. for $C_8H_8N_2O_2$: C, 58.53; H, 4.91; N, 17.06. Found: C, 58.44; H, 4.85; N, 16.96.

3,4-Dihydropyrido[4,3-*f*][1,4]oxazepine-5(2*H*)-thione (126). A mixture of 3,4-dihydropyrido[4,3-*f*][1,4]oxazepin-5(2*H*)-one (**125**, 1.54 g, 9.38 mmol) and Lawesson's reagents (5.69 g) in toluene (70 mL) was stirred at 90 °C for 2.5 h. The reaction mixture was treated with saturated $NaHCO_3$ aqueous solution. The mixture was extracted with chloroform. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting ethyl acetate and methanol and by recrystallization from ethyl acetate to afford 1.37 g of **126** (7.60 mmol, 81%) as yellow solids. mp 262 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.62 (2H, q, *J* = 5.5 Hz), 4.53 (2H, t, *J* = 5.3 Hz), 7.90 (1H, d, *J* = 5.1 Hz), 8.41 (1H, s), 8.43 (1H, d, *J* = 5.1 Hz), 8.55 (1H, s). Anal. Calcd. for $C_8H_8N_2OS$: C, 53.31; H, 4.47; N, 15.54. Found: C, 53.25; H, 4.43; N, 15.44.

3-(2,2,2-Trifluoroacetamido)-*N*-(2-(trifluoromethyl)benzyl)benzamide (127a). This compound was prepared from 3-amino-*N*-(2-(trifluoromethyl)benzyl)benzamide **69a** as described in the synthesis of **2a**, as white solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.67 (2H, d, *J* = 5.4 Hz), 7.46–7.87 (8H, m), 8.21 (1H, d, *J* = 1.5 Hz), 9.18 (1H, t, *J* = 5.7 Hz).

***N*-(2,6-Difluorobenzyl)-3-(2,2,2-trifluoroacetamido)benzamide (127b).** This compound was

prepared from *N*-2,6-difluorobenzyl-3-aminobenzamide **69b** as described in the synthesis of **2a**, as white solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.52 (2H, d, J = 4.8 Hz), 7.09 (2H, t, J = 8.0 Hz), 7.38–7.51 (2H, m), 7.68 (1H, d, J = 6.9 Hz), 7.78–7.81 (1H, m), 8.10 (1H, s), 8.93 (1 H, t-like), 11.39 (1H, s).

Benzyl 2-(2,2,2-trifluoro-*N*-(3-((2-(trifluoromethyl)benzyl)carbamoyl)phenyl)acetamido)acetate (128a). This compound was prepared from 3-(2,2,2-trifluoroacetamido)-*N*-(2-(trifluoromethyl)benzyl)benzamide **127a** as described in the synthesis of **3a**, as oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.44 (2H, s), 4.82 (2H, d, J = 5.7 Hz), 5.19 (2H, s), 6.46 (1H, t, J = 5.4 Hz), 7.26–7.81 (13H, m).

Benzyl 2-(*N*-(3-((2,6-difluorobenzyl)carbamoyl)phenyl)-2,2,2-trifluoroacetamido)acetate (128b). This compound was prepared from *N*-(2,6-difluorobenzyl)-3-(2,2,2-trifluoroacetamido)benzamide **127b** as described in the synthesis of **3a**, as white solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.52 (2H, d, J = 5.1 Hz), 4.60 (2H, s), 5.18 (2H, s), 7.09 (2H, t, J = 8.0 Hz), 7.35–7.42 (6H, m), 7.56 (2H, s), 7.92 (2H, s), 8.98 (1H, t-like).

General procedure for the preparation of 2-(2,2,2-trifluoro-*N*-(3-((2-(trifluoromethyl)benzyl)carbamoyl)phenyl)acetamido)acetic acid (129a). A mixture of benzyl 2-(2,2,2-trifluoro-*N*-(3-((2-(trifluoromethyl)benzyl)carbamoyl)phenyl)acetamido)acetate (**128a**, all amount, 26.5 mmol) and 10% palladium – on activated carbon (1.20 g) in methanol (200 mL) was

stirred at room temperature for 14 h under hydrogen (balloon). The reaction mixture was filtered and the filtrate was concentrated under reduced pressure to afford 12.0 g of **129a** (26.7 mmol, quantitative yield) as white foam. This product was used without further purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.43 (2H, s), 4.47 (2H, d, J = 5.4 Hz), 7.46–7.76 (6H, m), 8.00 (2H, s), 9.23 (1H, t, J = 5.5 Hz), around 13.0 (1H, br).

2-(*N*-(3-((2,6-Difluorobenzyl)carbamoyl)phenyl)-2,2,2-trifluoroacetamido)acetic acid (129b). This compound was prepared from benzyl 2-(*N*-(3-((2,6-difluorobenzyl)carbamoyl)phenyl)-2,2,2-trifluoroacetamido)acetate **128b** as described in the synthesis of **129a**, as white solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.40 (2H, s), 4.53 (2H, d, J = 4.8 Hz), 7.10 (1H, t, J = 7.9 Hz), 7.37–7.59 (3H, m), 7.92 (2H, s), 8.99 (1H, t, J = 4.9 Hz), CO_2H was not assigned.

General procedure for the preparation of *tert*-butyl 2-(2-(2,2,2-trifluoro-*N*-(3-((2-(trifluoromethyl)benzyl)carbamoyl)phenyl)acetamido)acetyl)hydrazinecarboxylate (130a). A mixture of 2-(2,2,2-trifluoro-*N*-(3-((2-(trifluoromethyl)benzyl)carbamoyl)phenyl)acetamido)acetic acid (**129a**, 4.49 g, 10.0 mmol), Boc-hydrazine (1.59 g), WSC•HCl (2.28 g) and HOBt•H_2O (0.15 g) in DMF (30 mL) was stirred at room temperature for 44 h. Ethyl acetate was added to the reaction mixture and then the mixture was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by

recrystallization from ethyl acetate to afford 3.36 g of **130a** as white solids. The mother liquor was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting hexane and ethyl acetate to afford 1.65 g of **130a** as white solids. Total: 5.01 g (8.91 mmol, 89% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (9H, s), 4.32–4.57 (2H, m), 4.89 (2H, d, J = 6.2 Hz), 6.48 (1H, s), 7.31–7.40 (1H, m), 7.45–7.68 (6H, m), 7.80–8.11 (3H, m).

***tert*-Butyl**

2-(2-(*N*-(3-((2,6-difluorobenzyl)carbamoyl)phenyl)-2,2,2-trifluoroacetamido)acetyl)hydrazinecarboxylate (130b). This compound was prepared from 2-(*N*-(3-((2,6-difluorobenzyl)carbamoyl)phenyl)-2,2,2-trifluoroacetamido)acetic acid **129b** as described in the synthesis of **130a**, as crude white solids. This product was used without further purification.

General procedure for the preparation of *tert*-butyl 2-(2-((3-((2-(trifluoromethyl)benzyl)carbamoyl)phenyl)amino)acetyl)hydrazinecarboxylate (131a). 8 N NaOH aqueous solution (5.6 mL) was added to a solution of *tert*-butyl 2-(2-(2,2,2-trifluoro-*N*-(3-((2-(trifluoromethyl)benzyl)carbamoyl)phenyl)acetamido)acetyl)hydrazine carboxylate (**130a**, 5.00 g, 8.89 mmol) in methanol (50 mL) at room temperature. The mixture was stirred at room temperature for 2.5 h. The reaction mixture was concentrated under reduced pressure. Ethyl acetate was added to the residue and then the mixture was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure.

The residue was purified by column chromatography on silica gel eluting hexane and ethyl acetate to afford 3.73 g of **131a** (8.00 mmol, 90%) as white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (9H, s), 3.96 (2H, d, *J* = 5.1 Hz), 4.42 (1H, t, *J* = 5.1 Hz), 4.88 (2H, d, *J* = 6.2 Hz), 6.34 (1H, s), 6.84 (1H, dd, *J* = 8.0, 1.4 Hz), 7.15 (1H, t, *J* = 1.8 Hz), 7.25–7.36 (2H, m), 7.41 (1H, d, *J* = 7.9 Hz), 7.45–7.55 (2H, m), 7.63 (1H, d, *J* = 7.7 Hz), 7.70 (1H, s), 8.18 (1H, d, *J* = 2.4 Hz).

***tert*-Butyl 2-(2-((3-((2,6-difluorobenzyl)carbamoyl)phenyl)amino)acetyl)hydrazinecarboxylate (131b).** This compound was prepared from *tert*-butyl 2-(2-(*N*-(3-((2,6-difluorobenzyl)carbamoyl)phenyl)-2,2,2-trifluoroacetamido)acetyl)hydrazinecarboxylate **130b** as described in the synthesis of **131a**, as white solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 (9H, s), 3.92 (2H, s), 4.45 (1H, br), 4.72 (2H, d, *J* = 5.7 Hz), 6.41 (1H, s), 6.77 (1H, dt, *J* = 6.6, 2.4 Hz), 6.88 (2H, t, *J* = 7.9 Hz), 7.07 (1H, s), 7.17–7.28 (4H, m), 8.21 (1H, d, *J* = 2.6 Hz).

General procedure for the preparation of 3-((2-hydrazinyl-2-oxoethyl)amino)-*N*-(2-(trifluoromethyl)benzyl)benzamide (132a). A solution of 4 N HCl in ethyl acetate (12 mL) was added to a solution of *tert*-butyl 2-(2-((3-((2-(trifluoromethyl)benzyl)carbamoyl)phenyl)amino)acetyl)hydrazinecarboxylate (**131a**, 3.72 g, 7.98 mmol) in ethyl aceate (12 mL) and ethanol (24 mL) at room temperature. The mixture was stirred at room temperature for 24 h. The reaction mixture was concentrated under reduced pressure. Ethyl acetate was added to the residue and then the mixture was washed with saturated sodium hydrogen carbonate aqueous solution, water and brine. The organic layer was dried over

anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by recrystallization from ethyl acetate and IPE to afford 2.54 g of **132a** (6.93 mmol, 87%) as white solids. mp 163 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.68 (2H, d, J = 6.0 Hz), 4.23 (2H, d, J = 3.4 Hz), 4.63 (2H, d, J = 5.7 Hz), 6.06 (1H, t, J = 5.9 Hz), 6.71–6.77 (1H, m), 7.08 (1H, s), 7.13 (1H, d, J = 7.8 Hz), 7.19 (1H, t, J = 7.6 Hz), 7.43–7.52 (2H, m), 7.65 (1H, t, J = 7.4 Hz), 7.73 (1H, d, J = 7.7 Hz), 8.92 (1H, t, J = 5.7 Hz), 9.09 (1H, s). Anal. Calcd. for $C_{17}H_{17}N_4O_2F_3$: C, 55.74; H, 4.68; N, 15.29. Found: C, 55.87; H, 4.78; N, 14.90.

***N*-(2,6-Difluorobenzyl)-3-((2-hydrazinyl-2-oxoethyl)amino)benzamide (132b).** This compound was prepared from *tert*-butyl 2-(2-((3-((2,6-difluorobenzyl)carbamoyl)phenyl)amino)acetyl)hydrazinecarboxylate **131b** as described in the synthesis of **132a**, as white solids. mp 165 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.66 (2H, d, J = 3.2 Hz), 4.48 (2H, d, J = 5.1 Hz), 4.59 (2H, s), 5.99 (1H, s), 6.68 (1H, dd, J = 7.9, 1.3 Hz), 6.98–7.15 (5H, m), 7.32–7.44 (1H, m), 8.66 (1H, t, J = 5.0 Hz), 9.14 (1H, s). Anal. Calcd. for $C_{16}H_{16}F_2N_4O_2$•0.2H_2O: C, 56.87; H, 4.89; N, 16.58. Found: C, 56.88; H, 4.76; N, 16.38.

General procedure for the preparation of 3-((5,6-dihydropyrido[4,3-*f*][1,2,4]triazolo[4,3-*d*][1,4]oxazepin-3-ylmethyl)amino)-*N*-(2-(trifluoromethyl)benzyl)benzamide (133a). A solution of sodium ethoxide in ethanol (20%, 668 mg) was added to a mixture of 3,4-dihydropyrido[4,3-*f*][1,4]oxazepine-5(2*H*)-thione (**126**, 354 mg, 1.96 mmol) and 3-((2-hydrazinyl-2-oxoethyl)amino)-*N*-(2-(trifluoromethyl)benzyl)benzamide (**132a**, 1.08

g) in toluene (40 mL) and ethanol (40 mL) at room temperature. The mixture was stirred at 100 °C for 24 h. The reaction mixture was treated with acetic acid and then the mixture was concentrated under reduced pressure. Ethyl acetate was added to the residue and then the mixture was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on basic silica gel eluting ethyl acetate and methanol, and by recrystallization from methanol and ethyl acetate to afford 66 mg of **133a** (0.13 mmol, 7%) as white solids. mp 144–148 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.51–4.54 (2H, m), 4.54–4.59 (4H, m), 4.64 (2H, d, J = 5.5 Hz), 6.45 (1H, t, J = 5.7 Hz), 6.92 (1H, dd, J = 7.9, 1.7 Hz), 7.14–7.18 (1H, m), 7.20–7.24 (1H, m), 7.27 (1H, t, J = 1.8 Hz), 7.47 (1H, t, J = 7.7 Hz), 7.51 (1H, d, J = 7.7 Hz), 7.63–7.68 (1H, m), 7.73 (1H, d, J = 7.7 Hz), 8.27–8.29 (1H, m), 8.30–8.33 (1H, m), 8.43 (1H, s), 8.93 (1H, t, J = 5.9 Hz). ^{13}C NMR (151 MHz, DMSO-d_6) δ 37.85, 38.99, 47.12, 68.35, 111.60, 115.13, 115.23, 120.74, 121.37, 124.42 (q, J = 274.2 Hz), 125.60 (q, J = 5.5 Hz), 125.90 (q, J = 31.0 Hz), 127.10, 127.89, 128.76, 132.53, 134.86, 137.72 (q, J = 1.1 Hz), 142.82, 143.44, 147.95, 149.93, 150.67, 154.22, 167.00. Anal. Calcd. for $C_{25}H_{21}F_3N_6O_2$•1.5H_2O: C, 57.58; H, 4.64; N, 16.12. Found: C, 57.66; H, 4.41; N, 16.07. MS (ESI/APCI) *m/z* 495.1 $[M + H]^+$. HPLC purity 98.1%.

