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FULL PAPER

A scaffold hopping strategy towards the identification of inhibitors of cyclin G associated kinase

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Abstract: We recently reported the discovery of isothiazolo[4,3-*b*]pyridine based inhibitors of cyclin G associated kinase (GAK) displaying low nanomolar binding affinity for GAK and demonstrating broad-spectrum antiviral activity. In order to come up with novel core structures that act as GAK inhibitors, a scaffold hopping approach was applied starting from two different isothiazolo[4,3-*b*]pyridines. In total, 13 novel 5,6- and 6,6-fused bicyclic heteroaromatic scaffolds were synthesized. Four of them displayed GAK affinity with Kd values in the low micromolar range that can serve as chemical starting points for the discovery of GAK inhibitors based on a different scaffold.

Introduction

Cyclin G associated kinase (GAK), also known as auxilin 2, is a cellular serine/threonine protein kinase first identified in 1997.[1] It is highly homologous to auxilin I, with the notable exception of the presence of an *N*-terminal kinase domain on GAK.[2] In contrast with auxilin I, GAK is ubiquitously expressed while auxilin I is solely expressed in neurons. Many biological effects have been attributed to GAK as a result of its abundance. It has been demonstrated that siRNA-mediated GAK knockdown results in mitotic arrest during metaphase and multipolar spindle formation.[3] Due to this mitotic involvement, GAK inhibition represents a possible drug target for the treatment of BXW7-deficient tumours, such as cholangiocarcinoma.[4] GAK was also identified as a potential target for the inhibition of prostate cancer growth in cells expressing androgen receptor splice variants.[5] Furthermore, GAK was found to be involved in osteosarcoma, which is the most frequent primary malignant bone tumour among children. In both osteosarcoma cell lines and tissue samples an overexpression of GAK is seen, compared to human osteoblasts. Its knockdown by siRNA decreased cell proliferation in both drug-sensitive and multidrug-resistant osteosarcoma cell lines, suggesting osteosarcoma growth and proliferation to be GAK dependent.[6]

In Parkinson's disease, the leucine rich repeat kinase 2 (LRRK2) is a major contributor to the illness. It is implicated in vesicle trafficking and was found to interact with GAK, where it plays a role in the dysfunction of LRRK2.[7]

GAK is a key regulator of clathrin-mediated endocytocis (CME) as it is one of the kinases known to phosphorylate Thr144 and Thr156 of the µ-subunits of adaptor protein complex (AP) 1 and AP2, respectively. While GAK-dependent phosphorylation of AP2 is important for its endocytic activity, GAK's phosphorylation of AP1 is implicated in *trans*-golgi network (TGN) to lysosome trafficking. This phosphorylation of APs enhances their binding to cargo proteins and helps the recruitment of clathrin to the membrane to form a clathrin coated vesicle (CCV).[2],[8]–[11] In analogy to auxilin I, GAK contains a *C*-terminal J-domain that acts as a cochaperone with the heat shock cognate 71 Kda protein (HSC70) to uncoat CCVs in order to recycle clathrin back to the cell surface.[2] Depletion of GAK inhibits CME by inhibiting the recruitment of clathrin and clathrin adaptors.[2],[12] Furthermore, we previously discovered that GAK's involvement in CME is required in both early and late stages of the lifecycle of distinct viruses, such as dengue virus (DENV), Ebola virus (EBOV), chikungunya virus (CHIKV), and hepatitis C virus (HCV). [10],[13],[14]

Recently, we reported the discovery of potent GAK inhibitors based on an isothiazolo[4,3-*b*]pyridine scaffold, of which compounds **1, 2** and **3** are the most promising representatives (Figure 1). These compounds display potent GAK affinity (Kd values in the range of 8-80 nM), are selective and are also endowed with broad-spectrum *in vitro* antiviral activity against HCV, DENV, EBOV and CHIKV.[15],[16]

Previous explorations of the structure-activity relationship (SAR) of isothiazolo[4,3-*b*]pyridines as GAK inhibitors focused on modifications of the functional groups at positions 3 and 6 of the scaffold.[15]–[17] In this manuscript, an alternative approach based on scaffold hopping was followed. Scaffold hopping is a widely applied medicinal chemistry strategy in which the core skeleton (the scaffold) of lead molecules is subjected to bioisosteric replacements, leading to the discovery of structurally novel compounds. There are different reasons to search for biologically active compounds based on alternative scaffolds. Examples include the development of analogues with improved activity and/or selectivity, altered physicochemical and ADMET properties, as well as obtaining molecules with a favourable intellectual property situation.[18]

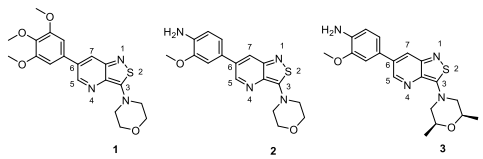


Figure 1. Previously reported isothiazolo[4,3-*b*]pyridines as GAK inhibitors.

A scaffold hopping exercise starts with an active compound and ends up with a novel chemotype by solely modifying the central core structure of the molecule. Two isothiazolo[4,3-*b*]pyridines

with a different substitution pattern were selected as the starting point for the scaffold hopping exercise (Figure 2). Compound **4**[16] bears a 3,4-dimethoxyphenyl residue at position 6, instead of its 3,4,5-trimethoxy (compound **2**) or 3-methoxy-4-amino (compound **2**) counterparts, and displays a Kd value of 52 nM for GAK. Despite the 5-fold lower GAK affinity of compound **4** compared to compounds **1** and **2**, it was selected as a reference compound because of the easy access and low cost of large amounts of 3,4-dimethoxyphenylboronic acid. For some of the scaffolds (such as the pyrazolo[1,5-*a*]pyrimidine, pyrrolo[3,2-*b*]pyridine, pyrazolo[4,3-*b*]pyridine and thieno[3,2-*b*]pyridine), the insertion of a morpholine residue via either a nucleophilic aromatic substitution, a palladium-catalyzed Buchwald reaction or a CuI/L-proline mediated amination was cumbersome. In contrast, the introduction of an aryl group via palladium-catalyzed Suzuki cross-coupling reaction proceeded smoothly. Therefore, compound **5**, characterized by the presence of a phenyl moiety at position 3 (instead of the morpholine residue of compound **4**) was used for comparison purposes.[19] Although, GAK affinity of compound **5** (Kd of 0.77 µM) is 15-fold lower than the corresponding 3-morpholino analogue **4**, the straightforward synthesis of different phenyl-substituted scaffold analogues justifies its use as a reference compound. As the substitution pattern is kept intact, the influence of scaffold modification on GAK affinity can be easily studied.

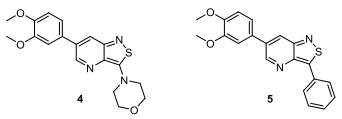
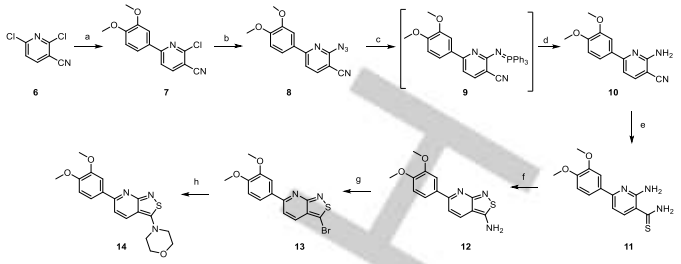


Figure 2. Reference GAK inhibitors for scaffold hopping.

Chemistry

Synthesis of isothiazolo[3,4-b]pyridine

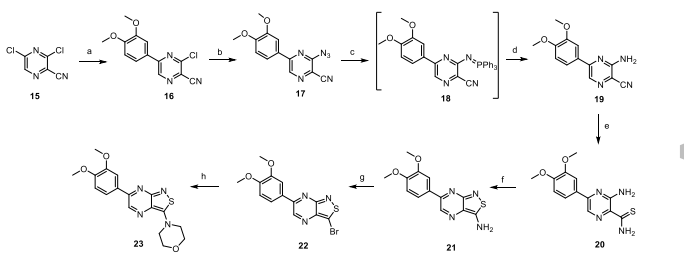
A palladium-catalyzed Suzuki coupling between 2,6-dichloronicotinonitrile **6** and 3,4-dimethoxyphenylboronic acid yielded compound **7** regioselectively (Scheme 1).[20] Nucleophilic displacement of the remaining chlorine at position 2 by ammonia in either water or methanol only led to the recovery of starting material. In contrast, the reaction with sodium azide proceeded smoothly, affording compound **8** in high yield. Staudinger reduction by treatment of compound **8** with triphenylphosphine formed the iminophosphorane intermediate **9**, which was subsequently submitted to an acidic hydrolysis yielding the 2-amino pyridine derivative **10**.[21] The cyano group of compound **10** was converted to its corresponding thioamide **11** using phosphorus pentasulfide. An oxidative ring closure using hydrogen peroxide in methanol allowed to construct the isothiazole moiety, furnishing isothiazolo[3,4-*b*]pyridine **12**. A Sandmeyer reaction using sodium nitrite, hydrogen bromide and CuBr yielded 3-bromo-6-(3,4-dimethoxyphenyl)isothiazolo[3,4-*b*]pyridine **13**. Finally, a nucleophilic aromatic substitution with morpholine afforded the desired isothiazolo[3,4-*b*]pyridine **14**.



Scheme 1. Synthesis of isothiazolo[3,4-*b*]pyridine. *Reagents and conditions:* a) 3,4-dimethoxyphenyl $B(OH)_2$, K_2CO_3, $Pd(PPh_3)_4$, H_2O, dioxane, 90°C (63%); b) NaN_3, DMF, 70°C (83%); c) PPh_3, pyridine, rt; d) 80% aq CH_3COOH, reflux (95%); e) P_2S_5, ethanol, reflux (71%); f) 30% aq. H_2O_2, MeOH, 0°C (83%); g) $NaNO_2$, HBr, CuBr, H_2O, 0°C to rt (25%); h) morpholine, EtOH, reflux (40%).

Synthesis of isothiazolo[3,4-b]pyrazine

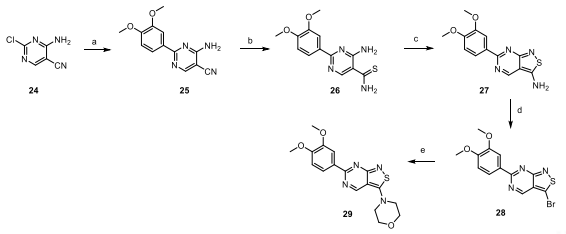
Applying the same aqueous Suzuki conditions that successfully yielded compound **7** (Scheme 1) to the synthesis of compound **16** resulted merely in the formation of different unidentified products. However, the regioselective introduction of a 3,4-dimethoxyphenyl residue on 3,5-dichloropyrazine-2-carbonitrile **15** was possible via an anhydrous Suzuki reaction using tripotassium phosphate as a base affording compound **16** (Scheme 2). The chlorine at position 3 was then exchanged by azide affording compound **17**. The azido moiety was subsequently converted to an amino group via Staudinger reduction with concomitant acidic hydrolysis of the iminophosphorane intermediate **18** yielding pyrazine **19**. Thionation of its cyano moiety yielding thioamide **20** was achieved with Lawesson's reagent (LR) rather than P_2S_5, as the work-up with LR was less labour intensive and consistently led to higher yields. The thioamide **20** was then submitted to an oxidative ring closure furnishing the isothiazolo[3,4-*b*]pyrazine **21**. Applying the typical Sandmeyer reaction conditions (as in Scheme 1) to convert the amino group into a bromine was unsuccessful, as a complex mixture of unidentified products was formed. An alternative Sandmeyer procedure using *tert*-butyl nitrite (*t*-BuONO) and $CuBr_2$ in dry acetonitrile yielded the desired product **22**.[22] Finally, nucleophilic substitution with morpholine yielded the title compound **23** in good yield.



Scheme 2. Synthesis of isothiazolo[3,4-*b*]pyrazine*. Reagents and conditions:* a) 3,4-dimethoxyphenyl- $B(OH)_2$, K_3PO_4, $Pd(PPh_3)_4$, dioxane, 90°C (74%); b) NaN_3, DMF, 70°C (83%); c) PPh_3, pyridine, rt; d) 80% aq CH_3COOH, reflux (95%); d) LR, ethanol, reflux (83%); f) 30% aq. H_2O_2, MeOH, 0°C (67%); g) *t*-BuONO, $CuBr_2$, CH_3CN, rt (32%); h) morpholine, EtOH, reflux (64%).

Synthesis of isothiazolo[3,4-d]pyrimidine

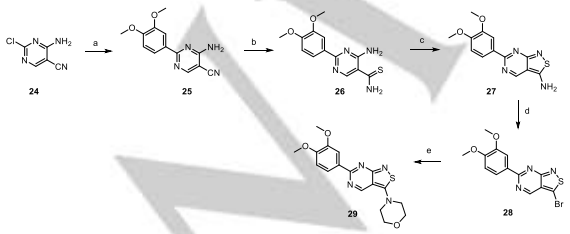
A Suzuki coupling on 4-amino-2-chloropyrimidine-5-carbonitrile **24** using the aforementioned reaction circumstances (Schemes 1 and 2) only resulted in the formation of side products. The presence of a free amino group is known to hamper Suzuki reactions and a common practice to circumvent this is by protecting the amino group.[23] However, additional protecting and deprotecting sequences are time consuming and lead to overall lower reaction yields. Therefore, alternative reaction conditions were explored. Performing the Suzuki reaction using tripotassium phosphate as a base, $Pd(OAc)_2$ as a catalyst and 1,1′-bis(di-*tert*-butylphosphino)ferrocene (D-t-BPF) as a ligand provided compound **25** efficiently (Scheme 3).[23] The subsequent steps are similar as in Schemes 1 and 2. Briefly, thioamide **26** was accessible via thionation of the cyano group of compound **25**. The isothiazolo moiety was then constructed via an oxidative ring closure yielding 3-amino-isothiazolo[3,4-*d*]pyrimidine **27**. Conversion of the amino group to a bromine **28**, followed by the introduction of a morpholine moiety, gave access to isothiazolo[3,4-*d*]pyrimidine **29**.



Scheme 3. Synthesis of isothiazolo[3,4-*d*]pyrimidine. *Reagents and conditions*: a) 3,4-dimethoxyphenyl- $B(OH)_2$, K_3PO_4, $Pd(OAc)_2$, D-t-BPF, dioxane (53%); b) LR, ethanol, reflux (60%); c) 30% aq. H_2O_2, MeOH, 0°C (84%); d) t-BuONO, CuB_{r2}, CH_3CN, rt (49%); e) morpholine, EtOH, reflux (65%).

Synthesis of pyrazolo[1,5-a]pyrimidine

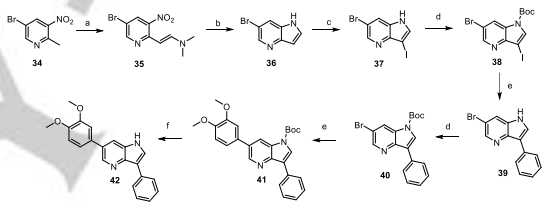
Condensation of 4-bromo-1H-pyrazol-3-amine 30 and 2-chloromalonaldehyde under acidic conditions afforded 3-bromo-6-chloropyrazolo[1,5-a]pyrimidine 31 (Scheme 4).[24] Two consecutive Suzuki reactions allowed to introduce both aromatic substituents regioselectively, as bromine is favoured over chlorine during the oxidative addition step.[25] The first Suzuki coupling yielded selectively the 3-phenyl-pyrazolo[1,5-a]pyrimidine 32. A subsequent Suzuki coupling under identical reaction conditions, except for using 3,4-dimethoxyphenylboronic acid, yielded the desired pyrazolo[1,5-a]pyrimidine 33 in good yield.



