Journal of Medicinal Chemistry

Article

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J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/jm3011349 • Publication Date (Web): 05 Nov 2012

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Core Refinement towards Permeable β-Secretase (BACE-1) Inhibitors with Low hERG Activity

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ABSTRACT

Using iterative design aided by predictive models for target affinity, brain permeability and hERG activity, novel and diverse compounds based on cyclic amidine and guanidine cores were synthesized with the goal of finding BACE-1 inhibitors as a treatment for Alzheimer's disease. Since synthetic feasibility had low priority in the design of the cores, an extensive synthetic effort was needed to make the relevant compounds. Syntheses of these compounds are reported, together with physicochemical properties and structure activity relationships based on *in vitro* data. Four crystal structures of diverse amidines binding in the active site are deposited and

discussed. Inhibitors of BACE-1 with 3 μM – 32 nM potencies in cells are shown, together with data on *in vivo* brain exposure levels for four compounds. The results presented show the importance of the core structure for the profile of the final compounds.

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease, with a huge and growing medical need.[1] One of the pathological hallmarks of an AD brain is the presence of amyloid plaques.[2] The main constituent of these plaques is amyloid-β (Aβ), which is produced by the sequential proteolytic cleavage of amyloid precursor protein (APP).[3] Beta-site APP cleaving enzyme (BACE-1) is responsible for the first step in the processing of APP, followed by the cleavage by the gamma-secretase complex (γ-secretase).[4-8] Early onset AD,[9] as well as protection from AD,[10] is associated with genetic alterations in APP, thus implicating that the amyloid pathway and alterations in the production of Aβ is important for disease. Taken together, this indicates that BACE-1 inhibition, to stop or reduce the production of Aβ, is an attractive approach to find a treatment for Alzheimer's disease. However, thirteen years after the cloning and identification of BACE-1, only a few BACE-1 inhibitors have been tested in the early stages of clinical trials.[11,12]

In the search for BACE-1 inhibitors, we have performed several lead generation screening campaigns using various methods, where fragment-based lead generation has been the most successful.[13] The majority of the interesting structural motifs resulting from these efforts have been based on amidine or guanidine core structures. These have been further developed into lead series through scaffold hopping and optimization of the substituents (Figure 1).

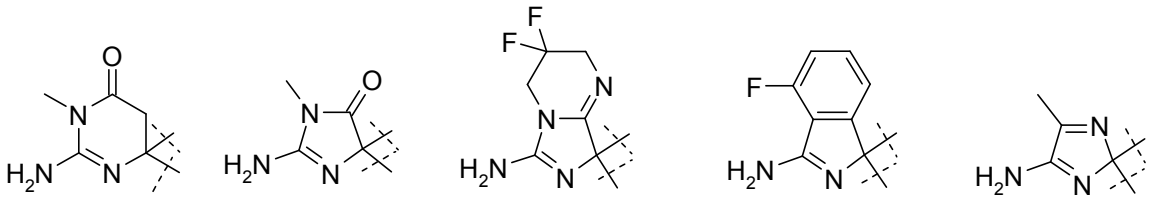


Figure 1. Examples of five amidine or guanidine core structures developed into lead series.[13-21]

Other groups have also presented amidine or guanidine lead series of BACE-1 inhibitors starting from hits identified from fragment-based or high throughput screens.[22-25] As previously described, the amidine or guanidine moiety interacts efficiently with the catalytic aspartates of BACE-1 via a hydrogen bonding network. This strong interaction has allowed modifications of other parts of the molecule to improve the overall property of the BACE-1 inhibitor.[26] Many of these reported amidines and guanidines have, after extensive optimization, shown good potencies in cellular assays and reduction of Aβ in plasma.[22-25] Recently, after overcoming problems with limited brain exposure, some compounds have also shown reduction of Aβ in brain.[20,21,27] The two hydrogen bond donors and the basic center on the amidine/guanidine core are however not optimal for high brain exposure and many of the reported amidines suffer from P-glycoprotein (P-gp)-mediated transport out of the brain, so called efflux.[28] A low efflux has been shown to be most important to achieve sufficient brain exposure *in vivo*.[29] Other properties of the amidine/guanidine inhibitors reported, which limit their exposure in brain, have been large size, high plasma protein binding or high clearance.[11]

In addition, the general properties of many of the described BACE-1 inhibitors, featuring a basic functionality and being quite lipophilic, coincides with properties correlated to inhibition of

the human ether-à-go-go related gene (hERG)-encoded potassium ion channel.[30] So far, only few data points from hERG inhibition have been reported for BACE-1 inhibitors.[20,31]

This work describes our continued research in amidine- and guanidine-based BACE-1 inhibitors. In light of the above mentioned challenges, we reasoned that the inherent properties of the cores would be crucial for the profile of the final test compounds, and consequently we focused on refining the amidine-containing core. Furthermore, we recognized the importance to focus on optimization of the core early in the lead generation phase, since the selection of the core is an important step, defining the framework for future lead optimization.[32]