***N*-(2,6-difluorobenzyl)-3-((5,6-dihydropyrido(4,3-*f*)(1,2,4)triazolo(4,3-*d*)(1,4)oxazepin-3-ylmethyl)amino)benzamide (133b).** This compound was prepared from 3,4-dihydropyrido[4,3-*f*][1,4]oxazepine-5(2*H*)-thione **126** and

N-(2,6-difluorobenzyl)-3-((2-hydrazinyl-2-oxoethyl)amino)benzamide **132b** as described in the synthesis of **133a**, as light yellow solids. mp 200 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.48–4.55 (8H, m), 6.37 (1H, t, J = 5.7 Hz), 6.87 (1H, dd, J = 8.1, 1.8 Hz), 7.04–7.08 (3H, m), 7.13–7.18 (2H, m), 7.35–7.40 (1H, m), 8.26–8.28 (1H, m), 8.29–8.33 (1H, m), 8.43 (1H, s), 8.66 (1H, t, J = 5.1 Hz). ^{13}C NMR (151 MHz, DMSO-d_6) δ 31.28 (t, J = 3.6 Hz), 37.85, 47.10, 68.34, 111.30 (dd, J = 20.5, 5.0 Hz), 111.61, 114.28 (t, J = 18.8 Hz), 114.88, 115.35, 120.74, 121.35, 128.56, 129.52 (t, J = 10.5 Hz), 134.94, 142.80, 143.42, 147.81, 149.91, 150.65, 154.20, 161.07 (dd, J = 248.2, 8.3 Hz), 166.43. Anal. Calcd. for $C_{24}H_{20}F_2N_6O_2$•0.5H_2O: C, 61.14; H, 4.49; N, 17.83. Found: C, 61.47; H, 4.51; N, 17.51. MS (ESI/APCI) *m/z* 463.1 $[M + H]^+$. HPLC purity 95.2%.

Pyrimidine-4-carboxylic acid. Selenium dioxide (89.4 g) was added to a solution of 4-methylpyrimidine (**134**, 50.0 g, 531 mmol) in pyridine (500 mL) at room temperature. The mixture was stirred at 60 °C for 2 h and at 85 °C for 4 h. The reaction mixture was stirred at room temperature for 16 h and then filtered. The filtrate was concentrated under reduced pressure. Water (150 mL) was added to the residue. The solids residue was collected, washed with water and dried to afford 24.8 g of pyrimidine-4-carboxylic acid (19%, 200 mmol) as brown solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.03 (1H, dd, J = 5.0, 1.4 Hz), 9.08 (1H, d, J = 5.0 Hz), 9.39 (1H, d, J = 1.4 Hz), CO_2H was not assigned.

Methyl pyrimidine-4-carboxylate (135). Conc. H_2SO_4 (1.2 mL) was added to a mixture of pyrimidine-4-carboxylic acid (24.8 g, 200 mmol) in methanol (240 mL) at room temperature. The

mixture was stirred at reflux for 16 h. The reaction mixture was treated with Et_3N (10 mL) at 0 °C. The resulting mixture was concentrated under reduced pressure. The resulting mixture was concentrated under reduced pressure. Ethyl acetate was added to the residue and then the mixture was washed with brine. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting ethyl acetate to afford 19.9 g of **135** (144 mmol, 72%) as light brown solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.05 (3H, s), 8.03 (1H, dd, J = 3.4, 1.0 Hz), 9.00 (1H, d, J = 3.4 Hz), 9.41 (1H, d, J = 1.0 Hz).

Pyrimidine-4-carbohydrazide (136). A mixture of methyl pyrimidine-4-carboxylate (**135**, 31.3 g, 227 mmol) and hydrazine hydrate (20 mL) in ethanol (300 mL) was stirred at reflux for 16 h. The reaction mixture was concentrated under reduced pressure. The solid residue was washed with ethanol and diethyl ether to afford 23.8 g of **136** (172 mmol, 76%) as light yellow solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.73 (2H, br), 7.98 (1H, dd, J = 5.2, 1.6 Hz), 9.05 (1H, d, J = 5.2 Hz), 9.29 (1H, d, J = 1.6 Hz), 10.30 (1H, br).

Ethyl 4-methyl-5-(pyrimidin-4-yl)-4*H*-1,2,4-triazole-3-carboxylate (137a). This compound was prepared from *N*-methylaminothiooxoacetate and pyrimidine-4-carbohydrazide **136** as described in the synthesis of **111**, as light yellow solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.37 (3H, t, J = 6.3 Hz), 4.29 (3H, s), 4.43 (2H, d, J = 6.3 Hz), 8.27 (1H, d, J = 5.1 Hz), 9.05 (1H, d, J = 5.1 Hz), 9.41 (1H, s).

Ethyl 4-propyl-5-(pyrimidin-4-yl)-4*H*-1,2,4-triazole-3-carboxylate (137b). This compound was prepared from ethyl 2-(propylamino)-2-thioxoacetate and pyrimidine-4-carbohydrazide **136** as described in the synthesis of **111**, as light yellow solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (3H, t, *J* = 7.2 Hz), 1.48 (3H, t, *J* = 7.2 Hz), 1.68–1.88 (2H, m), 4.34–4.14 (2H, m), 4.53 (2H, q, *J* = 7.2 Hz), 8.27 (1H, d, *J* = 5.1 Hz), 9.05 (1H, d, *J* = 5.1 Hz), 9.41 (1H, s).

(4-Methyl-5-(pyrimidin-4-yl)-4*H*-1,2,4-triazol-3-yl)methanol (138a). This compound was prepared from ethyl 4-methyl-5-(pyrimidin-4-yl)-4*H*-1,2,4-triazole-3-carboxylate **137a** as described in the synthesis of **112**, as white solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.07 (3H, s), 4.71 (2H, d, *J* = 5.7 Hz), 5.68 (1H, t, *J* = 4.8 Hz), 8.19 (1H, d, *J* = 3.9 Hz), 8.98 (1H, d, *J* = 5.4 Hz), 9.35 (1H, s).

(4-Propyl-5-(pyrimidin-4-yl)-4*H*-1,2,4-triazol-3-yl)methanol (138b). This compound was prepared from ethyl 4-propyl-5-(pyrimidin-4-yl)-4*H*-1,2,4-triazole-3-carboxylate **137b** as described in the synthesis of **112**, as light yellow solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.72 (3H, t, *J* = 7.2 Hz), 1.48–1.80 (2H, m), 4.12–4.22 (2H, m), 4.73 (2H, s), 5.71 (1H, br), 8.18 (1H, d, *J* = 3.9 Hz), 8.97 (1H, d, *J* = 5.4 Hz), 9.37 (1H, s).

3-(((4-Methyl-5-(pyrimidin-4-yl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)-*N*-(2-(trifluoromethyl)benzyl)benzamide (139a). This compound was prepared from (4-methyl-5-(pyrimidin-4-yl)-4*H*-1,2,4-triazol-3-yl)methanol **138a** and 3-amino-*N*-(2-(trifluoromethyl)benzyl)benzamide **69a** as described in the synthesis of **119a**, as white solids. ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.08 (3H, s), 4.59 (2H, d, *J* = 5.5 Hz), 4.64 (2H, d, *J* = 5.9

Hz), 6.52 (1H, t, *J* = 5.7 Hz), 6.94 (1H, dd, *J* = 8.1, 1.8 Hz), 7.15 (1H, d, *J* = 8.1 Hz), 7.20–7.23 (1H, m), 7.28 (1H, t, *J* = 1.8 Hz), 7.47 (1H, t, *J* = 7.5 Hz), 7.51 (1H, d, *J* = 7.7 Hz), 7.64–7.68 (1H, m), 7.73 (1H, d, *J* = 8.1 Hz), 8.18 (1H, dd, *J* = 5.1, 1.5 Hz), 8.93 (1H, t, *J* = 5.9 Hz), 8.97 (1H, d, *J* = 5.5 Hz), 9.34 (1H, d, *J* = 1.1 Hz). ^{13}C NMR (151 MHz, DMSO-d_6) δ 32.47, 37.76, 38.98, 111.55, 115.06, 115.18, 119.19, 124.43 (q, *J* = 274.2 Hz), 125.59 (q, *J* = 5.5 Hz), 125.92 (q, *J* = 29.9 Hz), 127.10, 127.90, 128.77, 132.54, 134.89, 137.73 (q, *J* = 1.1 Hz), 147.99, 150.46, 154.25, 155.43, 158.16, 158.31, 167.02. MS (ESI/APCI) *m/z* 467.2 $[M + H]^+$. HPLC purity 98.9%.

_N_-(2,6-Difluorobenzyl)-3-(((4-methyl-5-(pyrimidin-4-yl)-4_H_-1,2,4-triazol-3-yl)methyl)amino)benzamide (139b). This compound was prepared from 3-(((4-methyl-5-(pyrimidin-4-yl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)benzoic acid **141** and 2,6-difluorobenzylamine as described in the synthesis of **115h**, as white solids. ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.06 (3H, s), 4.49 (2H, d, *J* = 5.1 Hz), 4.56 (2H, d, *J* = 5.9 Hz), 6.44 (1H, t, *J* = 5.7 Hz), 6.89 (1H, dd, *J* = 8.1, 1.8 Hz), 7.04–7.08 (3H, m), 7.13–7.16 (1H, m), 7.17–7.19 (1H, m), 7.35–7.40 (1H, m), 8.17 (1H, dd, *J* = 5.1, 1.5 Hz), 8.67 (1H, t, *J* = 5.1 Hz), 8.97 (1H, d, *J* = 5.1 Hz), 9.33 (1H, d, *J* = 1.1 Hz). ^{13}C NMR (151 MHz, DMSO-d_6) δ 31.29 (t, *J* = 3.6 Hz), 32.45, 37.76, 111.31 (dd, *J* = 21.0, 5.5 Hz), 111.57, 114.29 (t, *J* = 18.8 Hz), 114.81, 115.30, 119.18, 128.57, 129.52 (t, *J* = 10.5 Hz), 134.97, 147.85, 150.43, 154.24, 155.41, 158.15, 158.29, 161.08 (dd, *J* = 247.7, 8.3 Hz), 166.46. MS (ESI/APCI) *m/z* 436.1 $[M + H]^+$. HPLC purity 95.8%.

3-(((4-Propyl-5-(pyrimidin-4-yl)-4_H_-1,2,4-triazol-3-yl)methyl)amino)-_N_-(2-(trifluoromethyl)ben

zyl)benzamide (139c). This compound was prepared from (4-propyl-5-(pyrimidin-4-yl)-4*H*-1,2,4-triazol-3-yl)methanol **138b** and 3-amino-*N*-(2-(trifluoromethyl)benzyl)benzamide **69a** as described in the synthesis of **119a**, as white solids. ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.85 (3H, t, *J* = 7.3 Hz), 1.72 (2H, dq, *J* = 15.1, 7.4 Hz), 4.52–4.56 (2H, m), 4.61 (2H, d, *J* = 5.5 Hz), 4.64 (2H, d, *J* = 5.5 Hz), 6.55 (1H, t, *J* = 5.5 Hz), 6.94 (1H, dd, *J* = 8.1, 1.8 Hz), 7.15 (1H, d, *J* = 7.7 Hz), 7.20–7.23 (1H, m), 7.27–7.29 (1H, m), 7.46–7.48 (1H, m), 7.51 (1H, d, *J* = 8.1 Hz), 7.65–7.67 (1H, m), 7.73 (1H, d, *J* = 7.7 Hz), 8.20 (1H, dd, *J* = 5.1, 1.5 Hz), 8.93 (1H, t, *J* = 5.9 Hz), 8.97 (1H, d, *J* = 5.5 Hz), 9.33 (1H, d, *J* = 1.5 Hz). ^{13}C NMR (151 MHz, DMSO-d_6) δ 10.67, 23.37, 37.83, 38.97, 46.12, 111.46, 114.99, 115.13, 119.08, 124.42 (q, *J* = 273.1 Hz), 125.59 (q, *J* = 5.5 Hz), 125.91 (q, *J* = 29.9 Hz), 127.09, 127.90, 128.79, 132.53, 134.90, 137.73, 147.97, 150.02, 154.17, 155.13, 158.28, 158.34, 167.00. MS (ESI/APCI) *m/z* 496.1 $[M + H]^+$. HPLC purity 97.1%.