Scheme 4. Synthesis of pyrazolo[1,5-*a*]pyrimidine. *Reagents and conditions*: a) 2-chloromalonaldehyde, MeOH, CH_3COOH, 70°C (57%); b) phenyl-$B(OH)_2$, K_2CO_3, $Pd(PPh_3)_4$, H_2O, dioxane (56%); c) 3.4-dimethoxyphenyl-$B(OH)_2$, K_2CO_3, $Pd(PPh_3)_4$, H_2O, dioxane (27%).

Synthesis of pyrrolo[3,2-b]pyridine

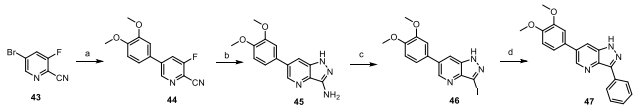
In analogy to the Lemgruber-Batcho synthesis of indoles, the synthesis of this scaffold started by reacting 5-bromo-2-methyl-3-nitropyridine **34** with *N,N*-dimethylformamide dimethyl acetal (DMF-DMA) to give enamine **35** (Scheme 5).[26] Reductive cyclization of enamine **35** using iron in acetic acid led to the formation of pyrrolo[3,2-*b*]pyridine **36**. Electrophilic iodination using *N*-iodosuccinimide (NIS) in THF yielded the 3-iodo congener **37** in low yield. Suzuki reaction using **37** as substrate, with the classical reaction conditions (Scheme 1) did not yield the desired product and only starting material was recovered. Protection of the pyrrole nitrogen prior to the palladium-catalyzed cross-coupling reaction turned out to be necessary. The pyrrole nitrogen was protected by reaction of **37** with di-*tert*-butyl carbonate (Boc_2O), yielding the Boc-protected derivative **38**. Suzuki coupling of **38** with phenylboronic acid proceeded with concomitant cleavage of the Boc group, yielding compound **39**. Reprotection with Boc (compound **40)** and subsequent Suzuki coupling furnished compound **41**. Remarkably, during this last Suzuki reaction, no Boc deprotection was observed, despite using the same reaction conditions as in step (e). Finally, acidic hydrolysis using a 4N HCl solution in dioxane yielded pyrrolo[3,2-*b*]pyridine **42** in moderate yield.



Scheme 5. Synthesis of pyrrolo[3,2-*b*]pyridine. *Reagents and conditions*. a) DMF-DMA, DMF, 90°C (98%); b) Fe, CH_3COOH, rt (36%); c) NIS, THF, rt (37%); d) Boc_2O, TEA, DMAP, THF, rt (95%); e) phenyl-$B(OH)_2$ (70%) or 3,4-dimethoxyphenyl-$B(OH)_2$ (15%), K_2CO_3, $Pd(PPh_3)_4$, H_2O, dioxane; f) 4N HCl in dioxane, reflux (47%).

Synthesis of pyrazolo[4,3-b]pyridine

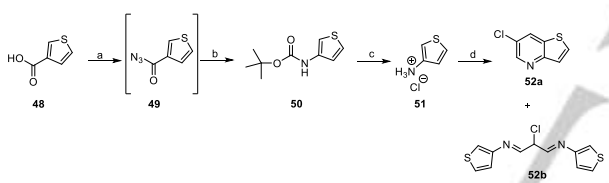
Suzuki coupling between 5-bromo-3-fluoropicolinonitrile **43** and 3,4-dimethoxyphenylboronic acid proceeded smoothly yielding compound **44** in excellent yield (Scheme 6). An aromatic nucleophilic substitution with hydrazine and consecutive ring closure gave access to the pyrazolo[4,3-*b*]pyridine scaffold **45**.[27] Several attempts to convert the amino group into a bromine via Sandmeyer reactions (using the conditions from Schemes 1 or 2) resulted in unidentified side products. Instead, a diazotation reaction using sodium nitrite, *para*-toluenesulphonic acid (*p*-TsOH) and potassium iodide was used[28], yielding the 3-iodo pyrazolo[4,3-*b*]pyridine analogue **46** in low yield. Finally, introduction of a phenyl moiety via a Suzuki coupling yielded the title compound **47** in moderate yield. Noteworthy, unlike in Scheme 5, the Suzuki coupling of pyrazolo[4,3-*b*]pyridine **46** did not require Boc protection of its pyrrole-like nitrogen for the reaction to be successful.



Scheme 6. Synthesis of pyrazolo[4,3-*b*]pyridine. *Reagents and conditions:* a) 3,4-dimethoxyphenyl- $B(OH)_2$, K_2CO_3, $Pd(PPh_3)_4$, H_2O, dioxane (86%); b) $NH_2NH_2.H_2O$, *n*-BuOH, 121°C (83%); c) *p*-TsOH, KI, $NaNO_2$, CH_3CN, rt (28%); d) phenyl-$B(OH)_2$, K_2CO_3, $Pd(PPh_3)_4$, H_2O, dioxane, MW, 120°C (23%).

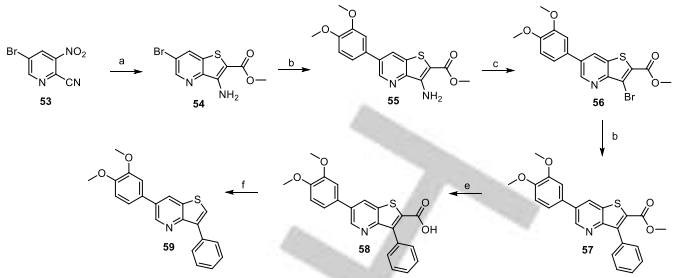
Synthesis of thieno[3,2-b]pyridine

The synthesis started from 3-amino-thiophene **51**, that was formed by reaction of thiophene-3-carboxylic acid **48** with diphenyl phosphoryl azide (DPPA) resulting in the acyl azide intermediate **49** (Scheme 7). This was subsequently submitted to a Curtius rearrangement by addition of dry *t*-BuOH yielding a Boc-protected 3-amino-thiophene **50**.[24] The use of dry *t*-BuOH was essential to prevent the formation of thiophene-3-carbamoylic acid. Deprotection of Boc using a 4N HCl solution in dioxane yielded 3-amino-thiophene hydrochloride **51**, which turned out to be UV-sensitive and needed to be protected from light. Reaction of **51** with 2-chloromalonaldehyde did not result in the formation of the desired thieno[3,2-*b*]pyridine scaffold **52a**, as mass spectral analysis showed 2-chloro-*N*1,*N*3-di(thiophen-3-yl)propane-1,3-diimine **52b** to be the main product. To avoid this side reaction a different synthetic route was chosen starting from the pyridine analogue **53** (Scheme 8).



Scheme 7. Attempted synthesis of thieno[3,2-*b*]pyridine. *Reagents and conditions:* a) DPPA, DIPEA, DMF, rt; b) *t*-BuOH, reflux (61%); c) 4N HCl in dioxane, reflux (100%); d) 2-chloromalonaldehyde, CH_3COOH/MeOH (2:3), 70°C.

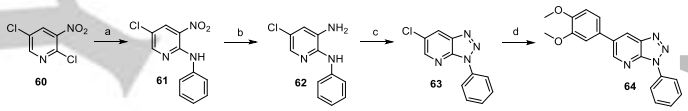
Reaction of pyridine **53** with ethyl 2-mercaptoacetate and potassium *tert*-butoxide (KOtBu) as a strong base afforded thieno[3,2-*b*]pyridine **54**.[29] Introduction of a 3,4-dimethoxyphenyl residue at position 6 of the scaffold required harsher reaction conditions compared to previous scaffolds. Higher temperature (110 °C) and a longer reaction time (~3 days) allowed to isolate the desired compound **55** in moderate yield. A classical Sandmeyer reaction using conditions from Scheme 2, followed by a Suzuki coupling, yielded product **57**. Hydrolysis of the ester using NaOH in EtOH yielded the carboxylic acid **58** as a pink precipitate. Refluxing compound **58** in a concentrated HCl solution was not able to achieve decarboxylation of the carboxylic acid group. Instead, only harsh conditions of metallic copper in quinoline at 200°C yielded the title compound **59**, albeit in low yield.[30]



Scheme 8. Synthesis of thieno[3,2-*b*]pyridine. *Reagents and conditions*. a) ethyl 2-mercaptoacetate, KOtBu, DMF (54%); b) 3,4-dimethoxyphenyl-$B(OH)_2$ (76%) or phenyl-$B(OH)_2$ (42%), K_3PO_4, $Pd(PPh_3)_4$, dioxane, 110°C; c) *t*-BuONO, $CuBr_2$, CH_3CN (47%); d) NaOH, EtOH, rt (89%); e) Cu, quinoline, 200°C (48%).

Synthesis of [1,2,3]triazolo[4,5-b]pyridine

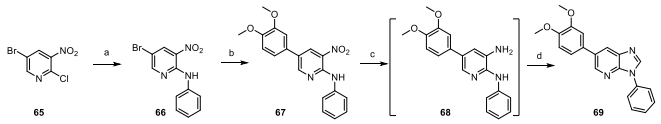
Nucleophilic displacement of the chlorine at position 2 of 2,5-dichloro-3-nitropyridine **60** by aniline furnished substitution product **61** (Scheme 9).[31] Catalytic reduction of the nitro group with Raney nickel under a hydrogen atmosphere yielded the 3-amino-pyridine **62**. Formation of the triazole ring was achieved by a diazotation reaction using sodium nitrite in acetic acid with concomitant cyclisation yielding the [1,2,3]triazolo[4,5-*b*]pyridine scaffold **63**. Finally, a Suzuki coupling afforded the target compound **64**.



Scheme 9. Synthesis of [1,2,3]triazolo[4,5-*b*]pyridine. *Reagents and conditions*: a) aniline, DIPEA, dioxane, 80°C (85%°; b) Raney nickel, H_2, THF, rt; c) $NaNO_2$, acetic acid, CH_2Cl_2, water (62%); d) 3,4-dimethoxyphenyl-$B(OH)_2$, Cs_2CO_3, $Pd(PPh_3)_4$, H_2O, DMF (78%).

Synthesis of imidazo[4,5-b]pyridine

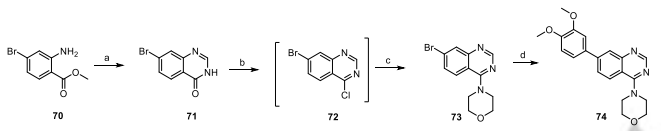
Initial attempts for the synthesis of the imidazo[4,5-*b*]pyridine scaffold followed a similar procedure as in Scheme 9. Reaction of compound **62** with triethyl orthoformate allowed to build up the imidazole ring and afforded 6-chloro-3-phenyl-imidazo[4,5-b]pyridine. Unfortunately, despite exploring different reaction conditions, the subsequent Suzuki coupling never yielded the desired compound. Therefore, an alternative synthetic route was developed in which the 3,4-dimethoxyphenyl residue was introduced early on in the synthetic sequence.[32] As the Suzuki coupling on the brominated pyridine analogue was consistently higher yielding, 5-bromo-2-chloro-3-nitropyridine **65** was selected as starting material. Introduction of an anilino moiety at position 2 by nucleophilic aromatic substitution afforded compound **66** (Scheme 10). Suzuki coupling formed the addition product **67** in good yield. For the reduction of the nitro group, we opted for zinc in acetic acid as this proceeded faster and cleaner than catalytic hydrogenation. The amino intermediate **68** was used as such without further purification for the formation of the imidazole moiety. Reaction of **68** with triethyl orthoformate afforded imidazo[4,5-*b*]pyridine **69** in low yield.



Scheme 10. Synthesis of imidazo[4,5-*b*]pyridine. *Reagents and conditions*: a) aniline, DIPEA, NMP, 120°C (78%); b) 3,4-dimethoxyphenyl-B(OH)$_2$, K_2CO_3, Pd(PPh$_3$)$_4$, H_2O, dioxane (78%); c) Zn, CH_3COOH; d) CH(OEt)$_3$, 130°C (35%).

Synthesis of quinazoline

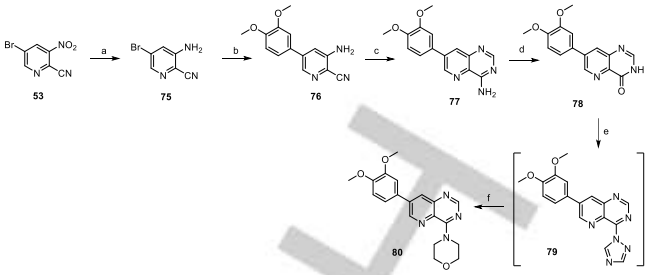
Condensation of methyl 2-amino-4-bromobenzoate **70** with triethyl orthoformate as a one-carbon fragment and ammonium acetate as nitrogen source allowed the isolation of 7-bromoquinazolin-4(3H)-one **71** (Scheme 11).[33] The tautomeric hydroxyl group of the lactam moiety was converted into a chlorine by treatment with phosphorus oxychloride, yielding a 4-chloro-quinazoline analogue **72**.[34] A nucleophilic aromatic substitution with morpholine yielded the 4-morpholino-quinazoline **73**. Coupling of **73** with 3,4-dimethoxyphenylboronic acid using the aforementioned conditions for the Suzuki reaction furnished quinazoline **74** in good yield.



Scheme 11. Synthesis of quinazoline. *Reagents and conditions* a) NH_4OAc, CH(OEt)$_3$, reflux (60%); b) $POCl_3$, reflux; c) morpholine, rt (57%); d) 3,4-dimethoxyphenyl-B(OH)$_2$, K_2CO_3, Pd(PPh$_3$)$_4$, H_2O, dioxane, 90°C (75%).

Synthesis of pyrido[3,2-d]pyrimidine

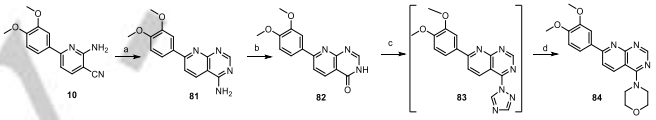
First, the nitro group of commercially available 3-nitro-5-bromopyridine-2-carbonitrile **53** was reduced by treatment with iron under acidic conditions, generating the desired aniline **75** as the major product (Scheme 12). However, due to the acid hydrolysis of the cyano group, a substantial amount (12%) of 3-amino-5-bromopicolinamide was also formed. These two compounds were easily separated by silica gel flash chromatography. Introduction of a 3,4-dimethoxyphenyl moiety on **75** via a Suzuki coupling yielded compound **76**. Construction of the pyrimidine moiety using triethyl orthoformate, as performed before for the synthesis of the quinazoline analogue, did not result in the isolation of the desired product. In contrast, heating of compound **76** in formamide (which functions both as a reagent and solvent) under microwave conditions furnished pyrido[3,2-*d*]pyrimidine 7**7** as a brown precipitate in excellent yield and the product was used as such without further purification.[35] Acid hydrolysis of the 4-amino group of **77** afforded pyrido[3,2-*d*]pyrimidin-4(3H)-one **78**. Phosphorus oxychloride mediated chlorination of compound **78** turned out to be cumbersome as no product was retrieved, even though starting material appeared to be fully consumed. Instead of chloride, 1,2,4-triazole was introduced as a leaving group successfully yielding intermediate **79**. Subsequent substitution with morpholine yielded the title compound **80** in moderate yield**.**



Scheme 12. Synthesis of pyrido[3,2-*d*]pyrimidine. *Reagents and conditions*: a) Fe, CH_3COOH, rt (56%); b) 3,4-dimethoxyphenyl-B(OH)$_2$, K_2CO_3, Pd(PPh$_3$)$_4$, H_2O, dioxane, 90°C (98%); c) formamide, 160°C, µW (74%); d) 6N HCl, reflux (66%); e) $POCl_3$, 1,2,4-triazole, DIPEA, CH_3CN; f) morpholine, dioxane, reflux (25%).