We thus set out to address target affinity, permeability and hERG activity in a multi-parameter optimization fashion.[33,34] Initially, new core structures were designed from the generic amidine structure in Figure 2. The design was aided by predictive chemistry, and a workflow based on three *in silico* models was established to aid in the prioritization of a number of core structures for synthesis.[35] First, a novel tool based on quantum mechanical calculations was developed to facilitate predictions of BACE-1 affinity.[36] Second, a predictive permeability tool was developed, with suggested limits for pKa and lipophilicity.[35] Third, to predict hERG activity, a QSAR model based on pKa and lipophilicity and size was established.[35]

The new core structures were designed iteratively, with the aim to achieve promising *in silico* profiles predicting that the final test compounds would have a balanced profile regarding the optimization parameters listed above. With the help of these predictive models, a physicochemical space of interest, spanned by limits in pKa and lipophilicity, was suggested. Unfortunately, these properties are inversely correlated when it comes to optimization of target activity, permeability and hERG activity.[35] In order to cover the interesting physicochemical space, aiming to find cores with the most well-balanced set of properties, a number of cores were

designed and selected for synthesis. More details on the selection workflow, predictions, and analyses of how the inherent physicochemical properties of the core structures relate to *in vitro* data for the test compounds, are described in a companion paper.[35]

The core structures were decorated with a selection of substituents from a pre-defined set (R1 and R2, Figure 2). These substituents were collected from a training set of previous in-house series and literature compounds, and were chosen to facilitate core-to-core comparisons of *in vitro* results for lead-like test compounds via matched pair analysis.[35] In addition, the R-groups were also selected for contributing equally to target potency, and carefully combined in order to keep lipophilicity of the test compounds lead-like, with cLogP between 1 and 3.5.[37] Thus, we envisaged quick assessments of whether the properties of the new cores were promising enough for further lead optimization efforts.

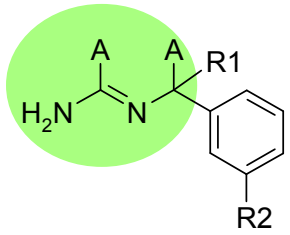


Figure 2. Amidine substructure with the "core" indicated on green background (A = any atom). The R1- and R2-substituents (and a phenyl group) added to each core were selected to allow for match pair analyses.[35] The R2-substituent in the *meta* position is a common feature of known BACE-1 inhibitors for reaching into the S3 sub-pocket of the active site. [19-20]

Synthetic feasibility of the cores was intentionally given a lower selection priority. The synthetic method developed for each core was used to synthesize just a few representative

compounds and the diversity of the cores made it difficult to use common intermediates. The core structures representing the synthesized compounds are presented in Figure 3.

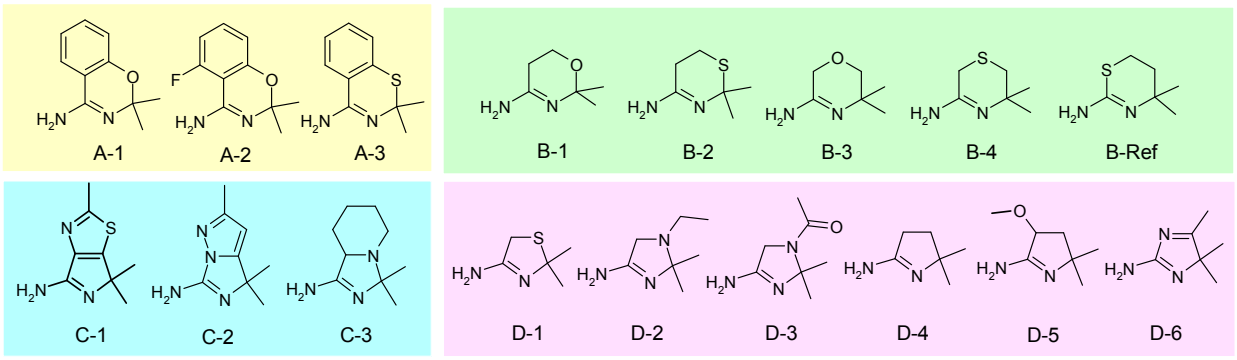


Figure 3. Core structures representing the compounds included in this report, divided into four structural subgroups; A: 6-membered bicyclic cores, B: 6-membered monocyclic cores, C: 5-membered bicyclic cores, D: 5-membered monocyclic cores. B-Ref represents a compound that have reached clinical Phase I studies.[23] During this work, five of the cores (A-3, B-1, B-2, B-3 and C-1) were independently published by others.[38,39,40]