***N*-(2,6-Difluorobenzyl)-3-(((4-propyl-5-(pyrimidin-4-yl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)be nzamide (139d).** This compound was prepared from (4-propyl-5-(pyrimidin-4-yl)-4*H*-1,2,4-triazol-3-yl)methanol **138b** and *N*-2,6-difluorobenzyl-3-aminobenzamide **69b** as described in the synthesis of **119a**, as white solids. ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.84 (3H, t, *J* = 7.3 Hz), 1.71 (2H, dq, *J* = 15.1, 7.4 Hz), 4.48 (2H, d, *J* = 5.1 Hz), 4.51–4.54 (2H, m), 4.58 (2H, d, *J* = 5.5 Hz), 6.48 (1H, t, *J* = 5.7 Hz), 6.89 (1H, dd, *J* = 8.1, 1.8 Hz), 7.03–7.09 (3H, m), 7.13–7.17 (1H, m), 7.18 (1H, s), 7.35–7.40 (1H, m), 8.20 (1H, dd,

J = 5.3, 1.3 Hz), 8.67 (1H, t, *J* = 5.0 Hz), 8.97 (1H, d, *J* = 5.5 Hz), 9.33 (1H, d, *J* = 1.1 Hz). ^{13}C NMR (151 MHz, DMSO-d_6) δ 10.66, 23.36, 31.29 (t, *J* = 3.6 Hz), 37.84, 46.11, 111.31 (dd, *J* = 20.5, 5.0 Hz), 111.50, 114.28 (t, *J* = 19.1 Hz), 114.75, 115.25, 119.07, 128.60, 129.52 (t, *J* = 10.2 Hz), 134.98, 147.84, 150.00, 154.18, 155.14, 158.28, 158.33, 161.07 (dd, *J* = 248.2, 8.3 Hz), 166.44. MS (ESI/APCI) *m/z* 464.1 $[M + H]^+$. HPLC purity 98.0%.

Ethyl 3-(((4-methyl-5-(pyrimidin-4-yl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)benzoate (140). This compound was prepared from (4-methyl-5-(pyrimidin-4-yl)-4*H*-1,2,4-triazol-3-yl)methanol **138a** and ethyl 3-aminobenzoate **13** as described in the synthesis of **119a**, as white solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.07 (3H, s), 4.52 (2H, d, *J* = 5.4 Hz), 6.20 (1H, t-like), 6.73 (1H, d, *J* = 6.9 Hz), 7.02 (1H, t, *J* = 7.7 Hz), 7.13 (1H, d, *J* = 7.5 Hz), 7.30 (1H, s), 8.16 (1H, d, *J* = 5.1 Hz), 8.96 (1H, d, *J* = 5.3 Hz), 9.32 (1H, s), CO_2H was not assigned.

3-(((4-Methyl-5-(pyrimidin-4-yl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)benzoic acid (141). This compound was prepared from ethyl 3-(((4-methyl-5-(pyrimidin-4-yl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)benzoate **140** as described in the synthesis of **34**, as light yellow solids. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.07 (3H, s), 4.52 (2H, d, *J* = 5.4 Hz), 6.20 (1H, brs), 6.73 (1H, d, *J* = 6.9 Hz), 7.12 (1H, t, *J* = 7.8 Hz), 7.13 (1H, d, *J* = 7.5 Hz), 7.30 (1H, s), 8.12–8.18 (1H, m), 8.95 (1H, d, *J* = 5.1 Hz), 9.32 (1H, s).

Biological Method

GRK kinase assays

GRK2 enzyme was purchased from Carnabiosciences (Kobe, Japan). GRK3, GRK5, GRK6, and GRK7 enzymes were purchased from Thermo Fischer (Massachusetts, USA). GRK1 enzyme was purchased from Sigma-Aldrich (Missouri, USA). GRK2, GRK3, GRK5, GRK6, and GRK7 kinase assays were performed by LANCE Ultra assay system (Perkin Elmer, Massachusetts, USA). GRK family kinases were incubated with test compounds for 60 min at room temperature in assay buffer (25 mM HEPES, 10 mM $MgCl_2$, 2 mM DTT, 0.01 % Tween-20, and 1 mM EGTA). Then, 8 or 800 μM ATP and 50 nM Ulight TopoIIα as an artificial substrate (Perkin Elmer) were added and incubated for 10 min at room temperature. 0.12 nM Eu anti-TopoIIα (Perkin Elmer), 0.01 % BSA, and 11 mM EDTA diluted in LANCE assay buffer (Perkin Elmer) were added to the each well. After 60 min incubation at room temperature, TR-FRET signal was measured by EnVision plate reader (Perkin Elmer). IC_{50} values of GRK2 kinase assay were calculated by nonlinear least squares regression method using XLfit software (IDBS, Guildford, UK).

GRK1 kinase assay was performed by LanthaScreen Eu kinase binding assay (Thermo Fischer). GST tagged GRK1 (Sigma-Aldrich) was incubated with Tb-labeled anti-GST antibody (CisBio, Massachusetts, USA) and test compound in assay buffer (25 mM HEPES, 10 mM $MgCl_2$, 2 mM DTT, 0.01 % Tween-20, and 1 mM EGTA). After 60 min incubation at room temperature, 2 nM Kinase Tracer 236 (ThermoFischer) was added and incubated for 10 min at room temperature.

TR-FRET signal was measured by EnVision plate reader (PerkinElmer).

ROCK2 assay

ROCK2 kinase assay was performed by KinEASE assay system in 384-well plates at room temperature. ROCK2 (Carnabiosciences) was incubated with 1 μM STK substrate 2-biotin (CisBio), 15 μM ATP, and test compounds for 30 min at room temperature in assay buffer (25 mM HEPES, 5 mM $MgCl_2$, 1 mM DTT, 0.01 % Tween-20, 0.01 % BSA, and 0.1 mM orthovanadate). Streptavidin-XL665 (CisBio) and STK 2 Antibody-Cryptate (CisBio) diluted in HTRF Detection buffer (CisBio) were added to the each well. After 60 min incubation, TR-FRET signal was measured by EnVision plate reader (PerkinElmer). IC_{50} values of ROCK2 kinase assay were calculated by nonlinear least squares regression method using XLfit software (IDBS, Guildford, UK).

PKCα assay

PKCα-dependent myelin basic protein (MBP) phosphorylation was measured by incubating 1 μg MBP (upstate), 20-300 ng human PKCα (or 3 ng PKCαactive, Upstate # 566-56701) and Lipid activator (5 μL, upstate #20-133) in 25 mM Hepes (50 μL), pH7.5, 0.5 μM ATP, 0.1 μCi [γ-^{32}P]ATP, 10 mM $MgCl_2$, 1 mM DTT at 30 °C for or 60 min. Reactions were stopped by the addition of an equal volume of 20% TCA (trichloroacetic acid) and the TCA precipitate were harvested to GF/C filter (Wattman). Free [γ-^{32}P]ATP was removed by washing in 250 mM phosphoric acid and the amount of [γ-^{32}P]ATP incorporated was measured by the TopCount (Packard) scintillation counter.

PKCα-dependent MBP phosphorylation was determined by the deference between the [γ-^{32}P]ATP incorporated in the absence and presence of the kinases.

cAMP accumulation assay

Human β2-adrenerigic receptor – green fluorescence protein fusion protein expressing HEK293 cells (HEK-B2, 5x10^6 cells/ml) were suspended in Dulbbecco's modified phosphate buffered saline with Ca^{++}, Mg^{++} (D-PBS (+)) contained 1 mM IBMX (3-ISOBUTYL-1-METHYLXANTHINE, WAKO chemicals) and 0.1% L-ascorbic acid sodium salt (WAKO chemicals) in the absence and presence of test compounds. After pre-incubation period of 20 min, 10 nM Isoproterenol (Sigma) was applied to the cells, following by the incubation at 37 °C for 20 min. Reactions were stopped by the addition of 20 μL of boiled 10 mM Tris-HCl, pH8.0, 1 mM EDTA and the cell suspension was centrifuged at 3000 rpm for 10 min. cAMP in the supernatants was measured by the cAMP ALPHA-screen kit (Perkinelmer).

Visualization of β2-adregenic receptor internalization

1000 cells of HEK-B2 cell line seeded into BD BioCoat Poly-D-Lysine Cellware 8 wells CultureSlides (BD Biosciences), and cultured at 37 ℃ for 48 h. Cultured cells were twice rinsed with D-PBS (+), added 0.5 mL/well of D-PBS (+) containg 0.1% ascorbic acid sodium salt, and incubated at 37 °C for 10 min. After incubation, added 50 μL of compound, and incubated at 37 °C for 20 min. After incubation, added 50 μL of 1 μM of isoproterenol, and incubated at 37 °C for 30 min. For reaction termination, cells were twice rinsed with D-PBS (+), and add. 0.5 mL of 3.7%

neutralized formalin, and incubated at room temperature for 10 min. Culture slides rinsed with D-PBS, and lasers scanning confocal images were collected with an inverted Zeiss LSM 510 microscope.

Crystal Structure Determination

Human GRK2 RH plus kinase domains (residues 22–538) were cloned into the pFastBacHTb vector, and expressed using strain Sf9 of the baculoviral expression system. The cell pellet from 10 L of cell culture was suspended into 800 mL lysis buffer (Buffer 1) consisting of 25 mM Tris (pH 7.6), 1 M NaCl, 25 mM Imidazole (pH 7.6), 0.5 mM TCEP, 3 Roche cOmplete Protease Inhibitor tablets and 20 U/mL benzonase. The lysate was run through a microfluidizer at 18,000 psi prior to being centrifuged for 1 hour at 14,000 RPM (Beckman, JA-14 rotor). The supernatant was subsequently filtered through cheesecloth and affinity purified using a Nickel HiTrap (Invitrogen) column. The protein was bound and equilibrated on the column with a buffer (Buffer 2) consisting of 25 mM Tris (pH 7.6), 1 M NaCl, 25 mM Imidazole, 0.5 mM TCEP. The column was washed with 100 column volumes of Buffer 2, after which the protein was eluted from the column using a gradient from 20% – 100% Buffer 3 (25 mM Tris (pH 7.6), 1 M NaCl, 300 mM Imidazole, 0.5 mM TCEP). Fractions containing GRK2 were pooled, TEV protease was added, and the mixture was dialyzed against 1 L of Buffer 2 containing only 10 mM Imidazole. After dialysis the GRK2-TEV protease solution was run over a second Nickel HiTrap column and the flow through was collected and concentrated for size exclusion chromatography. The protein was further purified by preparative size-exclusion

chromatography utilizing a Superdex 200 column equilibrated in (Buffer 4) 25 mM HEPES (pH 7.6), 150 mM NaCl, 0.5 mM TCEP. The monomeric peak was pooled and the protein concentrated to 14 mg/mL for crystallization. Crystals for **30i** were co-crystallized at 273 K in sitting drops by adding 50 nL of protein solution (12 mg/mL GRK2, 1 mM **30i**, 25 mM HEPES pH 7.6, 100 mM NaCl, 2 mM DTT) to 50 nL of reservoir solution (18% PEG MME 2000, 100 mM MES pH 5.9, 0.0175 M Ammonium Sulfate). The resulting crystals were briefly passed through a drop containing mother liquor plus 25% Ethylene Glycol before being flash frozen in liquid nitrogen and exposed to X-rays for data collection at beamline 5.0.2 at the ALS. Crystals for **115h** were grown at 273 K in sitting drops by adding 50 nL protein solution (14 mg/mL GRK2, 1 mM 4-(piperazin-1-yl)-1*H*-Indole, 25 mM HEPES pH 7.6, 150 mM NaCl, 0.5 mM TCEP) to 50 nL reservoir solution (18% PEG MME 2000, 0.1 M MES pH 5.6, and 0.0175 M Ammonium Sulfate). The resulting crystals were soaked in a drop containing mother liquor plus 5 mM **115h** and 30% PEG MME 2000 overnight before being flash frozen in liquid nitrogen and exposed to X-rays for data collection at beamile 14.1 at the SSRL. X-ray diffraction data set were collected from single cryogenically protected crystals, at the ALS beamline 5.0.2 on an ADSC Q315R detector, and at the SSRL beamline 14.1 on a MAR CCD 325 mm detector. GRK2 + **30i** crystals belong to the tetragonal space group P42212, and contain one enzyme molecule in the asymmetric unit. GRK2 + **115h** crystals belong to the triclinic space group P3121, and contain one enzyme molecule in the asymmetric unit. The data was reduced by the HKL2000 software package.[36] The structures were determined by the molecular replacement method

using MOLREP[37] of the CCP4 program suite. Multiple cycles of model building with XtalView[38] or COOT[39] and refinement with REFMAC[40] were performed to improve model quality. The coordinates and structure factors have been deposited in ProteinData Bank with the accession codes 5UVC (**30i**) and 5UUU (**115h**).

ASSOCIATED CONTENT

Supporting Information

Molecular formula strings and some data spreadsheet (CSV)

Accession Codes

The coordinates and structure factors have been deposited in ProteinData Bank with the accession codes 5UVC (**30i**) and 5UUU (**115h**). Authors will release the atomic coordinates and experimental data upon article publication.

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Author Contributions

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Data Collection and Processing: Dr. Gyorgy Snell (**30i** and **115h**), Mr. S. Weston Lane (**30i** and **115h**). Structure Solution and Refinement: Mr. Isaac D. Hoffman (**30i** and **115h**).