Synthesis of pyrido[2,3-d]pyrimidine

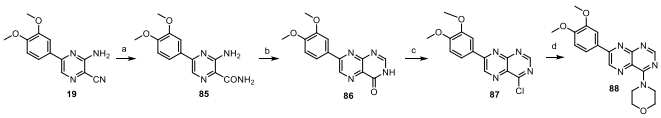
Starting from pyridine analogue **10** (synthesized in Scheme 1), the construction of the pyrimidine moiety and the introduction of morpholine was performed in analogy to Scheme 12, yielding the title compound **84** in moderate yield (Scheme 13).



Scheme 13. Synthesis of pyrido[2,3-*d*]pyrimidine. *Reagents and conditions*: a) formamide, 160°C, µW (46%); b) HCl 6N, reflux (60%); c) $POCl_3$, 1,2,4-triazole, DIPEA, CH_3CN; d) morpholine, dioxane, 60 °C (19%).

Synthesis of pteridine

Heating of compound **19** (available from Scheme 3) in formamide (as in Schemes 12 and 13) did not allow to form the pyrimidine moiety and only starting material was recovered. Alternatively, attempts to build up the pyrimidine ring using concentrated formic acid and concentrated sulphuric acid under microwave irradiation,[22],[36] solely led to the hydrolysis of the cyano group affording carboxamide **85** as a bright yellow precipitate (Scheme 14). The subsequent ring closure by treatment with formamide afforded pteridin-4(3*H*)-one **86**. Chlorination with phosphorus oxychloride yielded the 4-chloro-pteridine **87,** that was sufficiently stable to be purified by silica gel flash chromatography. Finally, introduction of morpholine yielded the target compound **88**.



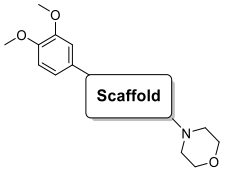
Scheme 14. Synthesis of pteridine. *Reagents and conditions*: a) HCOOH, H_2SO_4, 120°C, µW (78%); b) formamide, 160°C, µW (71%); c) $POCl_3$, toluene, reflux, rt (55%); d) morpholine, dioxane, reflux (97%).

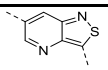
Biological evaluation and SAR

All compounds were tested for GAK binding affinity using the KINOME*scan*™ platform, that quantitatively measures the ability of a compound to compete with an immobilized active-site-

directed ligand.[37] In an initial series of compounds, the focus was on structural modification of the pyridine moiety of the isothiazolo[4,3-*b*]pyridine scaffold. Shuffling the position of the nitrogen yielded isothiazolo[3,4-*b*]pyridine **14**, displaying a 75-fold drop in GAK affinity, when compared to the parent isothiazolo[4,3-*b*]pyridine scaffold. Insertion of an additional nitrogen atom in the pyridine moiety of compound **14** yielded a pyrazine and pyrimidine analogue. The isothiazolo[3,4-*d*]pyrimidine **29** (Kd = 3.2 µM) is equally active as GAK ligand as isothiazolo[3,4-*b*]pyridine **14** (Kd = 3.9 µM), whereas isothiazolo[3,4-*b*]pyrazine **23** (Kd = 0.36 µM) is 10-fold more potent than isothiazolo[3,4-*b*]pyridine **14**, but still shows a 10-fold decreased GAK affinity when compared to the original isothiazolo[4,3-*b*]pyridine skeleton. Overall, the SAR of the pyridine moiety points towards an essential role of the nitrogen at position 4 of the scaffold.

Table 1. SAR of the pyridine moiety of the isothiazolo[4,3-b]pyridine scaffold.

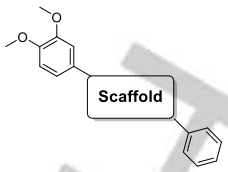


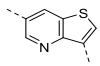
Compound#	Name scaffold	Scaffold	GAK affinity Kd (µM)[a]
4	isothiazolo[4,3-*b*]pyridine		0.052
14	isothiazolo[3,4-*b*]pyridine		3.9
23	isothiazolo[3,4-*b*]pyrazine		0.36
29	isothiazolo[3,4-*d*]pyrimidine		3.2

[a]Kd = dissociation constant. Values represent the average of two independent experiments.

To further evaluate the SAR with respect to GAK binding, the pyridine moiety was kept intact and the isothiazolo moiety was replaced by a number of five-membered heteroaromatic rings (Table 2). As mentioned before in the introduction, introducing morpholine turned out to be problematic and therefore, the 3-phenyl-isothiazolo[4,3-*b*]pyridine **5** (Kd of 0.77 µM) was selected as a reference compound. Neither the pyrrolo[3,2-*b*]pyridine **42**, pyrazolo[4,3-*b*]pyridine **47,** thieno[3,2-*b*]pyridine **59** or [1,2,3]triazolo[4,5-*b*]pyridine **64** were able to reach the GAK affinity value of the reference compound **5**. In contrast, pyrazolo[1,5-*a*]pyrimidine **33** and imidazo[4,5-*b*]pyridine **69** were the only scaffolds with a similar GAK affinity as isothiazolo[4,3-*b*]pyridine **5**.

Table 2. SAR of the isothiazolo moiety of the isothiazolo[4,3-*b*]pyridine scaffold

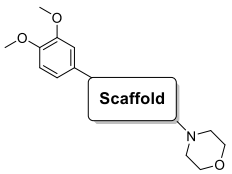


Compound#	Name scaffold	Scaffold	GAK affinity Kd (µM)[a]
5	isothiazolo[4,3-*b*]pyridine		0.77
33	pyrazolo[1,5-*a*]pyrimidine		0.69
42	pyrrolo[3,2-*b*]pyridine		4
47	pyrazolo[4,3-*b*]pyridine		1.7
59	thieno[3,2-*b*]pyridine		26
64	[1,2,3]triazolo[4,5-*b*]pyridine		>30
69	imidazo[4,5-*b*]pyridine		0.14

[a]Kd = dissociation constant. Values represent the average of two independent experiments.

Finally, the 6-5 bicyclic isothiazolo[4,3-*b*]pyridine core was replaced by a number of 6-6 bicyclic structures (Table 3). Keeping the pyridine moiety of isothiazolo[4,3-*b*]pyridine intact and replacing the isothiazole moiety by pyrimidine afforded the pyrido[3,2-*d*]pyrimidine **78**, which is endowed with a Kd value of 7.3 µM, and hence displays a 140-fold drop in GAK affinity when compared to the original isothiazolo[4,3-*b*]pyridine skeleton. Switching the position of the nitrogen in the pyridine ring (yielding pyrido[2,3-*d*]pyrimidine **84**) or insertion of an additional nitrogen (affording pteridine **88**) afforded scaffolds that were completely lacking GAK affinity (Kd of 20 µM or more). On the other hand, quinazoline **74** shows only a 10-fold drop in GAK affinity (Kd = 0.62 µM).

Table 3. SAR of 6-6 bicyclic scaffolds



Compound#	Name scaffold	Scaffold	GAK affinity Kd (μM)[a]
4	isothiazolo[4,3-*b*]pyridine		0.052
74	quinazoline		0.62
78	pyrido[3,2-*d*]pyrimidine		7.3
84	pyrido[2,3-*d*]pyrimidine		20
88	pteridine		25

[a]Kd = dissociation constant. Values represent the average of two independent experiments.

Conclusion

A number of 5,6- and 6,6- fused bicyclic heteroaromatic scaffolds that bear essential structural features for GAK affinity and that are based on the original isothiazolo[4,3-b]pyridine scaffolds **4** and **5** were synthesized. Four of the 13 novel scaffolds (isothiazolo[3,4-*b*]pyrazine **23**, pyrazolo[1,5-*a*]pyrimidine **33** imidazo[4,5-*b*]pyridine **69** and quinazoline **74**) displayed GAK affinity with Kd values under 1 μM. Although these compounds are not potent enough to serve as chemical tools to decipher GAK biology in a cellular context, they can serve as starting point for medicinal chemists to further improve the GAK binding affinity by modification of the decoration pattern.

Moreover, this manuscript also describes the synthesis of new scaffolds that are underrepresented in organic/medicinal chemistry. Examples include the isothiazolo[3,4-*b*]pyridine (206 known compounds according to SciFinder), isothiazolo[3,4-*b*]pyrazine (25 known derivatives according to SciFinder) and isothiazolo[3,4-*d*]pyrimidine (80 known analogues in SciFinder).

Experimental section

For all reactions, analytical grade solvents were used. All moisture-sensitive reactions were carried out in oven-dried glassware (125 °C). All microwave irradiation experiments were carried out in a dedicated CEM-Discover monomode microwave apparatus, operating at a frequency of 2.45 GHz with continuous irradiation power from 0 to 300 W utilizing the standard absorbance level (300 W maximum power). The reactions were carried out in 10 mL glass tubes, sealed with an aluminum/Teflon crimp top, which can be exposed to 250 °C and 20 bar internal pressure. The temperature was measured with an IR sensor on the outer surface of the process vial. After the irradiation period, the reaction vessel was cooled rapidly (60–120 s) to ambient temperature by gas jet cooling. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 MHz instrument (^{1}H NMR, 300 MHz; ^{13}C NMR, 75 MHz), 500 MHz instrument (^{1}H NMR, 500 MHz; ^{13}C NMR, 125 MHz) or a 600 MHz instrument (^{1}H NMR, 600 MHz; ^{13}C NMR, 150 MHz), using tetramethylsilane as internal standard for ^{1}H NMR spectra and DMSO-d_6 (39.5 ppm) or $CDCl_3$ (77.2 ppm) for ^{13}C NMR spectra. Abbreviations used are s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad. Coupling constants are expressed in Hz. High resolution mass spectra were acquired on a quadrupole orthogonal acceleration time-of-flight mass spectrometer (Synapt G2 HDMS, Waters, Milford, MA). Samples were infused at 3uL/min and spectra were obtained in positive or negative ionization mode with a resolution of 15000 (FWHM) using leucine enkephalin as lock mass. Precoated aluminum sheets (Fluka silica gel/TLC-cards, 254 nm) were used for TLC. Column chromatography was performed on silica gel 0.060 – 0.200 mm, 60 Å (Acros Organics). Purity of final compounds was verified to be >95% by HPLC analysis. HPLC conditions to asses purity were as follows: Shimadzu HPLC equipped with a LC-20AT pump, DGU-20A5 degasser, and a SPD-20A UV-VIS detector; Symmetry C18 column (5 μm, 4.6 mm × 150 mm); gradient elution of H_2O/CH_3CN from 95/5 or 70/30 to 5/95 over 25 minutes; flow rate 1 mL/min; wavelength, UV 254 nm. Preparative HPLC purifications were performed using a Phenomenex Gemini 110A column (C18, 10 μM, 21.2 mm x 250 mm).

Chemistry

2-Chloro-6-(3,4-dimethoxyphenyl)nicotinonitrile (7)

To a solution of 2,6-dichloronicotinonitrile **6** (2.0 g, 11.56 mmol) in dioxane (40 mL) and water (10 mL) were added 3,4-dimethoxyphenyl boronic acid (2.5 g, 13.87 mmol), potassium carbonate (3.2 g, 23.12 mmol), and $Pd(PPh_3)_4$ (134 mg, 0,12 mmol). The reaction mixture was stirred at 90 °C for 3 hours. The volatiles were evaporated and the crude residue was purified by silica gel flash column chromatography (using a mixture of dichloromethane/methanol 99:1) yielding the title compound (2.7 g, 63%). ^{1}H NMR (300 MHz, DMSO) δ 8.46 (d, *J* = 8.2 Hz, 1H), 8.21 (d, *J* = 8.3 Hz, 1H), 7.79 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.69 (d, *J* = 2.0 Hz, 1H), 7.12 (d, *J* = 8.6 Hz, 1H), 3.87 (s, 3H), 3.85 (s, 3H) ppm; ^{13}C NMR (75 MHz, DMSO) δ 159.71, 151.89, 150.95, 149.21, 144.37, 127.95, 121.18, 118.56, 115.65, 111.93, 110.33, 106.55, 55.79 ppm; HRMS m/z $[M+H]^+$ calcd for $C_{14}H_{11}Cl_1N_2O_2$: 275.0582, found: 275.0589.

2-Azido-6-(3,4-dimethoxyphenyl)nicotinonitrile (8)

To a solution of 2-chloro-6-(3,4-dimethoxyphenyl)nicotinonitrile **7** (2.0 g, 7.80 mmol) in DMF (25 mL) was added NaN_3 (812 mg, 12.48 mmol). The mixture was stirred at 70°C for 4 hours upon which a bright yellow precipitate formed. The mixture was allowed to cool to room temperature, the precipitate was filtered off and carefully washed with water to yield the title compound (1.8 g, 83%).

^{1}H NMR (300 MHz, DMSO) δ 8.78 – 8.55 (m, 1H), 7.96 – 7.65 (m, 3H), 7.47 – 7.19 (m, 1H), 3.91 (s, 3H), 3.88 (s, 3H) ppm; HRMS m/z $[M+H]^+$ calcd for $C_{14}H_{11}N_5O$: 282.0985, found: 282.0985.

2-Amino-6-(3,4-dimethoxyphenyl)nicotinonitrile (10)

To a solution of 2-azido-6-(3,4-dimethoxyphenyl)nicotinonitrile **8** (1.5 g, 5.33 mmol) in pyridine was added triphenyl phosphine (2.2 g, 8.53 mmol) and the mixture was stirred at room temperature overnight. After disappearance of the starting material the volatiles were evaporated and the crude residue was dissolved in an 80% solution of acetic acid in water and refluxed for 1 hour. The volatiles were evaporated *in vacuo* and the crude residue was purified by silica gel flash column chromatography (eluting with a gradient of dichloromethane/ethyl acetate in a ratio of 99:1 to 95:5) to yield the title compound (1.92 g, 95%).

^{1}H NMR (300 MHz, DMSO) δ 7.87 (d, *J* = 8.1 Hz, 1H), 7.67 (d, *J* = 1.8 Hz, 1H), 7.62 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.19 (d, *J* = 8.1 Hz, 1H), 7.01 (d, *J* = 8.5 Hz, 1H), 6.89 (bs, 2H), 3.84 (s, 3H), 3.81 (s, 3H) ppm; ^{13}C NMR (75 MHz, DMSO) δ 170.11, 159.71, 158.91, 150.82, 148.89, 142.83, 130.25, 120.31, 117.67, 111.55, 110.41, 108.16, 87.08, 55.65 ppm; HRMS m/z $[M+H]^+$ calcd for $C_{14}H_{13}N_3O_2$:256.1080, found: 256.1085.

2-Amino-6-(3,4-dimethoxyphenyl)pyridine-3-carbothioamide (11)

To a solution of 2-amino-6-(3,4-dimethoxyphenyl)nicotinonitrile **10** (500 mg, 1.96 mmol) in ethanol (25 mL) was added P_2S_5 (1.74 g, 7.84 mmol) and the mixture was stirred at reflux overnight. After disappearance of the starting material, the volatiles were evaporated and the crude residue was purified by silica gel flash column chromatography (eluting with a mixture of dichloromethane/acetone in a ratio of 90:10) to yield the title compound (400 mg, 71%).