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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Abbreviations Used

ADDP, 1,1'-(azodicarbonyl)dipiperidine; DIAD, diisopropyl azodicarboxylate; HOBt, 1-hydroxybenzotriazole; IPE, diisopropyl ether; TOSMIC, tosylmethyl isocyanide; WSC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (water-soluble carbodiimide); Z, benzyloxycarbonyl.

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Figure 1. Structure of Hit Compound **5** (hydrazone derivative) and Synthetic Plans

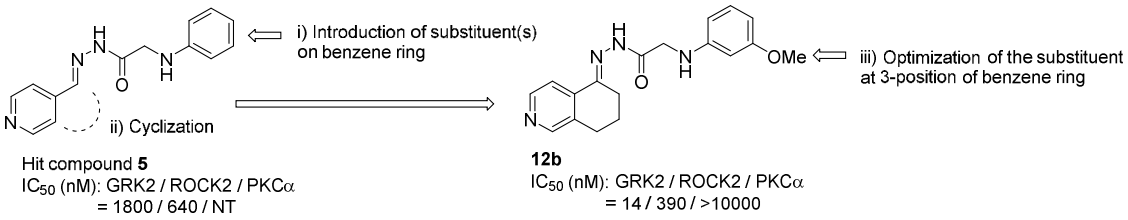
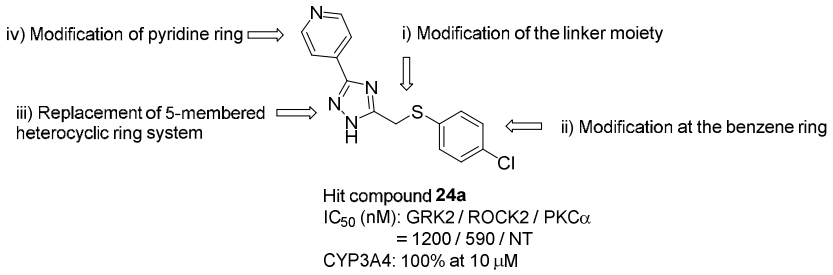
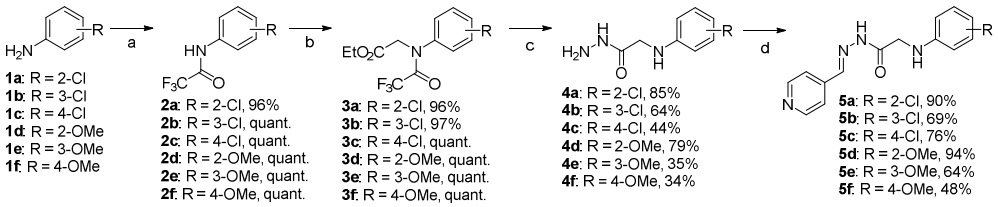


Figure 2. Structure of Hit Compound **24a** (1,2,4-triazole derivative) and Synthetic Plans



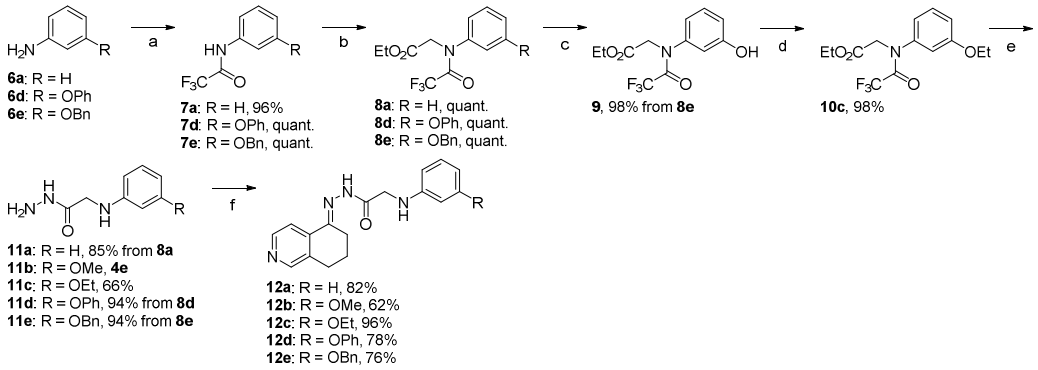
Hit compound **24a**
IC_{50} (nM): GRK2 / ROCK2 / PKCα
= 1200 / 590 / NT
CYP3A4: 100% at 10 μM

Scheme 1.[a]



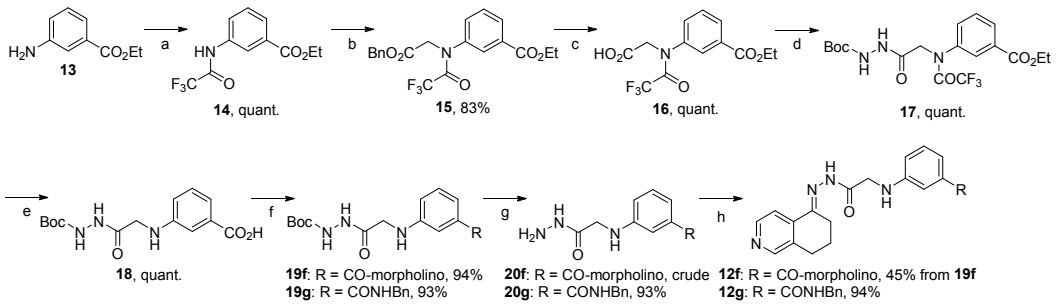
[a]Reagents and conditions: (a) TFAA, Et_3N, THF, 0 ºC to rt; (b) $BrCH_2CO_2Et$, K_2CO_3, DMF, rt; (c) $H_2NNH_2 \bullet H_2O$, EtOH, 90 ºC; (d) 4-pyridinecarboxaldehyde, MeOH, rt.

Scheme 2.[a]



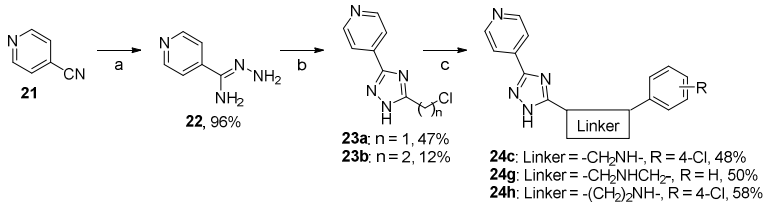
[a]Reagents and conditions: (a) TFAA, Et_3N, THF, 0 ºC to rt; (b) $BrCH_2CO_2Et$, K_2CO_3, DMF, rt; (c) 10% Pd/C, EtOH, H_2 (balloon), rt; (d) EtI, K_2CO_3, DMF, rt; (e) $H_2NNH_2 \bullet H_2O$, EtOH, 90 ºC; (f) 7,8-dihydroisoquinolin-5(6*H*)-one, EtOH, 90 ºC.

Scheme 3.[a]



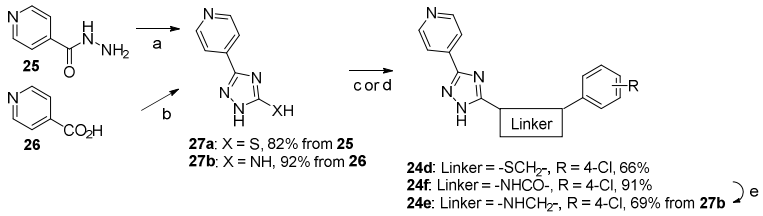
[a]Reagents and conditions: (a) TFAA, Et_3N, THF, 0 ºC to rt; (b) $BrCH_2CO_2Bn$, K_2CO_3, DMF, rt then TFAA, Et_3N, THF, 0 ºC to rt; (c) 10% Pd/C, EtOH, H_2 (balloon), rt; (d) $BocNHNH_2$, WSC•HCl, HOBt•H_2O, DMF, rt; (e) NaOH (aq.), EtOH, THF, rt; (f) corresponding amine, WSC•HCl, HOBt•H_2O, DMF, rt; (g) TFA, rt or HCl in AcOEt, EtOH, rt; (h) 7,8-dihydroisoquinolin-5(6*H*)-one, EtOH, 90 ºC.

Scheme 4.[a]



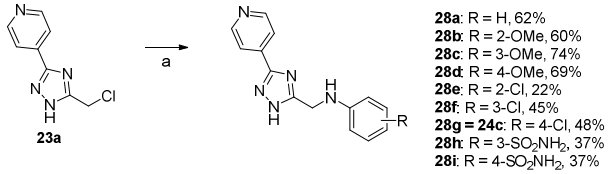
[a]Reagents and conditions: (a) H_2NNH_2, Et_2O, EtOH, rt; (b) $Cl(CH_2)_nCOCl$, DBU, 0 ºC to rt then AcOH, EtOH, reflux; (c) corresponding aniline or amine, DMF, 0 ºC.

Scheme 5.[a]



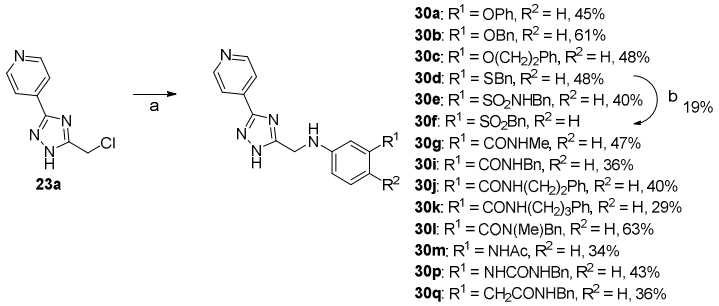
[a]Reagents and conditions: (a) NH_4SCN, 170 ºC then NaOH (aq.), reflux; (b) aminoguanidine hydrochloride, 190 ºC; (c) 4-Cl-C_6H_4-CH_2Cl, KOH, MeOH, 0 ºC to rt; (d) 4-Cl-C_6H_4-COCl, pyridine, 0 ºC to rt then 250 ºC; (e) BH_3•THF, THF, reflux.

Scheme 6.[a]



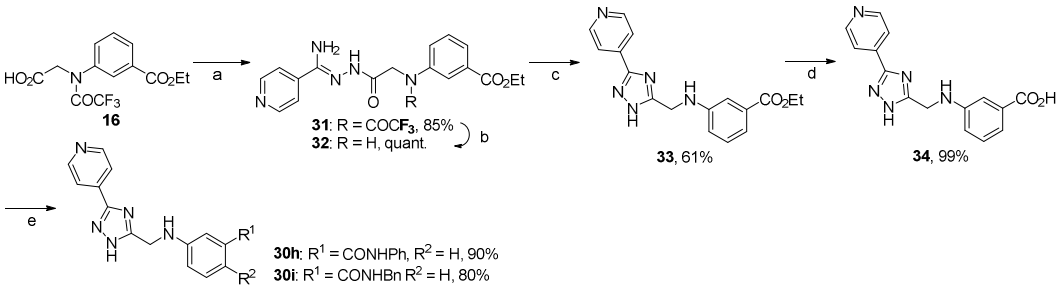
[a]Reagents and conditions: (a) corresponding aniline, DMF, 90 ºC.

Scheme 7.[a]



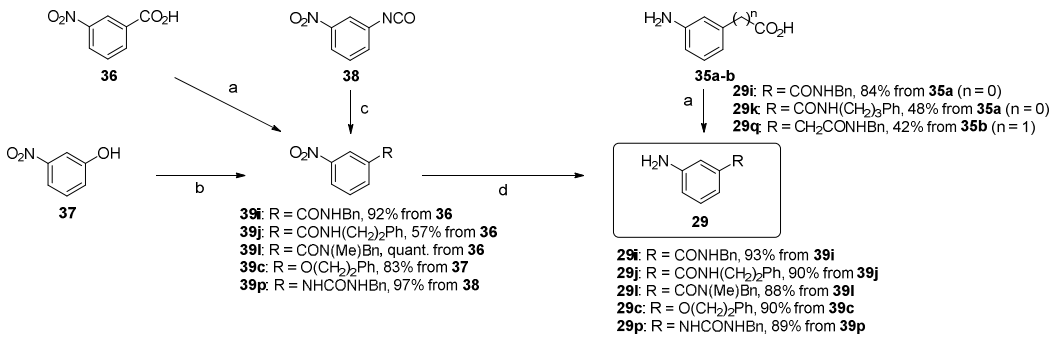
[a]Reagents and conditions: (a) corresponding aniline **29**, DMF, 90 ºC; (b) $NaIO_4$, MeOH, THF, H_2O, 90 ºC.

Scheme 8.[a]



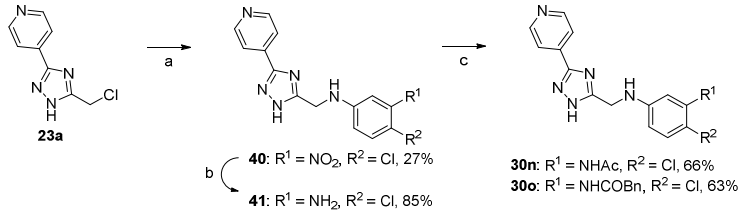
[a]Reagents and conditions: (a) **22**, WSC•HCl, HOBt•H_2O, DMF, rt; (b) NaOEt, EtOH, rt; (c) AcOH, EtOH, 90 ºC; (d) NaOH (aq.), EtOH, THF, rt; (e) corresponding aniline or amine, WSC•HCl, HOBt•H_2O, DMF, rt.