^{1}H NMR (300 MHz, DMSO) δ 9.70 (bs, 1H), 9.43 (bs, 1H), 7.75 – 7.57 (m, 3H), 7.22 – 7.12 (m, 3H), 7.04 (d, *J* = 8.4 Hz, 1H), 3.85 (s, 3H), 3.82 (s, 3H) ppm; ^{13}C NMR (75 MHz, DMSO) δ 198.24, 157.11, 156.33, 150.28, 148.87, 136.13, 130.94, 119.72, 115.66, 111.70, 110.22, 107.65, 55.69; HRMS m/z $[M+H]^+$ calcd for $C_{14}H_{15}N_3O_2S_1$: 290.0958, found: 290.0959.

6-(3,4-Dimethoxyphenyl)isothiazolo[3,4-*b*]pyridin-3-amine (12)

To a solution of 2-amino-6-(3,4-dimethoxyphenyl)pyridine-3-carbothioamide **11** (300 mg, 1.04 mmol) in methanol (5 mL) at 0°C was added dropwise a 30% aqueous H_2O_2 solution (354 µL, 4.15 mmol) and the mixture was stirred overnight at room temperature. After disappearance of the starting material, the mixture was cooled to 0°C and the precipitate was filtered off and washed with cold methanol to yield the title compound (250 mg, 83%).

^{1}H NMR (300 MHz, DMSO) δ 8.29 (d, *J* = 8.6 Hz, 1H), 8.13 (bs, 2H), 7.80 (s, 1H), 7.75 (d, *J* = 8.3 Hz, 1H), 7.50 (d, *J* = 8.8 Hz, 1H), 7.08 (d, *J* = 8.3 Hz, 1H), 3.88 (s, 3H), 3.84 (s, 3H) ppm; ^{13}C NMR (75 MHz, DMSO) δ 173.76, 164.69, 159.85, 150.93, 149.03, 131.91, 131.03, 120.78, 111.65, 110.94, 110.50, 109.72, 55.73, 55.68 ppm; HRMS m/z $[M+H]^+$ calcd for $C_{14}H_{13}N_3O_2S_1$: 288.0801, found: 288.0802.

3-Bromo-6-(3,4-dimethoxyphenyl)isothiazolo[3,4-*b*]pyridine (13)

A solution of 6-(3,4-dimethoxyphenyl)isothiazolo[3,4-*b*]pyridin-3-amine **12** (200 mg, 0.70 mmol) in HBr (5 mL) was stirred for 10 min at room temperature, then CuBr was added at once (200 g, 1.40 mmol). The resulting mixture was cooled to 0 °C and solution of sodium nitrite (146 g, 2.10 mmol) in H_2O (5 mL) was added dropwise over a period of 15 min. The reaction mixture was stirred for 4 h at 0 °C followed by overnight at room temperature. After disappearance of the starting material, the mixture was cooled to 0 °C and carefully neutralized using Na_2CO_3. The neutralised mixture was washed with ethyl acetate 3 times, organic phases were collected and concentrated *in vacuo*. The crude residue was purified by silica gel flash column chromatography (eluting with a mixture of hexane/acetone in a ratio of 80:20) yielding the title compound (60 mg, 25%).

HRMS m/z $[M+H]^+$ calcd for $C_{14}H_{11}Br_1N_2O_2S_1$: 350.9798, found: 350.9801

4-(6-(3,4-Dimethoxyphenyl)isothiazolo[3,4-*b*]pyridin-3-yl)morpholine (14)

To a solution of 3-bromo-6-(3,4-dimethoxyphenyl)isothiazolo[3,4-*b*]pyridine **13** (50 mg, 0.14 mmol) in ethanol (5 mL) was added morpholine (37 mg, 0.43 mmol). The reaction was stirred at reflux for 4 days. After disappearance of the starting material volatiles were evaporated *in vacuo* and the crude residue was purified by silica gel flash column chromatography (eluting with a mixture of dichloromethane/methanol in a ratio of 97:3) to yield the title compound (20 mg, 40%).

^{1}H NMR (300 MHz, DMSO) δ 8.41 (d, *J* = 9.1 Hz, 1H), 7.84 (d, *J* = 1.9 Hz, 1H), 7.79 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.58 (d, *J* = 9.2 Hz, 1H), 7.09 (d, *J* = 8.5 Hz, 1H), 3.98 – 3.81 (m, 10H), 3.54 (m, 4H) ppm; ^{13}C NMR (75 MHz, DMSO) δ 175.75, 166.13, 159.37, 151.12, 149.11, 132.68, 130.71, 120.92, 112.38, 111.68, 110.37, 109.96, 65.38, 55.76, 55.71, 50.66 ppm; HRMS m/z $[M+H]^+$ calcd for $C_{18}H_{19}N_3O_3S_1$: 358.1220, found: 358.1219.

3-Chloro-5-(3,4-dimethoxyphenyl)pyrazine-2-carbonitrile (16)

To a solution 3,5-dichloropyrazine-2-carbonitrile **15** (2.0 g, 11.50 mmol) in dioxane (50 mL) were added 3,4 dimethoxyphenylboronic acid (2.51 g, 13.80 mmol), tripotassium phosphate (4.88 g, 22.99 mmol), and $Pd(PPh_3)_4$ (132 mg, 0,12 mmol). The reaction mixture was stirred at 90 °C overnight. The volatiles were evaporated and the crude residue was purified by silica gel flash column chromatography (eluting with a mixture of dichloromethane/ethyl acetate in a ratio of 99:1) yielding the title compound (2.34 g, 74%).

^{1}H NMR (300 MHz, DMSO) δ 9.48 (s, 1H), 7.91 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.75 (d, *J* = 2.1 Hz, 1H), 7.18 (d, *J* = 8.6 Hz, 1H), 3.88 (s, 3H), 3.88 (s, 3H) ppm; HRMS m/z $[M+H]^+$ calcd for $C_{13}H_{10}Cl_1N_3O_2$: 276.0534, found: 276.0528.

3-Azido-5-(3,4-dimethoxyphenyl)pyrazine-2-carbonitrile (17)

To a solution of 3-chloro-5-(3,4-dimethoxyphenyl)pyrazine-2-carbonitrile **16** (2.34 g, 8.45 mmol) in DMSO (50 mL) was added NaN_3 (604 mg, 9.30 mmol). The mixture was stirred at 70°C overnight. After disappearance of the starting material the mixture was allowed to cool and water was added. The formed precipitate was filtered off and washed with water to yield the title compound (2.10 g, 83%).

HRMS m/z $[M+Na]^+$ calcd for $C_{13}H_{10}N_6O_2Na$: 305.0758, found: 305.0755.

3-Amino-5-(3,4-dimethoxyphenyl)pyrazine-2-carbonitrile (19)

To a solution of 3-azido-5-(3,4-dimethoxyphenyl)pyrazine-2-carbonitrile **17** (2.07 g, 7.00 mmol) in pyridine (30 mL) was added triphenyl phosphine (2.99 g, 11.40 mmol) and the mixture was stirred at room temperature overnight. After disappearance of the starting material, the volatiles were evaporated and the crude residue was dissolved in an 80% solution of acetic acid in water (20 mL) and refluxed for 1 hour. The volatiles were evaporated and the crude residue was purified by silica gel flash column chromatography (eluting with a gradient of dichloromethane/acetone in a ratio of 95:5) to yield the title compound (1.72 g, 95%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.47 (s, 1H), 7.63 – 7.56 (m, 2H), 7.01 – 6.93 (m, 1H), 5.27 (bs, 2H), 3.99 (s, 3H), 3.97 (s, 3H) ppm ^{13}C NMR (126 MHz, DMSO) δ 156.66, 153.39, 151.90, 149.48, 131.23, 127.86, 121.35, 117.03, 112.21, 110.74, 108.93, 56.11 ppm; HRMS m/z $[M+H]^+$ calcd for $C_{13}H_{12}N_4O_2$: 257.1033, found: 257.1032.

3-Amino-5-(3,4-dimethoxyphenyl)pyrazine-2-carbothioamide (20)

To a solution of 3-amino-5-(3,4-dimethoxyphenyl)pyrazine-2-carbonitrile **19** (700 mg, 2.73 mmol) in ethanol (20 mL) was added Lawesson reagent (2.21 g, 5.46 mmol) and the mixture was refluxed overnight. After disappearance of the starting material the volatiles were evaporated *in vacuo*. The residue was dissolved in water and extracted three times with dichloromethane. The combined organic layers were evaporated under reduced pressure and the crude residue was purified by silica gel silica gel flash column chromatography (using a mixture of dichloromethane/ethyl acetate in a ratio of 99:1 as mobile phase) yielding the title compound (660 mg, 83%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.47 (s, 1H), 7.65 – 7.55 (m, 2H), 7.02 – 6.93 (m, 1H), 3.99 (s, 3H), 3.97 (s, 3H) ppm; ^{13}C NMR (126 MHz, DMSO) δ 192.85, 155.01, 153.30, 151.59, 149.49, 128.39, 127.92, 125.29, 120.96, 112.19, 110.61, 56.10, 56.08 ppm.

6-(3,4-Dimethoxyphenyl)isothiazolo[3,4-*b*]pyrazin-3-amine (21)

To a solution of 3-amino-5-(3,4-dimethoxyphenyl)pyrazine-2-carbothioamide **20** (600 mg, 2.07 mmol) in methanol (5 mL) at 0°C was added dropwise a 30% aqueous H_2O_2 solution (703 µL, 8.27 mmol). The mixture was stirred overnight at room temperature. After disappearance of the starting material the volatiles were evaporated under reduced pressure and the crude residue was purified by silica gel flash column chromatography (eluting with a gradient of dichloromethane/acetone in a ratio of 9:1 to 8:2) to yield the title compound (400 mg, 67%).

^{1}H NMR (300 MHz, DMSO) δ 8.95 (s, 1H), 8.24 (s, 2H), 7.89 (d, *J* = 8.4 Hz, 1H), 7.82 (s, 1H), 7.13 (d, *J* = 8.5 Hz, 1H), 3.90 (s, 3H), 3.86 (s, 3H) ppm; HRMS m/z $[M+H]^+$ calcd for $C_{13}H_{12}N_4O_2S_1$: 289.0754, found: 289.0767

3-Bromo-6-(3,4-dimethoxyphenyl)isothiazolo[3,4-*b*]pyrazine (22)

To a solution of $CuBr_2$ (170 mg, 0,76) and *tert*-butylnitrite (93 mg, 0.90 mmol) in dry acetonitrile (5 mL) under argon atmosphere at 0°C was added 6-(3,4-dimethoxyphenyl)isothiazolo[3,4-*b*]pyrazin-3-amine **21** (200 mg, 0.67 mmol) and the mixture was stirred at 0°C for 5 hours. After disappearance of the starting material, volatiles were evaporated and the crude residue was purified by silica gel flash column chromatography (eluting with a mixture of dichloromethane/ethyl acetate in a ratio of 99:1) yielding the title compound (77 mg, 32%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.27 (s, 1H), 7.97 (s, 1H), 7.79 (d, *J* = 8.4 Hz, 1H), 7.01 (d, *J* = 8.4 Hz, 1H), 4.02 (s, 3H), 3.98 (s, 3H) ppm.

4-(6-(3,4-Dimethoxyphenyl)isothiazolo[3,4-*b*]pyrazin-3-yl)morpholine (23)

To a solution of 3-bromo-6-(3,4-dimethoxyphenyl)isothiazolo[3,4-*b*]pyrazine **22** (77 mg, 0.22 mmol) in ethanol (5 mL) was added morpholine (57 mg, 0.66 mmol) and the reaction was stirred at reflux overnight. After disappearance of the starting material, volatiles were evaporated *in vacuo* and the crude residue was purified by silica gel flash column chromatography (eluting with a mixture of dichloromethane/methanol in a ratio of 98:2) to yield the title compound (48 mg, 64%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.80 (s, 1H), 7.96 (d, *J* = 2.0 Hz, 1H), 7.73 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.99 (d, *J* = 8.5 Hz, 1H), 4.03 (s, 3H), 3.98 (s, 3H), 3.97 – 3.96 (m, 8H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.06, 160.31, 154.08, 152.07, 150.02, 136.81, 129.26, 125.65, 121.33, 111.10, 110.81, 66.40, 56.48, 56.34, 50.05 ppm; HRMS m/z $[M+H]^+$ calcd for $C_{17}H_{18}N_4O_3S_1$: 359.1172, found: 359.1161.

4-Amino-2-(3,4-dimethoxyphenyl)pyrimidine-5-carbonitrile (25)

A solution of 4-amino-2-chloropyrimidine-5-carbonitrile **24** (3.00 g, 19.50 mmol), tripotassium phosphate (8.28 g, 39 mmol) and 3,4-dimethoxyphenylboronic acid (3.67 g, 29.25 mmol) in dry dioxane (30 mL) was degassed and refilled with nitrogen gas two times. $Pd(OAc)_2$ (219 mg, 0.98 mmol) and D-t-BPF (501 mg, 0.98 mmol) were added and the mixture was degassed and refilled with nitrogen gas two times and stirred at reflux overnight. After disappearance of the starting material, the mixture was allowed to cool to room temperature and the solid was filtered off and thoroughly washed with ethyl acetate. The filtrate was concentrated *in vacuo* and the crude residue was purified by silica gel flash column chromatography (eluting with a mixture of dichloromethane/ethyl acetate in a ratio of 95:5) to yield the title compound (2.76 g, 53%).

^{1}H NMR (300 MHz, DMSO) δ 8.68 (s, 1H), 7.97 (d, *J* = 8.4 Hz, 1H), 7.91 – 7.80 (m, 3H), 7.09 (d, *J* = 8.5 Hz, 1H), 3.84 (s, 3H), 3.83 (s, 3H) ppm; ^{13}C NMR (126 MHz, DMSO) δ 164.65, 162.55, 161.47, 151.98, 148.55, 128.98, 122.15, 116.06, 111.33, 111.10, 86.80, 55.68, 55.49 ppm; HRMS m/z $[M+H]^+$ calcd for $C_{13}H_{12}N_4O_2$: 257.1033, found: 257.1038.

4-Amino-2-(3,4-dimethoxyphenyl)pyrimidine-5-carbothioamide (26)

To a solution 4-amino-2-(3,4-dimethoxyphenyl)pyrimidine-5-carbonitrile **25** (2.00 g, 7.80 mmol) in ethanol (30 mL) was added Lawesson's reagent (6.31 g, 15.60 mmol). The mixture was refluxed overnight. After disappearance of the starting material volatiles were evaporated *in vacuo* and the residue was dissolved in water and extracted three times with dichloromethane. The combined organic layers were evaporated under reduced pressure and the crude residue was purified by silica gel flash column chromatography (using a gradient of dichloromethane/acetone in a ratio of 85:15 to 75:15 as mobile phase) yielding the title compound (1.36 g, 60%).

^{1}H NMR (300 MHz, DMSO) δ 9.83 (s, 1H), 9.58 (s, 1H), 8.44 (s, 1H), 8.00 – 7.89 (m, 4H), 7.07 (d, *J* = 8.5 Hz, 1H), 3.83 (s, 6H) ppm; ^{13}C NMR (126 MHz, DMSO) δ 195.84, 163.00, 161.06, 153.48, 151.46, 148.56, 129.69, 121.53, 112.96, 111.36, 110.90, 55.68, 55.53 ppm.

6-(3,4-Dimethoxyphenyl)isothiazolo[3,4-*d*]pyrimidin-3-amine (27)

To a solution of 4-amino-2-(3,4-dimethoxyphenyl)pyrimidine-5-carbothioamide **26** (500 mg, 1.72 mmol) in methanol (5 mL) at 0°C was added dropwise a 30% aqueous H_2O_2 solution (585 µL, 6.89 mmol). The mixture was stirred overnight at room temperature. After disappearance of the starting material, the volatiles were evaporated *in vacuo* and the crude residue was purified by silica gel flash column chromatography (eluting with a gradient of dichloromethane/acetone 8:2) yielding the title compound (418 mg, 84%).

^{1}H NMR (300 MHz, DMSO) δ 9.38 (s, 1H), 8.87 (s, 2H), 8.09 (d, *J* = 8.4 Hz, 1H), 8.00 (s, 1H), 7.09 (d, *J* = 8.6 Hz, 1H), 3.87 (s, 3H), 3.84 (s, 3H) ppm; ^{13}C NMR (126 MHz, DMSO) δ 178.21, 165.37, 163.19, 156.33, 151.90, 149.04, 130.51, 122.50, 111.75, 111.50, 108.97, 56.05, 55.91 ppm; HRMS m/z $[M+H]^+$ calcd for $C_{13}H_{12}N_4O_2S_1$: 289.0754, found: 289.0747.

3-Bromo-6-(3,4-dimethoxyphenyl)isothiazolo[3,4-*d*]pyrimidine (28)

To a stirred solution of $CuBr_2$ (87 mg, 0.39) and *tert*-butylnitrite (46 mg, 0.45 mmol) in dry acetonitrile (10 mL) under argon atmosphere at 0°C was added 6-(3,4-dimethoxyphenyl)isothiazolo[3,4-*d*]pyrimidin-3-amine **27** (100 mg, 0.35 mmol) and the mixture was stirred at room temperature overnight. After disappearance of the starting material, volatiles were evaporated and the crude residue was purified by silica gel flash column chromatography (eluting with a mixture of dichloromethane/acetone in a ratio of 95:5) yielding the title compound (60 mg, 49%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.34 (s, 1H), 8.34 (d, *J* = 8.0 Hz, 1H), 8.22 (s, 1H), 7.00 (d, *J* = 8.3 Hz, 1H), 4.04 (s, 3H), 3.99 (s, 3H) ppm; HRMS m/z $[M+H]^+$ calcd for $C_{13}H_{10}Br_1N_3O_2S_1$: 351.9750, found: 351.9746.

4-(6-(3,4-Dimethoxyphenyl)isothiazolo[3,4-*d*]pyrimidin-3-yl)morpholine (29)

To a solution of 3-bromo-6-(3,4-dimethoxyphenyl)isothiazolo[3,4-*d*]pyrimidine **28** (50 mg, 0.14 mmol) in ethanol (5 mL) was added morpholine (37 mg, 0.43 mmol). The reaction was stirred at reflux overnight. After disappearance of the starting material, volatiles were evaporated *in vacuo* and the crude residue was purified by silica gel flash column chromatography (eluting with a mixture of dichloromethane/methanol in a ratio of 95:5) to yield the title compound (33 mg, 65%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.33 (s, 1H), 8.27 – 8.21 (m, 1H), 8.20 – 8.15 (m, 1H), 7.02 – 6.91 (m, 1H), 4.04 – 3.92 (m, 10H), 3.69 (d, *J* = 5.2 Hz, 4H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.64, 158.16, 153.06, 153.01, 149.38, 125.62, 123.05, 111.19, 111.07, 65.31, 56.20, 56.09, 50.89 ppm; HRMS m/z $[M+H]^+$ calcd for $C_{17}H_{18}N_4O_3S_1$: 359.1172, found: 359.1168.

3-Bromo-6-chloropyrazolo[1,5-*a*]pyrimidine (31)

To a solution of 4-bromo-1H-pyrazol-3-amine **30** (1.0 g, 6.17 mmol) in methanol (18 mL) and acetic acid (12 mL) was added 2-chloromalonaldehyde (690 mg, 6.48 mmol), and the mixture was stirred at 70°C for 2 hours. After disappearance of the starting material, the mixture was cooled to 0°C and the formed precipitate was filtered off and washed with methanol to yield the title compound (820 mg, 57%).

^{1}H NMR (300 MHz, DMSO) δ 9.62 (d, *J* = 2.2 Hz, 1H), 8.69 (d, *J* = 2.2 Hz, 1H), 8.42 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO) δ 150.27, 145.38, 143.30, 134.90, 117.44, 84.18 ppm; HRMS m/z $[M+H]^+$ calcd for $C_6H_3Br_1Cl_1N_3$: 231.9272, found: 231.9273.

6-Chloro-3-phenylpyrazolo[1,5-*a*]pyrimidine (32)

To a solution of 3-bromo-6-chloropyrazolo[1,5-*a*]pyrimidine **31** (400 mg, 1.72 mmol) in dioxane (15 mL) and water (5 mL) were added phenyl boronic acid (254 g, 2.08 mmol), potassium carbonate (476 mg, 3.44 mmol), and $Pd(PPh_3)_4$ (20 mg, 0,02 mmol). The reaction mixture was degassed and refilled with nitrogen gas and stirred at 90 °C overnight. After disappearance of the starting material, volatiles were evaporated and the crude residue was purified by silica gel flash column chromatography (using a mixture of heptane/acetone in a ratio of 90:10) to yield the title compound (220 mg, 56%).

^{1}H NMR (300 MHz, DMSO) δ 9.60 (d, *J* = 2.3 Hz, 1H), 8.80 (s, 1H), 8.72 (d, *J* = 2.3 Hz, 1H), 8.14 – 8.07 (m, 2H), 7.50 – 7.41 (m, 2H), 7.33 – 7.22 (m, 1H) ppm; HRMS m/z $[M+H]^+$ calcd for $C_{12}H_8Cl_1N_3$: 230.0479, found: 230.0471.

6-(3,4-Dimethoxyphenyl)-3-phenylpyrazolo[1,5-*a*]pyrimidine (33)

To a solution of 6-chloro-3-phenylpyrazolo[1,5-*a*]pyrimidine **32** (100 mg, 0.44 mmol) in dioxane (4 mL) and water (1 mL) were added 3,4-dimethoxyphenyl boronic acid (96 mg, 0.53 mmol), potassium carbonate (121 mg, 0.88 mmol), and $Pd(PPh_3)_4$ (4 mg, 0,003 mmol). The reaction mixture was degassed and refilled with nitrogen gas and stirred at 90 °C overnight. After disappearance of the starting material, volatiles were evaporated *in vacuo* and the crude residue was purified by silica gel flash column chromatography (using a mixture of dichloromethane/acetone in a ratio of 90:10) yielding the title compound (40 mg, 27%).

^{1}H NMR (300 MHz, DMSO) δ 9.51 (d, *J* = 2.3 Hz, 1H), 9.07 (d, *J* = 2.3 Hz, 1H), 8.78 (s, 1H), 8.22 – 8.15 (m, 2H), 7.50 – 7.40 (m, 4H), 7.30 – 7.23 (m, 1H), 7.10 (d, *J* = 8.4 Hz, 1H), 3.90 (s, 3H), 3.81 (d, *J* = 7.7 Hz, 3H) ppm; ^{13}C NMR (75 MHz, DMSO) δ 150.23, 149.84, 149.66, 143.45, 143.37, 132.68, 132.36, 129.14, 126.46, 126.32, 126.08, 122.11, 119.63, 112.80, 110.98, 109.34, 56.23, 56.11 ppm; HRMS m/z $[M+H]^+$ calcd for $C_{20}H_{17}N_3O_2$: 332.1393, found: 332.1403

(*E*)-2-(5-Bromo-3-nitropyridin-2-yl)-*N,N*-dimethylethen-1-amine (35)

To a solution of 5-bromo-2-methyl-3-nitropyridine **34** (1.00 g, 4.61 mmol) in dry DMF (10 mL) was added DMF-DMA (1.01 g, 9.22 mmol) dropwise. The mixture stirred at 90°C for 4 hours, upon which a dark red colour formed. After disappearance of the starting material, volatiles were

evaporated *in vacuo* yielding the title compound (1.23 g, 98%). The crude residue was used without further purification in the following reaction.
HRMS m/z $[M+H]^+$ calcd for $C_9H_{10}Br_1N_3O_2$: 272.0030, found: 272.0032

6-Bromo-1H-pyrrolo[3,2-*b*]pyridine (36)

A suspension of iron powder (1.13 g, 20.26 mmol) in glacial acetic acid (20mL) was stirred at 0°C for 10 minutes. Subsequently, a solution of (*E*)-2-(5-bromo-3-nitropyridin-2-yl)-*N*,*N*-dimethylethen-1-amine **35** (1.00 g, 3.68 mmol) in glacial acetic acid (10 mL) was added dropwise and the mixture was stirred at room temperature for 3 hours. After disappearance of the starting material, ethyl acetate was added to the mixture and it was filtrated using a paper filter. The filtered cake was washed thoroughly with ethyl acetate and the filtrate was evaporated *in vacuo*. The crude residue was purified by silica gel flash column chromatography (eluting with a mixture of dichloromethane/ethyl acetate in a ratio of 90:10) to yield the title compound (660 mg, 91%).
^{1}H NMR (300 MHz, DMSO) δ 11.46 (bs, 1H), 8.37 (d, *J* = 2.1 Hz, 1H), 8.03 – 8.00 (m, 1H), 7.69 – 7.66 (m, 1H), 6.60 – 6.57 (m, 1H) ppm; HRMS m/z $[M+H]^+$ calcd for $C_7H_5Br_1N_2$: 196.9709, found: 196.9709.

6-Bromo-3-iodo-1H-pyrrolo[3,2-*b*]pyridine (37)

To a solution of 6-bromo-1H-pyrrolo[3,2-*b*]pyridine **36** in dry THF (15 mL) was added *N*-iodosuccinimide at once and the mixture was stirred at room temperature for 3 hours. After disappearance of the starting material, volatiles were evaporated and the crude residue was purified by silica gel flash column chromatography (eluting with a gradient of dichloromethane/ethyl acetate in a ratio of 99.5:0.5 to 99:1) yielding the title compound (400 mg, 37%).
^{1}H NMR (300 MHz, DMSO) δ 8.43 (d, *J* = 2.0 Hz, 1H), 8.07 (d, *J* = 2.0 Hz, 1H), 7.85 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO) δ 143.72, 134.62, 129.17, 121.50, 113.07 ppm; HRMS m/z $[M+H]^+$ calcd for $C_7H_4Br_1I_1N_2$: 322.8678, found: 322.8670

***tert*-Butyl 6-bromo-3-iodo-1H-pyrrolo[3,2-*b*]pyridine-1-carboxylate (38)**

A mixture of 6-bromo-3-iodo-1H-pyrrolo[3,2-*b*]pyridine **37** (200 mg, 0.60 mmol), DMAP (8 mg, 0.06 mmol), TEA (80 mg, 0.8 mmol) and di-*tert*-butyl dicarbonate (158 mg, 0.72 mmol) was stirred in dry THF (6 mL) for 5 hours at room temperature. After disappearance of the starting material, volatiles were evaporated *in vacuo* and the crude residue was purified using silica gel flash column chromatography (eluting with a mixture of heptane/acetone in a ratio of 9:1) to yield the title compound (240 mg, 95%).
^{1}H NMR (300 MHz, DMSO) δ 8.66 (d, *J* = 2.0 Hz, 1H), 8.45 (d, *J* = 2.0 Hz, 1H), 8.20 (s, 1H), 1.63 (s, 9H) ppm; HRMS m/z $[M+H]^+$ calcd for $C_8H_4Br_1I_1N_2O_2$: 366.8576, found: 366.8572.

6-Bromo-3-phenyl-1H-pyrrolo[3,2-*b*]pyridine (39)

To a solution of *tert*-butyl 6-bromo-3-iodo-1H-pyrrolo[3,2-*b*]pyridine-1-carboxylate **38** (200 mg, 0.57 mmol) in dioxane (4 mL) and water (1 mL) were added phenylboronic acid (83 g, 0.68 mmol), potassium carbonate (158 mg, 1.14 mmol), and $Pd(PPh_3)_4$ (7 mg, 0,006 mmol). The reaction mixture was degassed and refilled with nitrogen gas and stirred at 90 °C overnight. After disappearance of the starting material, volatiles were evaporated *in vacuo* and the crude residue was purified by silica gel flash column chromatography (using a mixture of heptane/acetone in a ratio of 70:30) to yield the title compound (109 mg, 70%).
^{1}H NMR (300 MHz, DMSO) δ 11.72 (bs, 1H), 8.51 (d, *J* = 2.1 Hz, 1H), 8.25 – 8.18 (m, 3H), 8.08 (d, *J* = 2.1 Hz, 1H), 7.43 – 7.37 (m, 2H), 7.25 – 7.18 (m, 1H) ppm; HRMS m/z $[M+H]^+$ calcd for $C_{13}H_9Br_1N_2$:273.0022, found: 273.0019

***tert*-Butyl 6-bromo-3-phenyl-1H-pyrrolo[3,2-*b*]pyridine-1-carboxylate (40)**

A mixture of 6-bromo-3-iodo-1H-pyrrolo[3,2-*b*]pyridine **39** (70 mg, 0.26 mmol), DMAP (3 mg, 0.03 mmol), TEA (35 mg, 0.35 mmol) and di-*tert*-butyl dicarbonate (68 mg, 0.31 mmol) were stirred in dry THF (5 mL) for 5 hours. After disappearance of the starting material, volatiles were evaporated and the crude residue was purified by silica gel flash column chromatography (eluting with a mixture of heptane/acetone in a ratio of 9:1) to yield the title compound (65 mg, 67%).
^{1}H NMR (300 MHz, DMSO) δ 8.72 (s, 1H), 8.54 (s, 1H), 8.43 (s, 1H), 8.25 (d, *J* = 7.5 Hz, 2H), 7.52 – 7.43 (m, 2H), 7.39 – 7.30 (m, 1H), 1.68 (s, 9H) ppm; HRMS m/z $[M+H]^+$ calcd for $C_{14}H_9Br_1N_2O_2$: 316.9921, found: 316.9917.

***tert*-Butyl 6-(3,4-dimethoxyphenyl)-3-phenyl-1H-pyrrolo[3,2-*b*]pyridine-1-carboxylate (41)**

To a solution of *tert*-butyl 6-bromo-3-phenyl-1H-pyrrolo[3,2-*b*]pyridine-1-carboxylate **40** (65 mg, 0.17 mmol) in dioxane (4 mL) and water (1 mL) were added 3,4-dimethoxyphenylboronic acid (38 g, 0.21 mmol), potassium carbonate (47 mg, 0.34 mmol), and $Pd(PPh_3)_4$ (2 mg, 0.002 mmol). The reaction mixture was degassed and refilled with nitrogen gas and stirred at 90 °C overnight. The volatiles were evaporated *in vacuo* and the crude residue was purified by silica gel flash column chromatography (using a mixture of dichloromethane/ethyl acetate in a ratio of 90:10) to yield the title compound (11 mg, 15%).
^{1}H NMR (300 MHz, DMSO) δ 8.93 (d, *J* = 1.9 Hz, 1H), 8.54 (d, *J* = 1.7 Hz, 1H), 8.40 (s, 1H), 8.36 – 8.29 (m, 2H), 7.51 – 7.44 (m, 2H), 7.38 – 7.28 (m, 3H), 7.11 (d, *J* = 8.3 Hz, 1H), 3.88 (s, 3H), 3.83 (s, 3H), 1.70 (s, 9H) ppm; ^{13}C NMR (75 MHz, DMSO) δ 149.50, 149.07, 148.77, 144.93, 144.22, 132.16, 131.74, 130.50, 129.15, 128.59, 127.31, 127.14, 126.00, 119.68, 119.62, 119.42, 112.61, 110.74, 85.10, 55.76, 55.73, 27.72 ppm.