Scheme 9.[a]



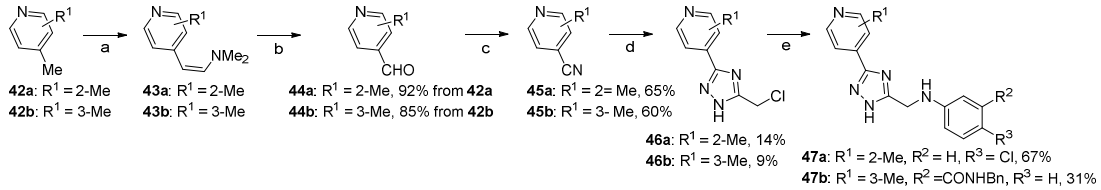
[a]Reagents and conditions: (a) corresponding amine, WSC•HCl, HOBt•H_2O, DMF, rt; (b) $Ph(CH_2)_2Br$, K_2CO_3, DMF, rt; (c) $BnNH_2$, THF, rt; (d) Fe, $CaCl_2$, 85% EtOH (aq.), 90 ºC.

Scheme 10.[a]



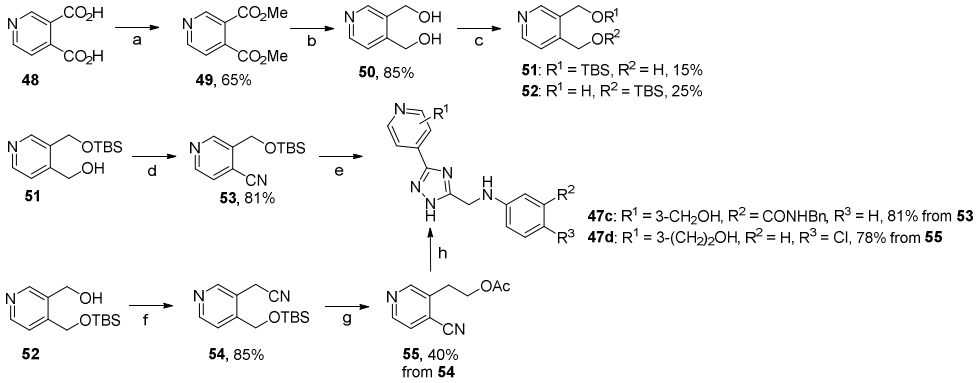
[a]Reagents and conditions: (a) corresponding aniline, DMF, 90 ºC; (b) Fe, $CaCl_2$, 85% EtOH (aq.), 90 ºC; (c) corresponding carboxylic acid, WSC•HCl, HOBt•H_2O, DMF, rt then K_2CO_3, MeOH, rt.

Scheme 11.[a]



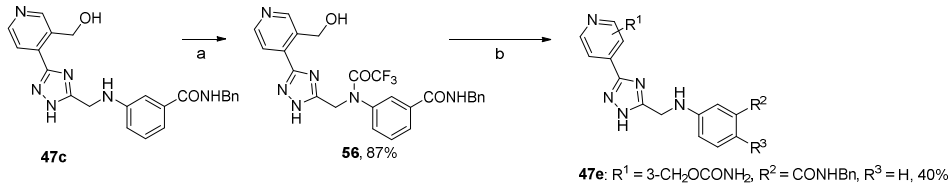
[a]Reagents and conditions: (a) 1. *n*-BuLi, THF, –78 ºC, 2. $MeNH_2$, THF, –78 ºC, 3. DMF, THF, –78 ºC; (b) $NaIO_4$, DCM, water, 0 ºC to rt; (c) NH_2OH•HCl, NaOH (aq.), CH_3CN, rt then Ac_2O, reflux; (d) 1. H_2NNH_2, 50 ºC, 2. $ClCH_2COCl$, DBU, THF, DCM, 0 ºC to rt, 3. EtOH, AcOH, 90 ºC; (e) corresponding aniline, 90 ºC.

Scheme 12.[a]



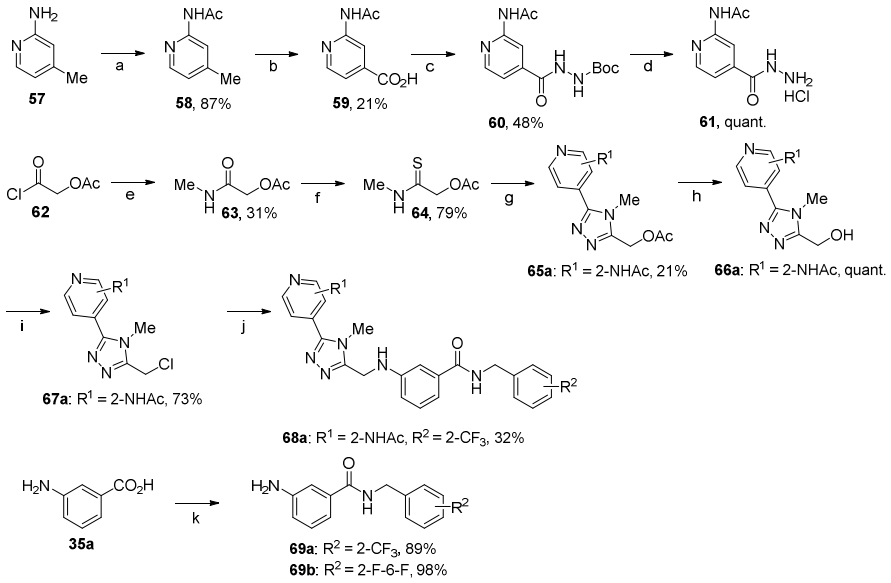
[a]Reagents and conditions: (a) c. HCl, MeOH, reflux; (b) $NaBH_4$, $CaCl_2$, EtOH, 0 ºC to rt; (c) TBSCl, imidazole, DMF, 0 ºC to rt; (d) 1. DMSO, $(COCl)_2$, DCM, THF, –78 ºC then Et_3N, –78 ºC to rt, 2. NH_2OH•HCl, NaOH (aq.), CH_3CN, rt, 3. CDI, reflux; (e) **20g**, NaH, EtOH, 90 ºC; (f) acetoncyanohydrin, ADDP, *n*-Bu_3P, THF, 0 ºC to rt; (g) 1. c. HCl, EtOH, reflux, 65%, 2. $NaBH_4$, $CaCl_2$, EtOH, 0 ºC to rt, 81%, 3. MnO_2, DCM, MeOH, rt, 4. NH_2OH•HCl, NaOH (aq.), CH_3CN, rt then Ac_2O, reflux, 76% in 3 steps; (h) **4c**, NaH, EtOH, 90 ºC.

Scheme 13.[a]



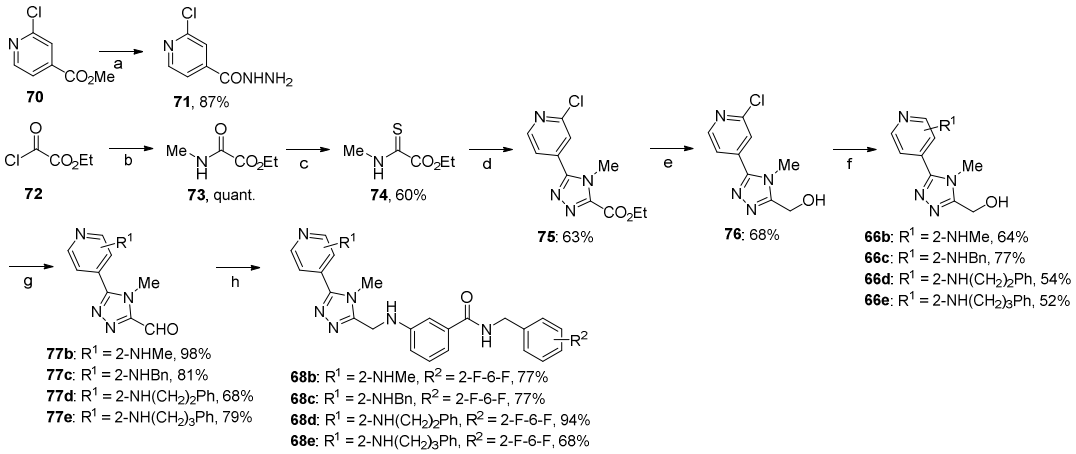
[a]Reagents and conditions: (a) TFAA, Et_3N, THF, 0 ºC to rt then MeOH, water, rt; (b) 1. Cl_3CONCO, CH_3CN, rt, 2. K_2CO_3, MeOH, water, rt.

Scheme 14.[a]



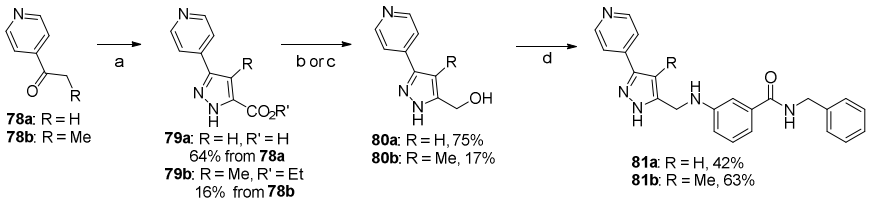
[a]Reagents and conditions: (a) Ac_2O, 70 ºC; (b) $KMnO_4$, water, 90 ºC; (c) $H_2NNHBoc$, WSC•HCl, HOBt•H_2O, Et_3N, DMF, rt; (d) 4N HCl in AcOEt, MeOH, rt; (e) $MeNH_2$, Et_3N, THF, 0 ºC to rt; (f) Lawesson's reagent, THF, 70 ºC; (g) 1. Et_3OBF_4, DCM, 0 ºC to rt, 2. **61**, Et_3N, *n*-BuOH, reflux; (h) 1N NaOH (aq.), MeOH, rt; (i) $SOCl_2$, DCM, rt; (j) **69a**, $NaHCO_3$, DMF, 90 ºC; (k) corresponding amine, WSC•HCl, HOBt•H_2O, DMF, rt.

Scheme 15.[a]



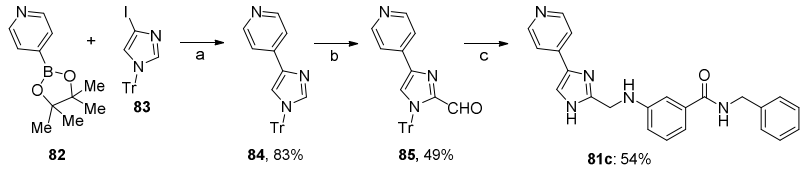
[a]Reagents and conditions: (a) $H_2NNH_2 \bullet H_2O$, EtOH, 90 ºC; (b) $MeNH_2$, pyridine, DCM, 0 ºC; (c) Lawesson's reagent, toluene, 90 ºC; (d) 1. Et_3OBF_4, DCM, 0 ºC to rt, 2. **71**, 130 ºC; (e) $NaBH_4$, $CaCl_2$, EtOH, 0 ºC; (f) corresponding amine, 150 ºC, sealed tube; (g) MnO_2, MeOH, rt; (h) 1. **69b**, MeOH, AcOH, rt, 2. $NaBH_4$, MeOH, 0 ºC to rt.

Scheme 16.[a]



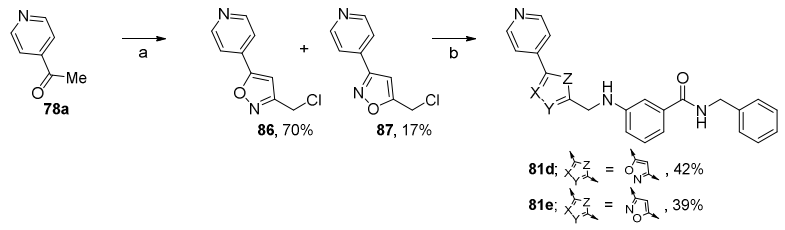
[a]Reagents and conditions: (a) LHMDS, $(CO_2Et)_2$, THF, –78 ºC, 2. $H_2NNH_2•H_2O$, 5.5 N HCl (aq.), 0 ºC to 85 ºC; (b) 1. MeONHMe•HCl, WSC•HCl, HOBt•H_2O, Et_3N, DMF, rt, 97%, 2. $LiAlH_4$, THF, –30 ºC to 0 ºC, quant., 3. $NaBH_4$, MeOH, 0 ºC to rt, 77%; (c) $LiAlH_4$, THF, –40 ºC; (d) 1. $SOCl_2$. rt, 2. **29i**, DMF, 90 ºC.

Scheme 17.[a]



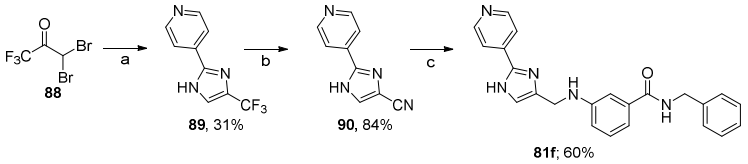
[a]Reagents and conditions: (a) $Pd(Ph_3P)_4$, K_3PO_4, DMF, 110 ºC; (b) 1. *n*-BuLi, THF, –78 ºC, 2. DMF, –78 ºC; (c) 1. $NaBH_4$, MeOH, 0 ºC, 2. $SOCl_2$, rt, 3. **29i**, DMF, 90 ºC.