6-(3,4-Dimethoxyphenyl)-3-phenyl-1H-pyrrolo[3,2-*b*]pyridine (42)

To a solution of *tert*-butyl 6-(3,4-dimethoxyphenyl)-3-phenyl-1H-pyrrolo[3,2-*b*]pyridine-1-carboxylate **41** (11 mg, 0.026 mmol) in methanol (2 mL) was added a 4N solution of HCl (2 mL) in dioxane and the mixture was stirred at 70°C for 4 hours. After disappearance of the starting material, volatiles were evaporated *in vacuo* and the crude residue was dissolved in water and washed three times with dichloromethane. Organic fractions were collected and concentrated *in vacuo* to yield the title compound (4 mg, 47%).
^{1}H NMR (300 MHz, DMSO) δ 12.97 (s, 1H), 8.84 – 8.78 (m, 1H), 8.70 (s, 1H), 8.53 – 8.44 (m, 1H), 7.92 (d, *J* = 7.4 Hz, 2H), 7.57 – 7.34 (m, 5H), 7.14 (d, *J* = 8.4 Hz, 1H), 3.91 (s, 3H), 3.84 (s, 3H) ppm; ^{13}C NMR (75 MHz, DMSO) δ 149.54, 149.47, 135.55, 133.46, 132.76, 131.62, 129.91, 128.97, 128.61, 127.52, 127.09, 123.88, 119.92, 112.62, 112.42, 111.26, 55.98, 55.84 ppm; HRMS m/z $[M+H]^+$ calcd for $C_{21}H_{18}N_2O_2$: 331.1441, found: 331.1442.

5-(3,4-Dimethoxyphenyl)-3-fluoropicolinonitrile (44)

To a solution 5-bromo-3-fluoropicolinonitrile **43** (5.00 g, 24.86 mmol) in dioxane (40 mL) and water (10 mL) were added 3,4-dimethoxyphenylboronic acid (5.432 g, 29.85 mmol), potassium carbonate (6.87 g, 49.72 mmol), and $Pd(PPh_3)_4$ (287 mg, 0.248 mmol). The reaction mixture was degassed and refilled with nitrogen gas and stirred at 90 °C overnight. After disappearance of the starting material, volatiles were evaporated *in vacuo* and the crude residue was purified by silica gel flash column chromatography (using a mixture of dichloromethane/ethyl acetate in a ratio of 99.5:0.5) to yield the title compound (5.54 g, 86%).
^{1}H NMR (300 MHz, DMSO) δ 9.05 (s, 1H), 8.58 – 8.42 (m, 1H), 7.54 – 7.47 (m, 2H), 7.13 (d, *J* = 8.4 Hz, 1H), 3.88 (s, 3H), 3.84 (s, 3H) ppm; ^{13}C NMR (126 MHz, DMSO) δ 162.61, 160.50, 150.70, 149.43, 145.35, 141.96, 126.22, 121.78, 121.64, 120.65, 118.51, 113.97, 112.24, 110.94, 55.88, 55.75 ppm**.** HRMS m/z $[M+H]^+$ calcd for $C_{14}H_{11}F_1N_2O_2$: 259.0877, found:259.0881.

6-(3,4-Dimethoxyphenyl)-1H-pyrazolo[4,3-*b*]pyridin-3-amine (45)

To a solution of 5-(3,4-dimethoxyphenyl)-3-fluoropicolinonitrile **44** (3.00 g, 11.61 mmol) in n-butanol (30 mL) was added hydrazine hydrate (2.91 g, 58.08 mmol) and the mixture was stirred at 121°C overnight. After disappearance of the starting material, volatiles were evaporated *in vacuo* and the crude residue was purified by silica gel flash column chromatography (eluting with a mixture of dichloromethane/methanol in a ratio of 95:5) to yield the title compound (2.6 g, 83%).
^{1}H NMR (300 MHz, DMSO) δ 11.66 (bs, 1H), 8.59 (s, 1H), 7.84 (s, 1H), 7.38 – 7.24 (m, 2H), 7.08 (d, *J* = 8.4 Hz, 1H), 5.39 (bs, 2H), 3.88 (s, 3H), 3.82 (s, 3H) ppm.

6-(3,4-Dimethoxyphenyl)-3-iodo-1H-pyrazolo[4,3-*b*]pyridine (46)

A solution of 6-(3,4-dimethoxyphenyl)-1H-pyrazolo[4,3-*b*]pyridin-3-fluoropicolinonitrile **45** (1.00 g, 3.70 mmol) and *p*-toluenesulfonic acid (2.11 g, 11.1 mmol) in acetonitrile was stirred for 10 minutes. Subsequently a solution of sodium nitrite (640 mg, 9.30 mmol) and potassium Iodide (1.54 g, 9.30 mmol) in water (10 mL) was added dropwise over 30 minutes and the mixture was stirred at room temperature for 5 hours. After

disappearance of the starting material, volatiles were evaporated *in vacuo* and the crude residue was purified by silica gel flash column chromatography (eluting with a mixture of dichloromethane/methanol in a ratio of 95:5) to yield the title compound (400 mg, 28%).
^{1}H NMR (300 MHz, DMSO) δ 9.19 – 9.11 (m, 1H), 8.89 – 8.85 (m, 1H), 7.68 – 7.61 (m, 2H), 7.56 (s, 1H), 7.22 – 7.14 (m, *J* = 7.7 Hz, 2H), 3.96 (s, 3H), 3.89 (s, 3H) ppm.

6-(3,4-Dimethoxyphenyl)-3-phenyl-1H-pyrazolo[4,3-*b*]pyridine (47)

To a solution 6-(3,4-dimethoxyphenyl)-3-iodo-1H-pyrazolo[4,3-*b*]pyridine **46** (100 mg, 0.26 mmol) in dioxane (4 mL) and water (1 mL) were added 3,4-dimethoxyphenylboronic acid (38 mg, 0.31 mmol), potassium carbonate (72 mg, 0.52 mmol), and $Pd(PPh_3)_4$ (60 mg, 0.0.052 mmol). The reaction mixture was stirred in a microwave at 120°C for 1 hour. After disappearance of the starting material, volatiles were evaporated *in vacuo* and the crude residue was purified by silica gel flash column chromatography (using a mixture of dichloromethane/methanol in a ratio of 98:2) to yield the title compound (20 mg, 23%).
^{1}H NMR (300 MHz, DMSO) δ 13.46 (s, 1H), 8.95 (s, 1H), 8.54 (d, *J* = 7.3 Hz, 2H), 8.20 (s, 1H), 7.60 – 7.33 (m, 5H), 7.12 (d, *J* = 8.4 Hz, 1H), 3.90 (s, 3H), 3.83 (s, 3H) ppm; ^{13}C NMR (75 MHz, DMSO) δ 149.53, 149.33, 145.10, 141.99, 137.58, 134.53, 133.30, 132.87, 130.41, 128.70, 128.05, 126.47, 120.00, 115.41, 112.69, 111.52, 55.93, 55.87 ppm; HRMS m/z $[M+H]^+$ calcd for $C_{20}H_{17}N_3O_2$: 332.1393, found: 332.1396.

***t*ert-Butyl thiophen-3-ylcarbamate (50)**

To a solution of thiophene-3-carboxylic acid **48** (2.00 g, 15.61 mmol) in dry dimethylformamide (25 mL) at 0 °C was added diphenylphosphoryl azide (4.30 g, 15.61 mmol) and the mixture was stirred at room temperature for 2 hours. After disappearance of the starting material, *tert*-butanol was added (15 mL, 156.10 mmol) and the mixture was stirred at reflux for 17 hours. After disappearance of the intermediate **49**, the volatiles were evaporated and the crude residue was purified by silica gel flash column chromatography (using a mixture of heptane/acetone in a ratio of 9:1) to yield the title compound (1.91 g, 61%).
^{1}H NMR (300 MHz, DMSO) δ 9.62 (bs, 1H), 7.42 – 7.34 (m, 1H), 7.19 – 7.12 (m, 1H), 7.02 – 6.95 (m, 1H), 1.47 (s, 9H) ppm; ^{13}C NMR (75 MHz, DMSO) δ 152.89, 137.48, 124.71, 121.26, 105.90, 28.25 ppm; HRMS m/z $[M+H]^+$ calcd for $C_5H_5N_1O_2S_1$: 144.0114, found: 144.0112.

3-Aminothiophene hydrochloride (51)

tert-Butyl thiophen-3-ylcarbamate **50** (500 mg, 2.50 mmol) was dissolved in a 4N solution of HCl in dioxane (10 mL) and the mixture was protected from light using aluminium foil and stirred at reflux for 3 hours. After disappearance of the starting material, volatiles were evaporated and the crude residue (340 mg, 100%) was protected from light and used as such without further purification in the following reaction.
^{1}H NMR (300 MHz, DMSO) δ 10.46 (bs, 2H), 7.76 – 7.63 (m, 1H), 7.63 – 7.51 (m, 1H), 7.15 – 7.10 (m, 1H) ppm; ^{13}C NMR (75 MHz, DMSO) δ 129.43, 128.02, 123.32, 118.50 ppm; HRMS m/z $[M+H]^+$ calcd for $C_4H_5N_1S_1$: 100.0215, found: 100.0227.

Ethyl 3-amino-6-bromothieno[3,2-*b*]pyridine-2-carboxylate (54)

To a solution of 5-bromo-3-nitropyridine-2-carbonitrile **53** (2.00 g, 21.93 mmol) and ethyl 2-mercaptoacetate (3.95 g, 32.89 mmol) in dimethylformamide (50 mL) was added potassium *tert*-butoxide (4.92, 43.86 mmol) portion wise. The mixture turned red and was stirred at room temperature for 3 hours. After disappearance of the starting material the volatiles were evaporated *in vacuo* and the crude residue was purified by silica gel flash column chromatography (eluting with a mixture of heptane /ethyl acetate in a ratio of 80:20) to yield the title compound (4.24 g, 54%).
^{1}H NMR (300 MHz, DMSO) δ 8.89 – 8.60 (m, 2H), 6.90 (bs, 2H), 4.30 (q, *J* = 7.0 Hz, 2H), 1.31 (t, *J* = 6.8 Hz, 3H) ppm; ^{13}C NMR (126 MHz, DMSO) δ 164.47, 147.92, 147.88, 145.40, 135.30, 134.49, 119.52, 98.47, 60.78, 14.85 ppm; HRMS m/z $[M+H]^+$ calcd for $C_{10}H_9Br_1N_2O_2S_1$: 300.9641, found: 300.9638.

Ethyl 3-amino-6-(3,4-dimethoxyphenyl)thieno[3,2-*b*]pyridine-2-carboxylate (55)

To a solution ethyl 3-amino-6-bromothieno[3,2-*b*]pyridine-2-carboxylate **54** (3.50 g, 11.62 mmol) in dioxane (50 mL) were added 3,4-dimethoxyphenylboronic acid (2.55 g, 13.95 mmol), potassium phosphate (4.90 g, 23.24 mmol), and $Pd(PPh_3)_4$ (671 mg, 0,58 mmol). The reaction mixture was stirred at reflux for 2 days. After disappearance of the starting material, volatiles were evaporated *in vacuo* and the crude residue was purified by silica gel flash column chromatography (eluting with a mixture of heptane/ethyl acetate in a ratio of 80:20) to yield the title compound (3.04 g, 76%).
^{1}H NMR (300 MHz, DMSO) δ 9.00 (d, *J* = 2.0 Hz, 1H), 8.66 (d, *J* = 2.0 Hz, 1H), 7.45 – 7.36 (m, 2H), 7.10 (d, *J* = 8.1 Hz, 1H), 6.89 (bs, 2H), 4.31 (q, *J* = 7.1 Hz, 2H), 3.89 (s, 3H), 3.82 (s, 3H), 1.32 (t, *J* = 7.1 Hz, 3H) ppm ^{13}C NMR (126 MHz, DMSO) δ 164.11, 147.57, 145.02, 134.92, 134.15, 133.21, 119.17, 118.62, 112.24, 110.49, 60.42, 55.69, 14.48 ppm; HRMS m/z $[M+H]^+$ calcd for $C_{18}H_{18}N_2O_4S_1$: 359.1060, found: 359.1054.

Ethyl 3-bromo-6-(3,4-dimethoxyphenyl)thieno[3,2-*b*]pyridine-2-carboxylate (56)

To a stirred solution of $CuBr_2$ (1.72 g, 7.67) and *tert*-butyl nitrite (789 mg, 7.66 mmol) in dry acetonitrile (25 mL) under argon atmosphere at 0°C was added ethyl 3-amino-6-(3,4-dimethoxyphenyl)thieno[3,2-*b*]pyridine-2-carboxylate **55** (2.5 g, 6.98 mmol) and the mixture was stirred at 0°C for 6 hours and then at room temperature overnight. After disappearance of the starting material, volatiles were evaporated *in vacuo* and the crude residue was purified by silica gel flash column chromatography (eluting with a mixture of heptane/ethyl acetate in a ratio of 70:30) yielding the title compound (1.38 g, 47%).
^{1}H NMR (300 MHz, DMSO) δ 9.20 (s, 1H), 8.88 (s, 1H), 7.48 – 7.35 (m, 2H), 7.12 (d, *J* = 8.1 Hz, 1H), 4.41 (q, *J* = 7.0 Hz, 2H), 3.89 (s, 3H), 3.83 (s, 3H), 1.37 (t, *J* = 7.0 Hz, 3H) ppm; HRMS m/z $[M+H]^+$ calcd for $C_{18}H_{16}Br_1N_1O_4S_1$: 422.0056, found: 422.0052.

Ethyl 6-(3,4-dimethoxyphenyl)-3-phenylthieno[3,2-*b*]pyridine-2-carboxylate (57)

To a solution ethyl 3-bromo-6-(3,4-dimethoxyphenyl)thieno[3,2-*b*]pyridine-2-carboxylate **56** (1.00 g, 2.40 mmol) in dioxane (25 mL) were added phenylboronic acid (340 mg, 2.80 mmol), tripotassium phosphate (1.02 g, 4.80 mmol), and $Pd(PPh_3)_4$ (280 mg, 0.24 mmol). The reaction mixture was stirred at reflux overnight. After disappearance of the starting material, volatiles were evaporated *in vacuo* and the crude residue was purified by silica gel flash column chromatography (eluting with a mixture of heptane/acetone in a ratio of 80:20) yielding the title compound (422 mg, 42%).
^{1}H NMR (600 MHz, DMSO) δ 9.08 (d, *J* = 2.1 Hz, 1H), 8.87 (d, *J* = 2.1 Hz, 1H), 7.52 – 7.45 (m, 5H), 7.43 (d, *J* = 2.0 Hz, 1H), 7.40 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.11 (d, *J* = 8.4 Hz, 1H), 4.21 (q, *J* = 7.1 Hz, 2H), 3.88 (s, 3H), 3.82 (s, 3H), 1.14 (t, *J* = 7.1 Hz, 3H) ppm; ^{13}C NMR (151 MHz, DMSO) δ 161.90, 152.13, 149.46, 149.43, 147.58, 142.51, 134.65, 133.54, 133.33, 131.34, 130.60, 129.12, 128.12, 128.02, 127.45, 119.75, 112.43, 110.90, 61.49, 55.78, 55.70, 13.80 ppm; HRMS m/z $[M+H]^+$ calcd for $C_{24}H_{21}N_1O_4S_1$: 420.1264, found: 420.1261.