Scheme 18.[a]



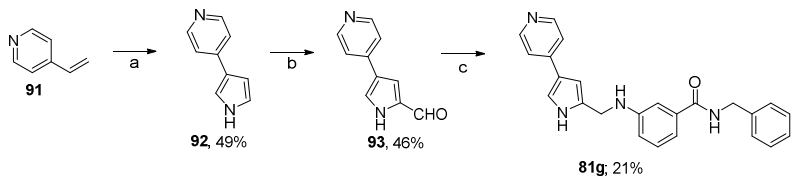
[a]Reagents and conditions: (a) 1. LHMDS, $(CO_2Et)_2$, THF, –78 ºC, 2. H_2NOH•HCl, HCl (aq.), EtOH, 0 ºC to 85 ºC, 3. $LiAlH_4$, THF, –30 ºC, 4. $SOCl_2$, rt; (b) **29i**, DMF, 90 ºC.

Scheme 19.[a]



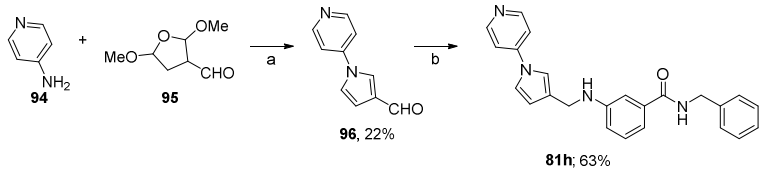
[a]Reagents and conditions: (a) 1. NaOAc, water, 110 ºC, 2. 4-pyridinecarboaldehyde, 25% NH_3 (aq.), MeOH, rt; (b) 5% NH_3 (aq.), MeOH, 60 ºC; (c) DIBALH, THF, –78 ºC to rt, 42%, 2. $NaBH_4$, MeOH, 0 ºC, 98%, 3. $SOCl_2$, rt, 4. **29i**, DMF, 90 ºC.

Scheme 20.[a]



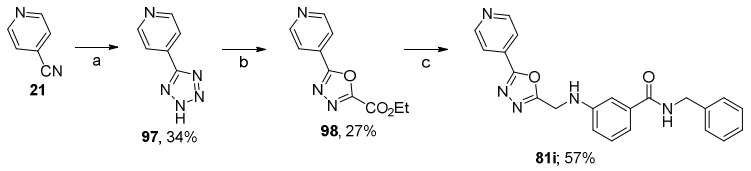
[a]Reagents and conditions: (a) TOSMIC, NaO*t*-Bu, DMSO, rt; (b) $POCl_3$, DMF, 0 ºC to 130 ºC; (c) **29i**, $NaBH(OAc)_3$, MeOH, AcOH, rt.

Scheme 21.[a]



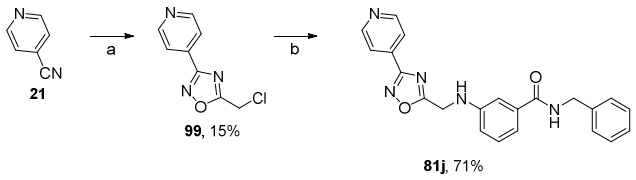
[a]Reagents and conditions: (a) AcOH, 90 ºC; (b) **29i**, MeOH, AcOH, rt.

Scheme 22.[a]



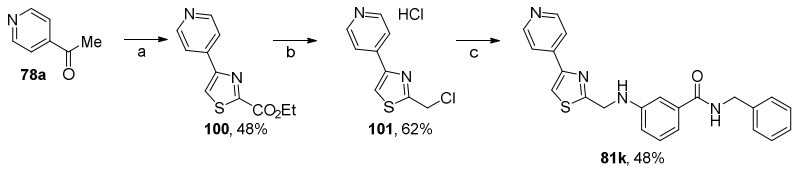
[a]Reagents and conditions: (a) NaN_3, NH_4Cl, DMF, 150 ºC; (b) $ClCOCO_2Et$, 2,4,6-collidine, toluene, 120 ºC; (c) 1. $NaBH_4$, $CaCl_2$, EtOH, 0 ºC, 77%, 2. $SOCl_2$, rt, quant., 3. **29i**, DMF, 90 ºC.

Scheme 23.[a]



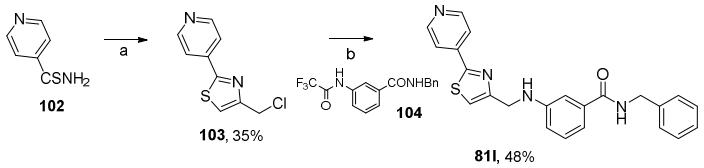
[a]Reagents and conditions: (a) 1. $HONH_2$ (aq.), EtOH, 70 ºC, 98%, 2. $(ClCH_2CO)_2O$, toluene, 120 ºC, 15%; (b) **29i**, DMF, 90 ºC.

Scheme 24.[a]



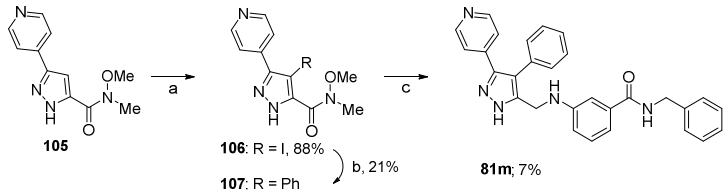
[a]Reagents and conditions: (a) 1. Br_2, HBr, AcOH, 90 ºC, 81%, 2. ethyl thiooxamate, EtOH, reflux, 59%; (b) 1. DIBALH, toluene, 0 ºC, 57%, 2. $SOCl_2$, rt, 92%; (c) **29i**, DMF, 90 ºC, 48%.

Scheme 25.[a]



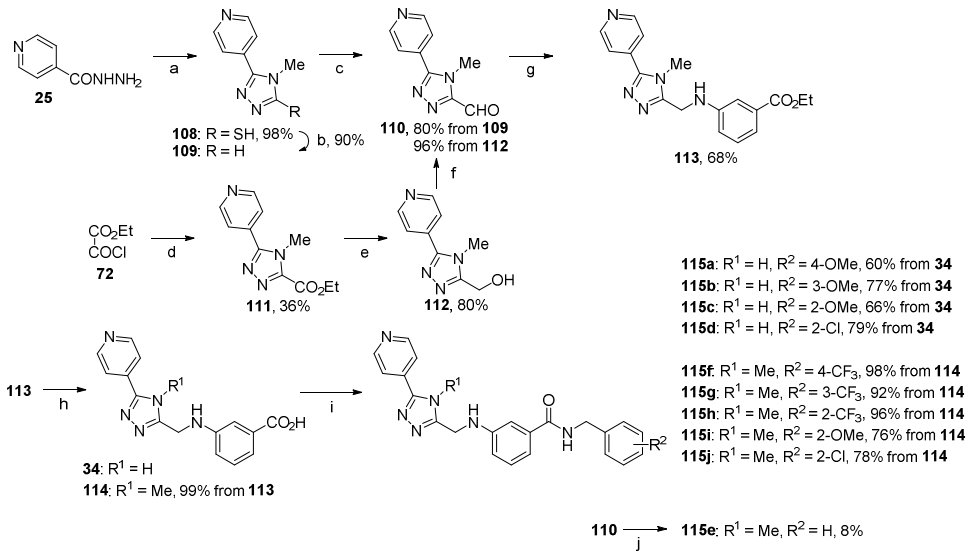
[a]Reagents and conditions: (a) 1,3-dichloroacetone, EtOH, 90 ºC; (b) 1. **104**, K_2CO_3, DMF, 80 ºC, 2. K_2CO_3, water, rt.

Scheme 26.[a]



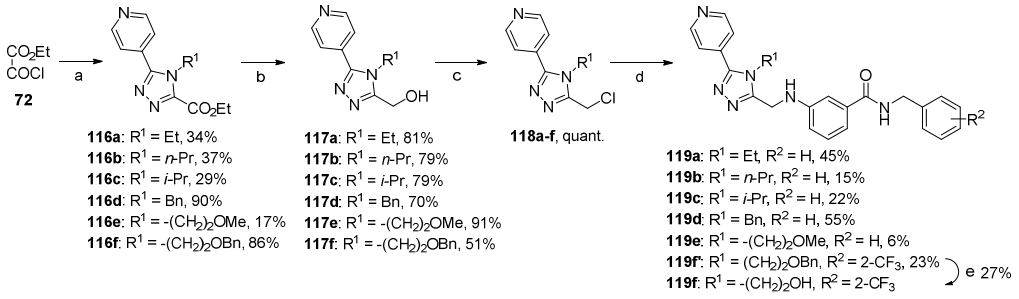
[a]Reagents and conditions: (a) I_2, CAN, CH_3CN, 95 ºC; (b) $PhB(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, toluene, EtOH, water, 100 ºC; (c) 1. $LiAlH_4$, THF, 0 ºC, crude, 2. **29i**, $NaBH(OAc)_3$, AcOH, MeOH, rt.

Scheme 27.[a]



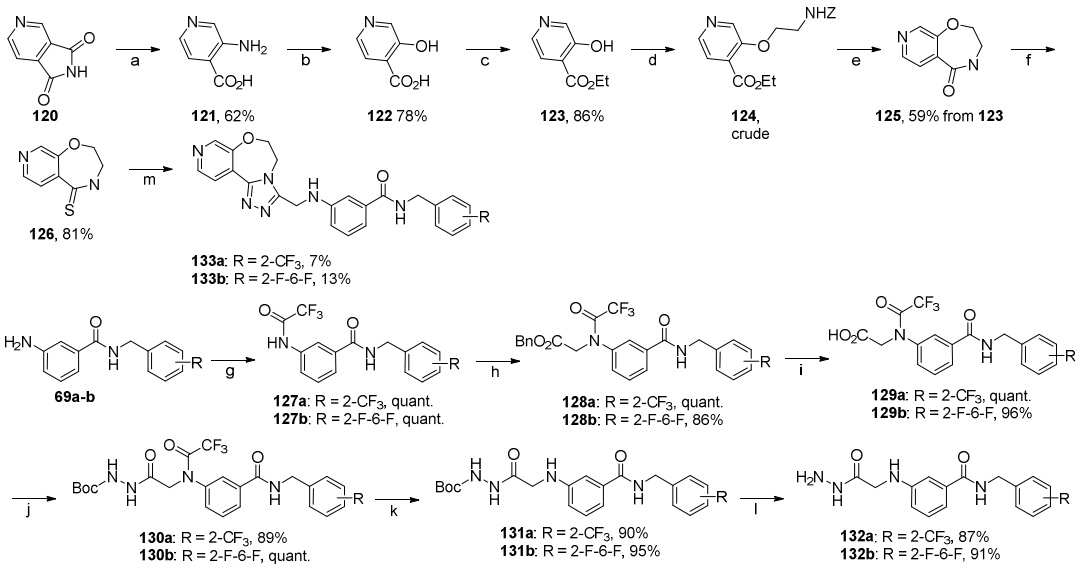
[a]Reagents and conditions: (a) 1. MeNCS, EtOH, 90ºC, 99%, 2. $NaHCO_3$ (aq.), 120 ºC, 99%; (b) HNO_3, 120 ºC; (c) 1. *n*-BuLi, THF, –78 ºC, 2. DMF, –78ºC; (d) 1. $MeNH_2$, pyridine, DCM, 0 ºC, quant., 2. Lawesson's reagent, toluene, 90 ºC, 60%, 3. Et_3OBF_4, DCM, 0 ºC to rt, 4. **25**, toluene, 130 ºC, 60% in 2 steps; (e) $NaBH_4$, $CaCl_2$, EtOH, 0 ºC; (f) MnO_2, MeOH, rt; (g) 1. **13**, MeOH, AcOH, rt, 2. $NaBH_4$, MeOH, 0 ºC to rt; (h) NaOH (aq.), THF, MeOH, rt; (i) corresponding amine, WSC•HCl, HOBt•H_2O, DMF, rt; (j) 1. **29i**, MeOH, AcOH, rt, 2. $NaBH_4$, MeOH, 0 ºC to rt.

Scheme 28.[a]



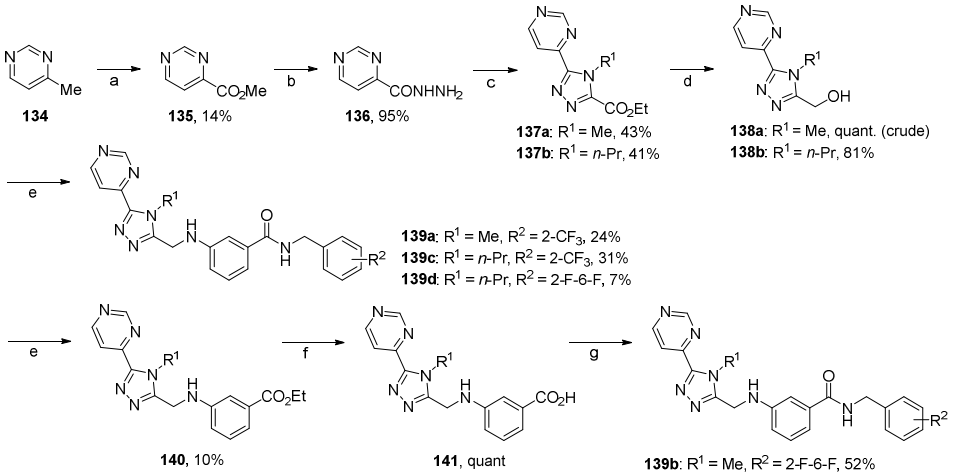
[a]Reagents and conditions: (a) 1. corresponding amine, pyridine, DCM, 0 ºC, 2. Lawesson's reagent, toluene, 90 ºC, 3. Et_3OBF_4, DCM, 0 ºC to rt, 4. **25**, toluene, reflux; (b) $NaBH_4$, $CaCl_2$, EtOH, THF, 0 ºC; (c) $SOCl_2$, rt; (d) **29i** or **69a**, DMF, 90 ºC; (e) HCO_2NH_4, 10% Pd/C, MeOH, water, 80 ºC.