6-(3,4-Dimethoxyphenyl)-3-phenylthieno[3,2-*b*]pyridine-2-carboxylic acid (58)

To a solution of ethyl 6-(3,4-dimethoxyphenyl)-3-phenylthieno[3,2-*b*]pyridine-2-carboxylate **57** (200 mg, 0.48 mmol) in ethanol (10 mL) was added a solution of NaOH (192 mg, 4.8 mmol) in water (2 mL) and the mixture was stirred at room temperature for 4 hours. After disappearance of the starting material, the mixture was neutralised using a 6 N HCl solution in water, upon which a precipitate formed. The precipitate was filtered off and washed with water to yield the title compound (168 mg, 89%).
HRMS m/z $[M+H]^+$ calcd for $C_{22}H_{17}N_1O_4S_1$: 392.0951, found: 392.0941

6-(3,4-Dimethoxyphenyl)-3-phenylthieno[3,2-*b*]pyridine (59)

To a solution of 6-(3,4-dimethoxyphenyl)-3-phenylthieno[3,2-*b*]pyridine-2-carboxylic acid **58** (100 mg, 0.25 mmol) in quinoline (1 mL) was added copper (11 mg, 0.18 mmol) and the reaction was stirred at 200°C overnight. After disappearance of the starting material, the mixture was diluted with water and washed three times with dichloromethane. Organic phases were collected and concentrated *in vacuo* and the crude residue was purified by silica gel flash column chromatography (eluting with a mixture of heptane/acetone 80:20) to yield the title compound (42 mg, 48%).
^{1}H NMR (300 MHz, DMSO) δ 9.10 (d, *J* = 2.1 Hz, 1H), 8.84 (d, *J* = 2.1 Hz, 1H), 8.37 (s, 1H), 8.19 – 8.08 (m, 2H), 7.54 – 7.36 (m, 5H), 7.10 (d, *J* = 8.4 Hz, 1H), 3.89 (s, 3H), 3.82 (s, 3H) ppm; ^{13}C NMR (75 MHz, DMSO) δ 151.54, 149.47, 149.15, 146.00, 135.21, 134.51, 134.32, 131.19, 129.70,

128.57, 128.50, 128.23, 127.70, 119.49, 112.50, 110.87, 55.80, 55.74 ppm; HRMS m/z $[M+H]^+$ calcd for $C_{21}H_{17}N_1O_2S_1$: 348.1053, found: 348.1053.

5-Chloro-3-nitro-*N*-phenylpyridin-2-amine (61)

To a mixture of 2,5-dichloro-3-nitropyridine **60** (1.93 g, 10 mmol) and aniline (2.8 mL, 30 mmol) in 1,4-dioxane (100 mL) was added DIPEA (0.52 mL, 35 mmol) and the reaction was stirred at 80 °C overnight. After disappearance of the starting material, the reaction was cooled to room temperature and diluted with ethyl acetate, washed with water, dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue was purified by silica gel flash column chromatography (eluting with a gradient of ethyl acetate/Methanol in a ratio of 100:0 to 95:5) to yield the title compound (2.12 g) in 85% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 10.05 (s, 1H), 8.51 (d, *J* = 2.5 Hz, 1H), 8.42 (d, *J* = 2.5 Hz, 1H), 7.65 – 7.56 (m, 2H), 7.47 – 7.35 (m, 2H), 7.28 – 7.16 (m, 1H).

5-Chloro-*N*^{2}-phenylpyridine-2,3-diamine (62)

A mixture of 5-chloro-3-nitro-*N*-phenylpyridin-2-amine **61** (0.5 g, 2 mmol) and Raney nickel (0.1 g) in THF (20 mL) was degassed and filled with hydrogen gas. The reaction was allowed to stir at room temperature for two hours. After disappearance of the starting material the mixture was filtered, concentrated *in vacuo* and used in the following reaction without further purification.

6-Chloro-3-phenyl-3H-[1,2,3]triazolo[4,5-*b*]pyridine (63)

To a solution of 5-chloro-*N*^{2}-phenylpyridine-2,3-diamine **62** in a mixture of glacial acetic acid (0.56 mL, 10 mmol), water (0.5 mL) and dichloromethane (0.5 mL) at 0°C was added sodium nitrite (0.18 g, 2.6 mmol) dropwise. After 20 minutes of stirring, the mixture was diluted with dichloromethane, washed with water and dried over Na_2SO_4.. Organic phases were collected and concentrated *in vacuo*. The crude residue was purified by silica gel flash column chromatography (eluting with gradient of pentane/ethyl acetate in a ratio of 100:0 to 60:40) to yield the title compound (0.28 g, 62 %)

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.66 (d, *J* = 2.2 Hz, 1H), 8.39 (d, *J* = 2.2 Hz, 1H), 8.26 – 8.19 (m, 2H), 7.63 – 7.54 (m, 2H), 7.50 – 7.43 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.95, 143.72, 138.23, 136.19, 129.61, 128.60, 128.16, 127.71, 121.36.

6-(3,4-Dimethoxyphenyl)-3-phenyl-3H-[1,2,3]triazolo[4,5-*b*]pyridine (64)

To a solution of 6-chloro-3-phenyl-3H-[1,2,3]triazolo[4,5-*b*]pyridine **63** (120 mg, 0.52 mmol) in dimethylformamide (8 mL) and water (2 mL) were added 3,4-dimethoxyphenyl boronic acid (227 mg, 1.24 mmol), Cesium carbonate (542 mg, 1.61 mmol) and $Pd(PPh_3)_4$ (60 mg, 0.052 mmol). The reaction mixture was degassed and refilled with nitrogen gas and stirred at 100 °C overnight. After disappearance of the starting material, volatiles were evaporated and the crude residue was dissolved in ethyl acetate and washed with water. Collected organic phases were dried over Na_2SO_4, concentrated *in vacuo* and the crude residue was purified using silica gel flash column chromatography (eluting with a gradient of pentane/ethyl acetate in a ratio of 100:0 to 70:30) to yield the title compound (134 mg, 78%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.96 (d, *J* = 2.1 Hz, 1H), 8.51 (d, *J* = 2.1 Hz, 1H), 8.36 – 8.28 (m, 2H), 7.66 – 7.56 (m, 2H), 7.52 – 7.43 (m, 1H), 7.20 (dd, *J* = 8.2, 2.1 Hz, 1H), 7.14 (d, *J* = 2.1 Hz, 1H), 7.01 (d, *J* = 8.3 Hz, 1H), 3.99 (s, 3H), 3.95 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.50, 149.85, 149.72, 144.63, 138.34, 136.65, 134.26, 130.16, 129.62, 128.34, 125.75, 121.41, 120.25, 112.11, 111.00, 56.21, 56.17; HRMS: $[M + H]^+$ calcd for $C_{19}H_{16}N_4O_2$: 333.1346; found, 333.1345.

5-Bromo-3-nitro-*N*-phenylpyridin-2-amine (66)

To a solution of 5-Bromo-2-chloro-3-nitropyridine **65** (2.37g, 10 mmol) in NMP (5 mL) was added aniline (1.4 mL, 15 mmol) and DIPEA (5 mL, 30 mmol) and the mixture was stirred at 120°C for 1.5 h. After disappearance of the starting material, the reaction was allowed to cool to room temperature, diluted with water and washed with ethyl acetate. Organic phases were collected, washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by silica gel flash column chromatography (eluting with a mixture of pentane/ethyl acetate in a ratio of 90:10) to yield the title compound (2.29 g, 78%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 10.04 (s, 1H), 8.63 (s, 1H), 8.48 (d, *J* = 2.1 Hz, 1H), 7.59 (d, *J* = 8.0 Hz, 2H), 7.39 (t, *J* = 7.8 Hz, 2H), 7.27 – 7.13 (m, 1H) ppm; ^{13}C NMR (126 MHz, DMSO) δ 155.29, 148.38, 138.04, 136.94, 129.23, 128.70, 124.59, 123.14, 106.20 ppm.

5-(3,4-Dimethoxyphenyl)-3-nitro-*N*-phenylpyridin-2-amine (67)

To a solution of 5-bromo-3-nitro-*N*-phenylpyridin-2-amine **66** (600 mg, 2.0 mmol), in dioxane (16 mL) and water (4 mL) were added (3,4-dimethoxyphenyl)boronic acid (408 mg, 2.2 mmol), sodium carbonate (912 mg, 8.6 mmol) and $Pd(PPh_3)_4$ (120 mg, 0.1 mmol). The reaction mixture was degassed and refilled with nitrogen gas and stirred at 80 °C overnight. After disappearance of the starting material, volatiles were evaporated *in vacuo* and the crude residue was dissolved in ethyl acetate and washed with water. Collected organic phases were dried over Na_2SO_4, concentrated *in vacuo* and the crude residue was purified using silica gel flash column chromatography (eluting with a mixture of Pentane/Ethyl acetate in a ratio of 75:25) to yield the title compound (360 mg, 78%).

6-(3,4-Dimethoxyphenyl)-3-phenyl-3H-imidazo[4,5-*b*]pyridine (69)

To a solution of 5-(3,4-dimethoxyphenyl)-3-nitro-*N*-phenylpyridin-2-amine **68** (360 mg, 1 mmol) in glacial acetic acid (5 mL), was added zinc (2.2 g, 34 mmol) and the mixture was stirred at reflux for 3 hours. After disappearance of the starting material, the reaction was allowed to cool to room temperature and filtrated through celite, washed with acetic acid and concentrated *in vacuo*. The crude residue was subsequently suspended in triethyl orthoformate (10 mL) and stirred at 130°C for 2 h. After disappearance of the starting material the volatiles were evaporated *in vacuo* and the crude residue was dissolved in ethyl acetate, washed with water, saturated sodium bicarbonate and brine. Collected organic phases were dried over $MgSO_4$ and concentrated *in vacuo*. The crude residue was purified using silica gel flash column chromatography (eluting with a mixture of ethyl acetate/pentane in a ratio of 6:4) to give the title compound which was further purified by crystallisation from methanol (116 mg, 35%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.68 (d, *J* = 2.0 Hz, 1H), 8.38 (s, 1H), 8.29 (d, *J* = 2.1 Hz, 1H), 7.84 – 7.77 (m, 2H), 7.65 – 7.56 (m, 2H), 7.51 – 7.43 (m, 1H), 7.20 (dd, *J* = 8.2, 2.1 Hz, 1H), 7.15 (d, *J* = 2.1 Hz, 1H), 7.01 (d, *J* = 8.3 Hz, 1H), 3.98 (s, 3H), 3.95 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.77, 149.27, 146.24, 144.29, 143.76, 136.36, 133.16, 131.70, 130.01, 128.07, 126.43, 123.64, 120.08, 112.17, 111.21, 56.24; HRMS: $[M + H]^+$ calcd for $C_{20}H_{17}N_3O_2$, 392.1393; found, 392.1393.

7-Bromoquinazolin-4-ol (71)

To a solution of methyl 2-amino-4-bromobenzoate **70** (1.00 g, 4.35 mmol) in triethyl orthoformate (20 mL) was added ammonium acetate (419 mg, 5.43 mmol) and the mixture was stirred at reflux overnight. After disappearance of the starting material the volatiles were evaporated *in vacuo* and the crude residue was dissolved in water. The formed precipitate was filtered off, yielding the title compound (600 mg, 60%).

^{1}H NMR (300 MHz, DMSO) δ 12.38 (bs, 1H), 8.14 (s, 1H), 8.03 (d, *J* = 8.5 Hz, 1H), 7.89 (d, *J* = 1.8 Hz, 1H), 7.69 (dd, *J* = 8.5, 1.8 Hz, 1H).

7-Bromo-4-chloroquinazoline (72)

7-Bromoquinazolin-4-ol **71** (200 mg, 0,88 mmol) was suspended in phosphorus oxychloride (3,0 mL) and stirred at 125°C overnight. After disappearance of the starting material, the solvent was evaporated *in vacuo* and the crude residue was used as such without further purification in the following reaction.

4-(7-Bromoquinazolin-4-yl)morpholine (73)

To a solution of crude residue containing 7-bromo-4-chloroquinazoline **72** in dioxane was added morpholine (958 µL, 11 mmol) and the mixture was stirred at room temperature for 3 hours. After disappearance of the starting material volatiles were evaporated *in vacuo* and the crude residue was purified by silica gel flash column chromatography (eluting with a mixture of dichloromethane/methanol 95:5) yielding the title compound (148 mg, 57%).

^{1}H NMR (300 MHz, DMSO) δ 8.83 (s, 1H), 8.15 – 8.03 (m, 2H), 7.82 (dd, *J* = 9.0, 1.9 Hz, 1H), 4.21 – 4.00 (m, 4H), 3.91 – 3.72 (m, 5H).

4-(7-(3,4-Dimethoxyphenyl)quinazolin-4-yl)morpholine (74)

To a solution of 4-(7-bromoquinazolin-4-yl)morpholine **73** (100 mg, 0.34 mmol) in dioxane (4 mL) and water (1 mL) were added 3,4 dimethoxyphenyl boronic acid (74 mg, 0.41 mmol), potassium carbonate (94 mg, 0.68 mmol), and $Pd(PPh_3)_4$ (12 mg, 0,01 mmol). The reaction mixture was degassed, refilled with nitrogen and stirred at 90 °C overnight.

The volatiles were evaporated *in vacuo* and the crude residue was purified by silica gel flash column chromatography (eluting with a mixture of dichloromethane/methanol in a ratio of 99:1) yielding the title compound (90 mg, 75%).

^{1}H NMR (300 MHz, DMSO) δ 8.66 (s, 1H), 8.10 – 8.03 (m, 2H), 7.90 – 7.83 (m, 1H), 7.43 – 7.37 (m, 2H), 7.11 (d, *J* = 8.9 Hz, 1H), 3.90 (s, 3H), 3.83 (s, 3H), 3.81 – 3.73 (m, 8H); ^{13}C NMR (75 MHz, DMSO) δ 163.68, 154.18, 152.06, 149.59, 149.38, 144.17, 131.28, 125.90, 124.57, 124.41, 119.72, 114.52, 112.38, 110.80, 66.17, 55.78, 55.75, 49.81; HRMS m/z $[M+H]^+$ calcd for $C_{20}H_{21}N_3O_3$: 352.16555, found 352.1660.

3-Amino-5-bromopyridine-2-carbonitrile (75)

To a stirred solution of iron powder (4.900 g, 87.7 mmol) in 50 mL of acetic acid at room temperature, was added 5-bromo-3-nitropyridine-2-carbonitrile **53** (10 g, , 43.9 mmol) at once and the reaction was stirred for 3 hours. After completion, ethyl acetate was added to the mixture and the precipitate was filtered off. The filtered cake was washed thoroughly with ethyl acetate and the filtrate was evaporated *in vacuo*. Crude was purified by silica gel flash column chromatography (eluting with dichloromethane) to yield the title compound (4.845 g 56%).

^{1}H NMR (300 MHz, DMSO) δ 8.08 – 7.66 (m, 1H), 7.55 – 7.26 (m, 1H), 6.58 (bs, 2H); HRMS m/z $[M+H]^+$ calcd for $C_6H_4BrN_3$: 197.96618, found 197.9656.

3-Amino-5-(3,4-dimethoxyphenyl)picolinonitrile (76)

To a solution 3-amino-5-bromopyridine-2-carbonitrile **75** (1.0 g, 5.05 mmol) in dioxane (40 mL) and water (10 mL) were added 3,4 dimethoxyphenylboronic acid (1.11 g, 6.06 mmol), potassium carbonate (1.38 g, 10.1 mmol), and $Pd(PPh_3)_4$ (58 mg, 0,05 mmol). The reaction mixture was stirred at 90 °C overnight. The volatiles were evaporated and the crude residue was purified by silica gel flash column chromatography (eluting with a mixture of dichloromethane/acetone in a ratio of 90:10) yielding the title compound (1.27 g, 98%).

^{1}H NMR (300 MHz, DMSO) δ 8.22 (d, *J* = 1.9 Hz, 1H), 7.42 (d, *J* = 1.9 Hz, 1H), 7.24 – 7.20 (m, 2H), 7.12 – 7.05 (m, 1H), 3.85 (s, 3H), 3.81 (s, 3H); ^{13}C NMR (75 MHz, DMSO) δ 149.87, 149.34, 148.80, 139.62, 137.72, 128.63, 119.65, 117.34, 113.03, 112.34, 110.51, 55.76 ppm.