Scheme 29.[a]



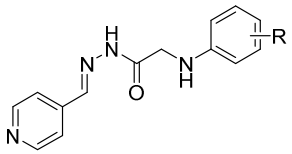
[a]Reagents and conditions: (a) Br_2, NaOH (aq.), 0 ºC to 85 ºC; (b) $NaNO_2$, c. H_2SO_4, H_2O, 60 ºC to 85 ºC; (c) c. H_2SO_4, EtOH, 95 ºC; (d) $HO(CH_2)_2NHZ$, DIAD, Ph_3P, THF, 0 ºC to rt; (e) 10% Pd/C, H_2 (balloon), EtOH, rt; (f) Lawesson's reagent, toluene, 90 ºC; (g) TFAA, Et_3N, THF, 0 ºC; (h) $BrCH_2CO_2Bn$, K_2CO_3, DMF, 0 ºC to rt; (i) 10% Pd/C, H_2 (balloon), MeOH, rt; (j) $BocNHNH_2$, WSC•HCl, HOBt•H_2O, DMF, rt; (k) NaOH (aq.), MeOH, rt; (l) HCl in AcOEt, EtOH, rt; (m) **132a-b**, NaOEt, EtOH, toluene, 100 ºC.

Scheme 30.[a]



[a]Reagents and conditions: (a) 1. SeO_2, pyridine, 60 ºC then 85 ºC, 19%, 2. MeOH, H_2SO_4, reflux, 72%; (b) $H_2NNH_2 \bullet H_2O$, EtOH, reflux; (c) thioimidate, toluene, reflux; (d) $NaBH_4$, $CaCl_2$, EtOH, THF, 0 ºC (e) 1. $SOCl_2$, DCM, rt, 2. **69a-b** or **13**, DMF, 90 ºC; (f) NaOH (aq.), EtOH, 70 ºC (g) corresponding amine, WSC•HCl, HOBt•H_2O, Et_3N, DMF, rt.

Table 1. Biological Activities of Acyclic Hydrazone Derivatives.[a]



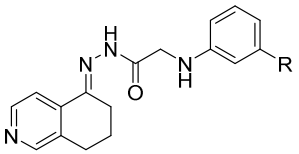
Compound	R	GRK2 IC_{50} (nM) (95% CI)	ROCK2 IC_{50} (nM) (95% CI)	PKCα IC_{50} (nM)
Hit 5	H	1800 (1500-2300)	640 (510-790)	NT[b]
5a	2-Cl	>10000	2600 (2200-3200)	NT
5b	3-Cl	700 (550-880)	300 (250-370)	NT
5c	4-Cl	5600 (4300-7300)	540 (420-700)	NT
5d	2-OMe	>10000	7400 (5900-9300)	NT
5e	3-OMe	49 (38-65)	62 (46-84)	NT
5f	4-OMe	>10000	3700	NT

(2900-4700)

[a]95% confidence intervals (CI) conducted in duplicate (n = 2).

[b]NT means "not tested".

Table 2. Biological Activities of Cyclic Hydrazone Derivatives.[a]

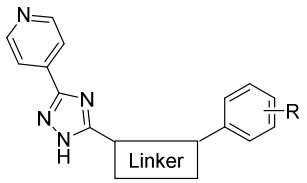


Compound	R	GRK2 IC_{50} (nM) (95% CI)	ROCK2 IC_{50} (nM) (95% CI)	PKCα IC_{50} (nM)
12a	H	1100 (720-1800)	1800 (1400-2300)	NT[b]
12b	OMe	14 (12-17)	390 (350-430)	>10000
12c	OEt	120 (100-150)	5700 (4100-7900)	>10000
12d	OPh	42 (36-49)	6200 (4600-8300)	>10000
12e	OBn	53 (48-58)	>10000	>10000
12f	CO-morpholino	>10000	>10000	NT
12g	CONHBn	20 (17-22)	58 (46-73)	>10000

[a]95% confidence intervals (CI) conducted in duplicate (n = 2).

[b]NT means "not tested".

Table 3. Biological Activities of 4-Pyridyl-1,2,4-Triazole Derivatives[a]



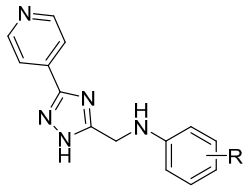
Compound	Linker	R	GRK2 IC_{50} (nM) (95% CI)	ROCK2 IC_{50} (nM) (95% CI)	PKCα IC_{50} (nM)	CYP3A4 % (at 10 μM)
Hit 24a	$-CH_2S-$	4-Cl	1200 (1000-1500)	590 (520-670)	NT[b]	100
Hit 24b	$-CH_2O-$	3-Cl	4400 (3300-5700)	1700 (1400-2100)	NT	86
24c	$-CH_2NH-$	4-Cl	41 (32-54)	94 (82-110)	>10000	97
24d	$-SCH_2-$	4-Cl	5700 (1600-20000)	1900 (1500-2400)	NT	97
24e	$-NHCH_2-$	4-Cl	980 (820-1200)	270 (220-320)	NT	96
24f	-NHCO-	4-Cl	>10000	>10000	NT	27
24g	$-CH_2NHCH_2-$	H	>10000	5100	NT	74

				(3900-6600)		
24h	-$(CH_2)_2NH$-	4-Cl	>10000	900 (680-1200)	NT	93

[a]95% confidence intervals (CI) conducted in duplicate (n = 2).

[b]NT means "not tested".

Table 4. Biological Activities of 4-Pyridyl-1,2,4-Triazole Derivatives[a]

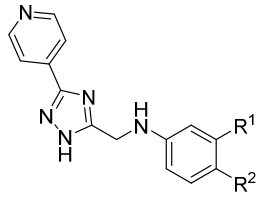


Compound	R	GRK2 IC_{50} (nM) (95% CI)	ROCK2 IC_{50} (nM) (95% CI)	PKCα IC_{50} (nM)	CYP3A4 % (at 10 μM)
28a	H	100 (88-120)	630 (510-770)	>10000	94
28b	2-OMe	1200 (870-1700)	1800 (1400-2300)	>10000	93
28c	3-OMe	160 (130-210)	720 (530-990)	>10000	92
28d	4-OMe	440 (250-750)	280 (240-340)	>10000	95
28e	2-Cl	250 (210-150)	1700 (1500-2000)	>10000	100
28f	3-Cl	140 (120-150)	380 (320-450)	>10000	93

28g (24c)	4-Cl	41 (32-54)	94 (82-110)	>10000	97
28h	3-SO_2NH_2	44 (32-61)	160 (140-180)	>10000	25
28i	4-SO_2NH_2	1200 (900-1600)	360 (320-410)	>10000	20

[a]95% confidence intervals (CI) conducted in duplicate (n = 2).

Table 5. Biological Activities of 4-Pyridyl-1,2,4-Triazole Derivatives[a]



Compound	R^1	R^2	GRK2 IC_{50} (nM) (95% CI)	ROCK2 IC_{50} (nM) (95% CI)	PKCα IC_{50} (nM)	CYP3A4 % (at 10 μM)
28a	H	H	100 (88-120)	630 (510-770)	>10000	94
28c	OMe	H	160 (130-210)	720 (530-990)	>10000	92
30a	OPh	H	300 (340-390)	>10000	>10000	100
30b	OBn	H	360 (260-510)	1400 (1100-1800)	>10000	97
30c	$O(CH_2)_2Ph$	H	280 (200-370)	2700 (1900-3800)	>10000	96
30d	SBn	H	290 (240-350)	1200 (900-1600)	>10000	100

30e	SO_2NHBn	H	100 (84-130)	98 (87-110)	NT[b]	83
30f	SO_2Bn	H	1800 (1500-2100)	1100 (910-1300)	NT	86
30g	CONHMe	H	65 (55-77)	220 (180-260)	>10000	66
30h	CONHPh	H	54 (44-66)	31 (27-37)	680	94
30i	CONHBn	H	6.1 (4.9-7.4)	24 (21-27)	6300	91
30j	$CONH(CH_2)_2Ph$	H	38 (32-44)	47 (43-50)	>10000	95
30k	$CONH(CH_2)_3Ph$	H	23 (18-30)	100 (87-130)	>10000	94
30l	CON(Me)Bn	H	700 (600-830)	2200 (1800-2800)	>10000	79
30m	NHAc	H	65 (53-81)	70 (62-80)	NT	77
30n	NHAc	Cl	130	74	8300	80

			(110-160)	(60-91)		
30o	NHCOBn	Cl	12 (10-14)	18 (16-20)	1700	90
30p	NHCONHBn	H	19 (16-22)	30 (26-35)	2700	92
30q	$CH_2CONHBn$	H	320 (240-420)	480 (420-550)	>10000	92

[a]95% confidence intervals (CI) conducted in duplicate (n = 2).

[b]NT means "not tested".

Figure 3. Section of the X-ray crystal structure of human GRK2 in complex with the small molecule inhibitor **30i**

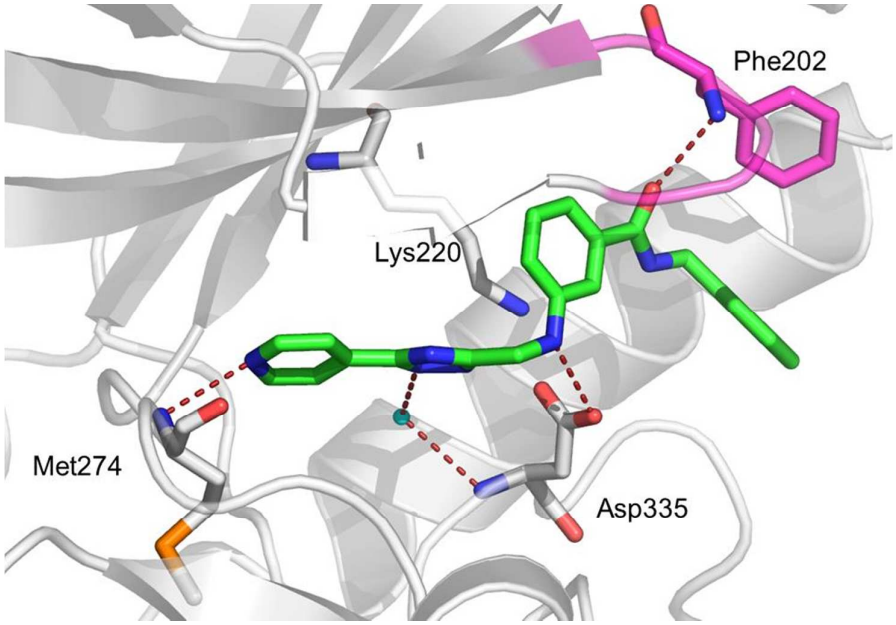
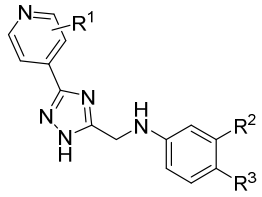


Table 6. Biological Activities of 4-Pyridyl-1,2,4-Triazole Derivatives[a]



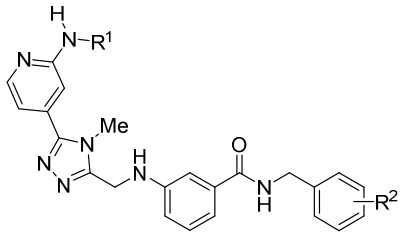
Compound	R^1	R^2	R^3	GRK2 IC_{50} (nM) (95% CI)	ROCK2 IC_{50} (nM) (95% CI)	PKCα IC_{50} (nM)	CYP3A4 % (at 10 μM)
24c	H	H	Cl	41 (32-54)	94 (82-110)	>10000	97
30i	H	CONHBn	H	6.1 (4.9-7.4)	24 (21-27)	6300	91
47a	2-Me	H	Cl	>10000	7100 (5400-9500)	>10000	47
47b	3-Me	CONHBn	H	42 (34-52)	94 (80-110)	>10000	88
47c	3-CH_2OH	CONHBn	H	130 (100-150)	540 (460-630)	>10000	100
47d	3-$(CH_2)_2OH$	H	Cl	3800 (2500-5800)	>10000	NT[b]	100

47e	3-CH_2OCONH_2	CONHBn	H	560 (460-680)	2300 (2000-2600)	NT	63

[a]95% confidence intervals (CI) conducted in duplicate (n = 2).

[b]NT means "not tested".