7-(3,4-Dimethoxyphenyl)pyrido[3,2-*d*]pyrimidin-4-amine (77)

3-Amino-5-(3,4-dimethoxyphenyl)picolinonitrile **76** (200 mg) was suspended in formamide (4 mL) and heated in a microwave for 10 minutes at 160 °C. The reaction was allowed to cool to room temperature and the formed precipitate was filtered off and washed with water to yield the title compound (164 mg, 74 %).

^{1}H NMR (300 MHz, DMSO) δ 9.12 (d, *J* = 2.1 Hz, 1H), 8.45 (s, 1H), 8.30 (d, *J* = 2.1 Hz, 1H), 7.93 (bs, 2H), 7.50 – 7.39 (m, 2H), 7.10 (d, *J* = 8.1 Hz, 1H), 3.90 (s, 3H), 3.83 (s, 3H); ^{13}C NMR (75 MHz, DMSO) δ 161.91, 156.56, 149.87, 149.51, 147.32, 144.88, 139.76, 131.01, 129.79, 128.67, 120.14, 112.39, 111.11, 55.88, 55.75 ppm; HRMS m/z $[M+H]^+$ calcd for $C_{15}H_{14}N_4O_2$: 283.1189, found: 383.1188.

7-(3,4-Dimethoxyphenyl)pyrido[3,2-*d*]pyrimidin-4-ol (78)

7-(3,4-Dimethoxyphenyl)pyrido[3,2-*d*]pyrimidin-4-amine **77** (120 mg, 0.43 mmol) was suspended in a 6 N HCl solution (5 mL) and stirred at reflux for 1 hour. The reaction was allowed to cool to room temperature and the formed precipitate was filtered off to yield the title compound (85 mg, 66%). HRMS m/z $[M+H]^+$ calcd for $C_{15}H_{13}N_3O_3$:284.1030, found: 284.1029.

7-(3,4-Dimethoxyphenyl)-4-(1H-1,2,4-triazol-1-yl)pyrido[3,2-*d*]pyrimidine (79)

A solution of phosphorus oxychloride (230 mg, 1.50 mmol), triazole (207 mg, 3.0 mmol) and DIPEA (116 mg, 0.90 mmol) in acetonitrile (5 mL) was stirred for 20 minutes at room temperature when 7-(3,4-dimethoxyphenyl)pyrido[3,2-*d*]pyrimidin-4-ol **78** (85 mg, 0.30 mmol) was added and the mixture was stirred for 48 hours at room temperature. After disappearance of the starting material, the precipitate was filtered off and the filtrate was washed with a 10% solution of HCl. Organic phases were collected and concentrated *in vacuo*. The crude residue was used as such without further purification in the following reaction.

4-(7-(3,4-Dimethoxyphenyl)pyrido[3,2-*d*]pyrimidin-4-yl)morpholine (80)

To a solution of 7-(3,4-dimethoxyphenyl)-4-(1H-1,2,4-triazol-1-yl)pyrido[3,2-*d*]pyrimidine **79** (0.35 mmol) in dioxane (5 mL) was added morpholine (152 mg, 1.75 mmol) and the mixture was stirred at reflux for 1 hour. After disappearance of the starting material, the volatiles were evaporated *in vacuo* and the crude residue was purified by silica gel flash column chromatography (eluting with a mixture of dichloromethane/methanol in a ratio of 95:5) yielding the title compound (26 mg 25%).

^{1}H NMR (300 MHz, DMSO) δ 9.18 (d, *J* = 2.3 Hz, 1H), 8.56 (s, 1H), 8.36 (d, *J* = 2.3 Hz, 1H), 7.52 – 7.47 (m, 2H), 7.12 (d, *J* = 9.0 Hz, 1H), 4.49 – 4.39 (m, 4H), 3.90 (s, 3H), 3.83 (s, 3H), 3.81 – 3.76 (m, 4H) ppm; ^{13}C NMR (75 MHz, DMSO) δ 158.58, 154.97, 149.99, 149.54, 147.24, 145.83, 138.64, 131.77, 131.35, 128.17, 120.06, 112.44, 110.92, 66.52, 55.86, 55.77, 47.80 ppm; HRMS m/z $[M+H]^+$ calcd for $C_{19}H_{20}N_4O_3$: 353.1608, 353.1601.

7-(3,4-Dimethoxyphenyl)pyrido[2,3-*d*]pyrimidin-4-amine (81)

2-Amino-6-(3,4-dimethoxyphenyl)nicotinonitrile **10** (400 mg) was suspended in formamide (4 mL) and heated in a microwave for 10 minutes at 160 °C. The reaction was allowed to cool to room temperature and the precipitate was filtered off and washed with water to yield the title compound (200 mg, 46%).

^{1}H NMR (300 MHz, DMSO) δ 8.69 (d, *J* = 8.6 Hz, 1H), 8.52 (s, 1H), 8.15 (d, *J* = 8.7 Hz, 1H), 8.02 (bs, 1H), 7.93 – 7.83 (m, 2H), 7.13 (d, *J* = 9.0 Hz, 1H), 3.90 (s, 3H), 3.86 (s, 3H) ppm; ^{13}C NMR (75 MHz, DMSO) δ 163.01, 161.23, 159.06, 158.68, 151.15, 149.14, 134.21, 130.56, 120.86, 117.87, 111.78, 110.56, 107.50, 55.75, 55.72 ppm; HRMS m/z $[M+H]^+$ calcd for $C_{15}H_{14}N_4O_2$:283.1189, found: 283.1189.

7-(3,4-Dimethoxyphenyl)pyrido[2,3-*d*]pyrimidin-4-ol (82)

7-(3,4-Dimethoxyphenyl)pyrido[2,3-*d*]pyrimidin-4-amine **81** (200 mg, 0.71 mmol) was suspended in a 6 N HCl solution (5 mL) and stirred at reflux for 2 hours. The reaction was allowed to cool to room temperature and the precipitate was filtered off and washed with water to yield the title compound (120 mg, 60%).

^{1}H NMR (300 MHz, DMSO) δ 8.69 (s, 1H), 8.53 (d, *J* = 8.4 Hz, 1H), 8.21 (d, *J* = 8.4 Hz, 1H), 7.91 – 7.80 (m, 2H), 7.15 (d, *J* = 8.3 Hz, 1H), 3.89 (s, 3H), 3.86 (s, 3H) ppm; ^{13}C NMR (75 MHz, DMSO) δ 161.38, 160.93, 151.50, 149.99, 149.18, 136.81, 132.19, 131.67, 131.55, 129.58, 128.99, 128.83, 121.09, 119.34, 111.94, 110.62, 55.83 ppm; HRMS m/z $[M+H]^+$ calcd for $C_{15}H_{13}N_3O_3$: 284.1030, found: 284.1025.

7-(3,4-Dimethoxyphenyl)-4-(1H-1,2,4-triazol-1-yl)pyrido[2,3-*d*]pyrimidine (83)

A solution of phosphorus oxychloride (268 mg, 1.73 mmol), triazole (242 mg, 3.5 mmol) and DIPEA (136 mg, 1.05 mmol) in acetonitrile (5 mL) was stirred for 20 minutes at room temperature when 7-(3,4-dimethoxyphenyl)pyrido[3,2-*d*]pyrimidin-4-ol **82** (100 mg, 0.35 mmol) was added. The mixture was stirred for 48 hours at room temperature. After disappearance of the starting material, the precipitate was filtered of and the filtrate was washed with a 10% solution of HCl. Organic phases were collected and concentrated *in vacuo*. The crude residue was used as such in the following reaction.

4-(7-(3,4-Dimethoxyphenyl)pyrido[2,3-*d*]pyrimidin-4-yl)morpholine (84)

To a solution of 7-(3,4-dimethoxyphenyl)-4-(1H-1,2,4-triazol-1-yl)pyrido[2,3-*d*]pyrimidine **83** (0.35 mmol) in dioxane (5 mL) was added morpholine (152 mg, 1.75 mmol) and the mixture was stirred at reflux for 1 hour. The volatiles were evaporated *in vacuo* and the crude residue was purified by silica gel flash column chromatography (eluting with a mixture of dichloromethane/methanol in a ratio of 95:5) yielding the title compound (20 mg, 19%).

^{1}H NMR (300 MHz, DMSO) δ 8.71 (s, 1H), 8.43 (d, *J* = 8.8 Hz, 1H), 8.05 (d, *J* = 8.7 Hz, 1H), 7.92 – 7.80 (m, 2H), 7.13 (d, *J* = 8.2 Hz, 1H), 3.90 (s, 3H), 3.86 (s, 3H), 3.85 – 3.75 (m, 8H) ppm; ^{13}C NMR (75 MHz, DMSO) δ 163.99, 160.90, 159.98, 157.06, 151.36, 149.19, 135.87, 130.21, 121.00, 117.33, 111.83, 110.49, 108.67, 66.13, 55.77, 55.72, 49.48 ppm; HRMS m/z $[M+H]^+$ calcd for $C_{19}H_{20}N_4O_3$:353.1608, found:353.1597.

3-Amino-5-(3,4-dimethoxyphenyl)pyrazine-2-carboxamide (85)

To a solution of 3-amino-5-(3,4-dimethoxyphenyl)pyrazine-2-carbonitrile **19** (500 mg, 1.95 mmol) in concentrated formic acid (3 mL) was added concentrated sulphuric acid (140 µL). The mixture was heated in a microwave for 20 minutes at 100°C. After disappearance of the starting material the mixture was allowed to cool and the formed bright yellow

precipitate was filtered off and washed with water to yield the title compound (417 mg, 78%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.26 (s, 1H), 7.63 (d, *J* = 2.0 Hz, 1H), 7.58 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.97 (d, *J* = 8.4 Hz, 1H), 4.00 (s, 3H), 3.96 (s, 3H) ppm; ^{13}C NMR (75 MHz, DMSO) δ 168.73, 154.58, 152.77, 150.98, 149.10, 128.35, 127.88, 123.66, 120.34, 111.83, 110.23, 55.73 ppm; HRMS m/z $[M+H]^+$ calcd for $C_{13}H_{14}N_4O_3$: 275.1139, found: 275.1164.

7-(3,4-Dimethoxyphenyl)pteridin-4(3H)-one (86)

3-Amino-5-(3,4-dimethoxyphenyl)pyrazine-2-carbonitrile **85** (400 mg, 1.56 mmol) was suspended in formamide (10 mL) and heated in a microwave at 160°C for 20 minutes. The reaction was allowed to cool to room temperature, the precipitate was filtered off and washed with water to yield the title compound (315 mg, 71%).

HRMS m/z $[M+H]^+$ calcd for $C_{14}H_{12}N_4O_3$: 285.0982, found: 285.0982.

4-Chloro-7-(3,4-dimethoxyphenyl)pteridine (87)

To a solution of 7-(3,4-dimethoxyphenyl)pteridin-4(3H)-one **86** (300 mg, 1.05 mmol) in dry toluene were added DIPEA (407 mg, 3.15 mmol) and phosphorus oxychloride (483 mg, 3.15 mmol) and the mixture was stirred at reflux overnight. After disappearance of the starting material, the volatiles were evaporated and the crude residue was purified by silica gel flash column chromatography (eluting with a gradient of dichloromethane/ethyl acetate in a ratio of 97:3 to 95:5) yielding the title compound (179 mg, 55%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.50 (s, 1H), 9.23 (s, 1H), 8.02 (d, *J* = 2.1 Hz, 1H), 7.86 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.02 (d, *J* = 8.5 Hz, 1H), 4.02 (s, 3H), 3.98 (s, 3H) ppm; HRMS m/z $[M+H]^+$ calcd for $C_{14}H_{11}Cl_1N_4O_2$: 303.0643, found: 303.0645.

4-(7-(3,4-Dimethoxyphenyl)pteridin-4-yl)morpholine (88)

To a solution of 4-chloro-7-(3,4-dimethoxyphenyl)pteridine **87** (100 mg, 0.35 mmol) in dioxane (5 mL) was added morpholine (152 mg, 1.75 mmol) and the mixture was stirred at reflux for 4 hours. After disappearance of the starting material, volatiles were evaporated and the crude residue was dissolved in water and washed with dichloromethane. The organic phases were collected and concentrated *in vacuo* to yield the title compound (120 mg, 97 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.11 (s, 1H), 8.73 (s, 1H), 8.01 (d, *J* = 2.1 Hz, 1H), 7.78 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.00 (d, *J* = 8.5 Hz, 1H), 4.58 – 4.43 (m, 4H), 4.02 (s, 3H), 3.97 (s, 3H), 3.92 – 3.86 (m, 4H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.85, 158.52, 155.88, 155.43, 152.32, 150.02, 138.60, 128.29, 126.40, 121.21, 111.09, 110.71, 67.33, 56.41, 56.23, 48.24 ppm; HRMS m/z $[M+H]^+$ calcd for $C_{18}H_{19}N_5O_3$: 354.1561, found: 354.1557.

GAK binding assay

Kd values for GAK were determined as previously described [27]. Briefly, the DNA-tagged GAK, an immobilized ligand on streptavidin-coated magnetic beads, and the test compound are combined. When binding occurs between GAK and a test compound, no binding can occur between GAK and the immobilized ligand. Upon washing, the compound-bound, DNA-tagged GAK is washed away. The beads carrying the ligands are then resuspended in elution buffer and the remaining kinase concentration measured by qPCR on the eluate. Kd values are determined using dose-response curves.

Abbreviations

AP: Adaptor protein complex; Boc_2O: Di-*tert*-butyldicarbonate; CCV: Clathrin coated vesicle; CHIKV: Chikungunya virus; DMF-DMA: *N*,*N*-Dimethylformamide dimethylacetal; DPPA: Diphenyl phosphoryl azide; D-t-BPF: 1,1′-Bis(di-*tert*-butylphosphino)ferrocene; EBOV: Ebola virus; DENV: Dengue virus; GAK: Cyclin G-associated kinase; HCV: hepatitis C virus; Kd: Dissociation constant; KOtBu: Potassium *tert*-butyloxide; LR: Lawesson's reagent; LRRK2: leucine rich repeat kinase 2; NIS: N-Iodosuccinimide; *p*-TsOH: *para*-toluenesulphonic acid; *t*-BuONO: *tert*-butyl nitrite; TGN: *trans*-Golgi network

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Keywords: cyclin G associated kinase • medicinal chemistry • nitrogen heterocycles • scaffold hopping • isothiazolo[4,3-*b*]pyridine

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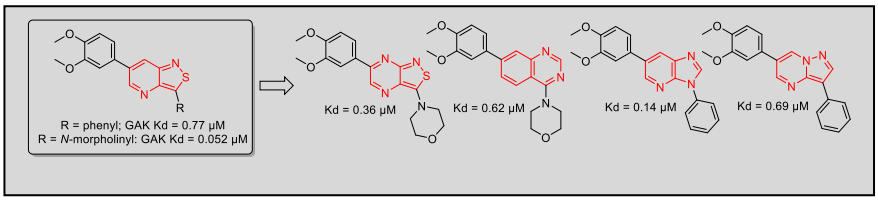

Starting from a cyclin G associated kinase (GAK) inhibitor based on an isothiazolo[4,3-*b*]pyridine skeleton, a scaffold hopping strategy was applied, resulting in the synthesis of 13 novel scaffolds. Four of these scaffolds (isothiazolo[3,4-*b*]pyrazine, pyrazolo[1,5-*a*]pyrimidine, imidazo[4,5-*b*]pyridine and quinazoline) display potent GAK affinity with Kd values of less than 1 µM. These heterocycles can be used as starting points for the discovery of novel GAK inhibitors based on a different chemotype.