Table 7. Biological Activities of 4-Pyridyl-1,2,4-Triazole Derivatives[a]

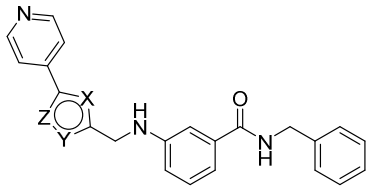


Compound	R^1	R^2	GRK2 IC_{50} (nM) (95% CI)	ROCK2 IC_{50} (nM) (95% CI)	PKCα IC_{50} (nM)	CYP3A4 % (at 10 μM)
68a	Ac	2-CF_3	170 (130-200)	>10000	NT[b]	56
68b	Me	2-F-6-F	62 (52-75)	7400 (6400-8600)	NT	29
68c	Bn	2-F-6-F	67 (57-78)	>10000	>10000	47
68d	$(CH_2)_2Ph$	2-F-6-F	250 (88-700)	>10000	NT	69
68e	$(CH_2)_3Ph$	2-F-6-F	110 (95-130)	>10000	2500	64

[a]95% confidence intervals (CI) conducted in duplicate (n = 2).

[b]NT means "not tested".

Table 8. Biological Activities of 4-Pyridyl 5-membered heterocyclic ring system [a]



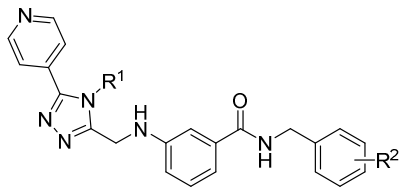
Compound		GRK2 IC_{50} (nM) (95% CI)	ROCK2 IC_{50} (nM) (95% CI)	PKCα IC_{50} (nM)	CYP3A4 % (at 10 μM)
30i		6.1 (4.9-7.4)	24 (21-27)	6300	91
81a		14 (10-18)	60 (54-66)	7200	99
81b		9.9 (8.1-12)	23 (20-27)	4800	96
81c		21 (16-27)	7.3 (6.7-8.0)	3600	100
81d		24 (20-30)	18 (16-19)	2500	84
81e		46	130	8300	92

		(30-68)	(120-150)		
81f		460 (360-580)	160 (140-180)	NT[b]	93
115e		45 (35-57)	550 (480-630)	>10000	26
81g		130 (96-160)	52 (46-60)	>10000	100
81h		1700 (1400-2200)	340 (260-440)	NT	95
81i		67 (54-84)	320 (270-370)	>10000	43
81j		200 (150-250)	350 (260-470)	>10000	86
81k		160 (130-200)	22 (19-25)	>10000	92
81l		470 (380-580)	120 (100-140)	>10000	87
81m		510 (360-740)	170 (130-210)	NT	87

[a]95% confidence intervals (CI) conducted in duplicate (n = 2).

[b]NT means "not tested".

Table 9. Biological Activities of 4-Pyridyl (4-Alkyl or 1*H*)-1,2,4-triazole Derivatives [a]



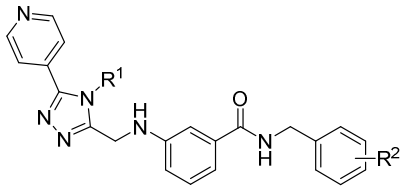
Compound	R^1	R^2	GRK2 IC_{50} (nM) (95% CI)	ROCK2 IC_{50} (nM) (95% CI)	PKCα IC_{50} (nM)	CYP3A4 % (at 10 μM)	HEK-B2 EC_{50} (μM)
30i	H	H	6.1 (4.9-7.4)	24 (21-27)	6300	91	41
115a	H	4-OMe	17 (14-20)	27 (24-35)	7100	91	NH[b]
115b	H	3-OMe	12 (10-14)	20 (18-23)	3200	95	NT
115c	H	2-OMe	3.0 (2.5-3.6)	85 (75-96)	3300	95	76
115d	H	2-Cl	3.9 (3.3-4.6)	50 (47-54)	1100	97	120
115e	Me	H	45 (37-55)	550 (480-630)	>10000	26	1900

115f	Me	4-CF_3	45 (37-55)	620 (550-700)	>10000	36	930
115g	Me	3-CF_3	39 (30-50)	530 (460-610)	>10000	44	220
115h	Me	2-CF_3	18 (15-21)	1400 (1100-1600)	8100	44	10
115i	Me	2-OMe	44 (34-57)	1600 (1400-1900)	>10000	60	NT
115j	Me	2-Cl	12 (9.2-16)	1600 (980-1500)	8700	54	33

[a]95% confidence intervals (CI) conducted in duplicate (n = 2).

[b]NT means "not tested".

Table 10. Biological Activities of 4-Pyridyl-4-alkyl-1,2,4-triazole derivatives [a]



Compound	R^1	R^2	GRK2 IC_{50} (nM) (95% CI)	ROCK2 IC_{50} (nM) (95% CI)	PKCα IC_{50} (nM)	CYP3A4 % (at 10 μM)	HEK-B2 EC_{50} (μM)
30i	H	H	6.1 (4.9-7.4)	24 (21-27)	6300	91	41
115e	Me	H	45 (37-55)	550 (480-630)	>10000	26	1900
115h	Me	2-CF_3	18 (15-21)	1400 (1100-1600)	8100	44	10
119a	Et	H	7.5 (6.3-8.9)	76 (71-82)	2900	50	87
119b	*n*-Pr	H	32 (30-35)	110 (100-120)	5700	67	26
119c	*i*-Pr	H	140 (99-190)	260 (240-280)	4800	50	>30 (ND)[c]

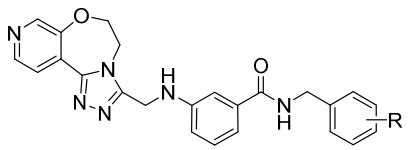
119d	Bn	H	370 (280-490)	3200 (2800-3700)	NT[b]	87	NT
119e	$(CH_2)_2OMe$	H	16 (13-19)	320 (290-360)	7400	57	45
119f	$(CH_2)_2OH$	2-CF_3	5.5 (4.7-6.5)	320 (280-360)	7600	79	12

[a]95% confidence intervals (CI) conducted in duplicate (n = 2).

[b]NT means "not tested".

[c]ND means "not determined".

Table 11. Biological Activities of Fused Pyridine-Triazole Derivatives [a]

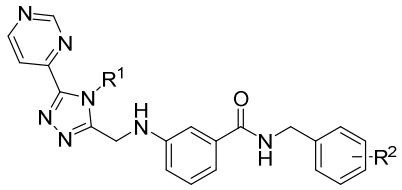


Compound	R	GRK2 IC_{50} (nM) (95% CI)	ROCK2 IC_{50} (nM) (95% CI)	PKCα IC_{50} (nM)	CYP3A4 % (at 10 μM)	HEK-B2 EC_{50} (μM)
133a	2-CF_3	1.8 (1.6-2.0)	49 (45-54)	NT[b]	83	1.5
133b	2-F-6-F	1.7 (1.5-1.8)	190 (160-220)	NT	49	23

[a]95% confidence intervals (CI) conducted in duplicate (n = 2).

[b]NT means "not tested".

Table 12. Biological Activities of 4-Pyrimidyl-1,2,4-Triazole Derivatives [a]



Compound	R^1	R^2	GRK2 IC_{50} (nM) (95% CI)	ROCK2 IC_{50} (nM) (95% CI)	PKCα IC_{50} (nM)	CYP3A4 % (at 10 μM)	HEK-B2 EC_{50} (μM)
139a	Me	2-CF_3	7.8 (7.0-8.8)	720 (620-830)	>10000	63	7.4
139b	Me	2-F-6-F	6.8 (5.3-8.7)	1900 (1500-2300)	>10000	24	>30 (ND)[c]
139c	*n*-Pr	2-CF_3	1.9 (1.7-2.1)	140 (120-160)	1200	81	3.0
139d	*n*-Pr	2-F-6-F	1.2 (1.0-1.4)	190 (170-220)	250	51	NT[b]

[a]95% confidence intervals (CI) conducted in duplicate (n = 2).

[b]NT means "not tested".

[c]ND means "not determined".

Table 13. Selectivity of selected GRK2 inhibitors for GRK family

Compound	GRK1 IC_{50} (nM) (95% CI)	GRK2 IC_{50} (nM) (95% CI)	GRK3 IC_{50} (nM) (95% CI)	GRK5 IC_{50} (nM) (95% CI)	GRK6 IC_{50} (nM) (95% CI)	GRK7 IC_{50} (nM) (95% CI)
30g	>30000	65 (55-77)	21 (16-30)	>30000	>30000	>30000
30i	>30000	6.1 (4.9-7.4)	1.8 (1.3-2.5)	5700 (4200-7800)	>30000	8800 (5700-14000)
115h	3100 (2200-4200)	18 (15-21)	5.4 (3.4-8.5)	2300 (850-6500)	>30000	25000 (13000-51000)
119a	>30000	7.5 (6.3-8.9)	3.3 (2.1-5.1)	21000 (18000-24000)	>30000	>30000
133a	270 (140-540)	1.8 (1.6-2.0)	0.65 (0.59-0.71)	1500 (1000-2200)	>30000	2400 (1400-4300)
81b	>30000	9.9 (8.1-12)	4.3 (2.9-6.2)	>30000	>30000	18000 (9400-36000)

Figure 4. Section of the X-ray crystal structure of human GRK2 in complex with the small molecule inhibitor **115h**

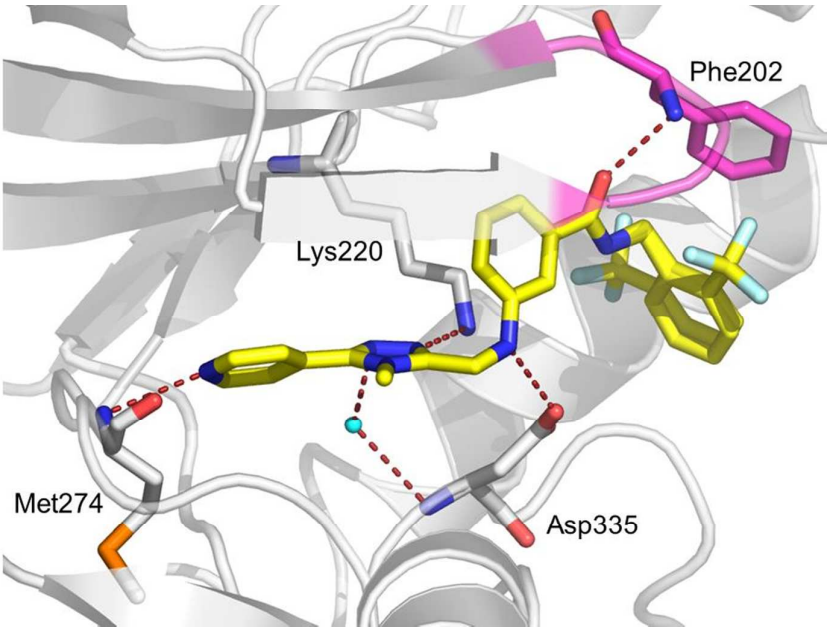


Figure 5. Retardation of the internalization of β-adrenergic receptors by using GRK2 inhibitors (**30i** and **115h**)

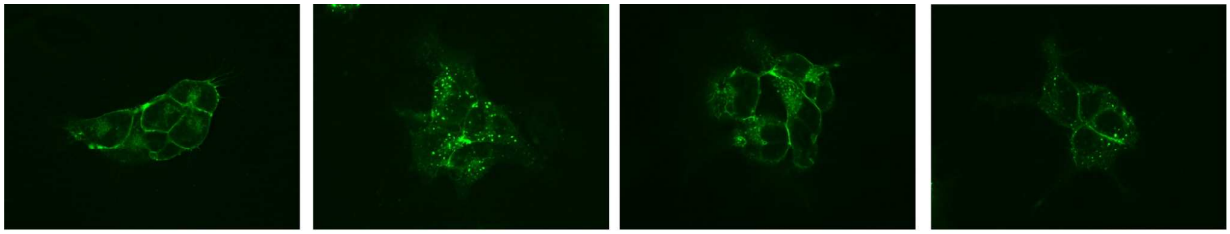
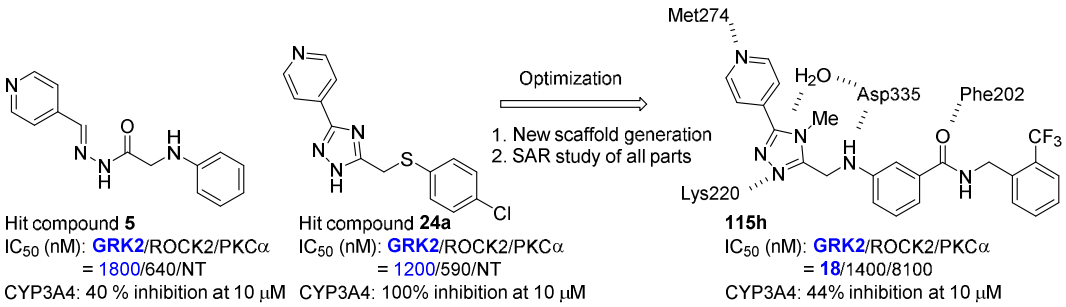


Table of Contents graphic



Hit compound **5**
IC_{50} (nM): **GRK2**/ROCK2/PKCα
= 1800/640/NT
CYP3A4: 40 % inhibition at 10 μM

Hit compound **24a**
IC_{50} (nM): **GRK2**/ROCK2/PKCα
= 1200/590/NT
CYP3A4: 100% inhibition at 10 μM

115h
IC_{50} (nM): **GRK2**/ROCK2/PKCα
= **18**/1400/8100
CYP3A4: 44% inhibition at 10 μM