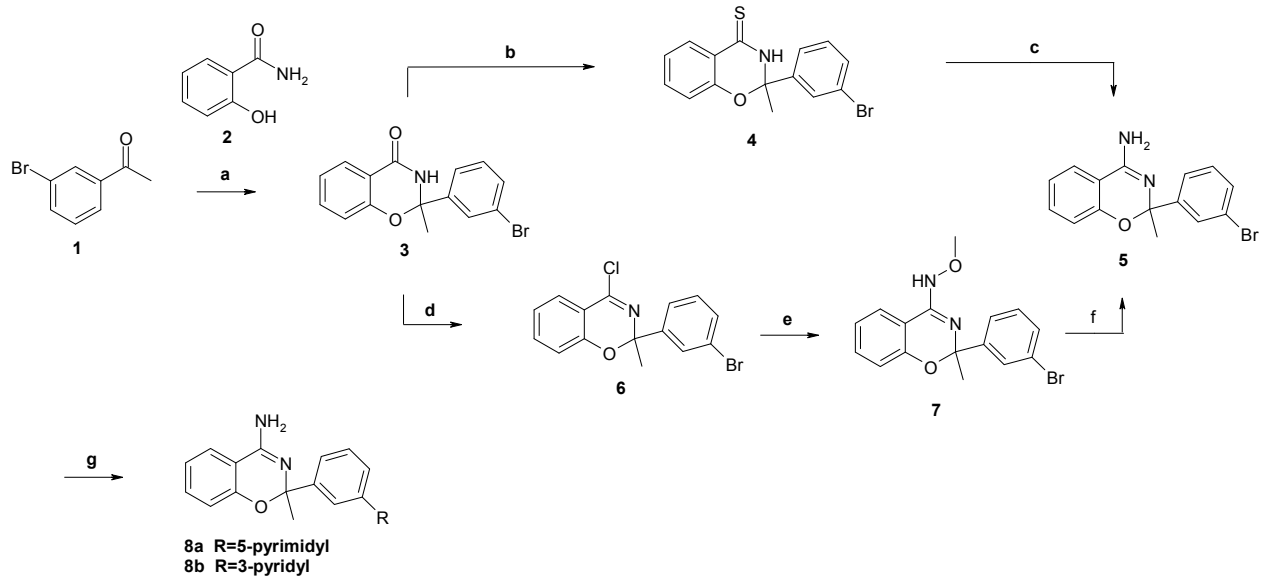
SYNTHETIC CHEMISTRY

Within the iterative design cycles using the predictive in silico models, more than ten cores were selected for synthesis.[35] The synthesis of compounds representing cores of subtype A were initiated prior to the setup of the predictive workflow.[41] Herein we describe the successful synthesis of compounds representing all of these cores, divided into their subgroups as described in Figure 3.

6-Membered bicyclic amidines (core sub-type A). Our synthetic efforts started by preparing core types A-1 and A-2.[41] Attempts for improvements of this core sub-type, to decrease the

lipophilicity and lower the pKa were inspired by previous work.[20,21] The synthesis of two examples (**8a** and **8b**) is described in detail in Scheme 1. The first step involved a condensation of commercially available 1-(3-bromophenyl)ethanone (**1**) and 2-hydroxybenzamide (**2**) under acidic conditions, continuously distilling off the formed water using a Dean-Stark trap to form the lactam **3**. Thioamide **4** could then be obtained by treating lactam **3** with P_2S_5 in pyridine. Amidine **5** was then obtained by treating the thioamide **4** with ammonia in methanol in the presence of a mild oxidant (*t*-BuOOH). However, the thiolactam **4** was somewhat prone to hydrolysis which made the conversion to the amidine **5** hard to control, so a preferred alternative route to amidine **5** was developed as outlined in Scheme 1. Thus, the lactam **3** was converted to the chloroimidate **6** and the amidine **5** was then obtained in two steps, via the hydroxylamine **7**. The final compounds could then be obtained by a Suzuki-Miyaura coupling.

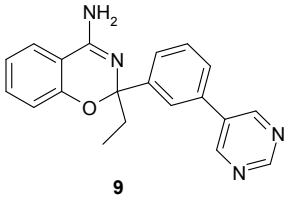
Scheme 1. Synthesis of core sub-types A-1 and A-2[a]



[a]Reagents and conditions: (a) *p*-toluenesulfonic acid monohydrate, toluene, reflux, Dean-Stark. (b) P_2S_5, Pyridine, 120 °C (c) NH_3 (7 M in MeOH), *t*-BuOOH (aq) (70%). (d) PCl_5, $POCl_3$, 50

°C (e) $MeONH_3Cl$, DIPEA, DMF, 90 °C. (f) Zinc, AcOH, rt. (g) Pyrimidine-5-ylboronic acid or pyridine-3-ylboronic acid, bis(triphenylphosphine)palladium(II)chloride, Na_2CO_3 (1 M aq), DME:H_2O (2:1), 80 °C.

To evaluate the effect of the size of the non-aromatic substituent, compound **9**, with a slightly larger R1-group, was synthesized according to Scheme 1 starting from 1-(3-bromophenyl)propan-1-one. It was also interesting to introduce an electron withdrawing fluoride on the fused phenyl ring of the core to lower the pKa. The syntheses of two such examples (**10a** and **10b**) were performed according to Scheme 1.



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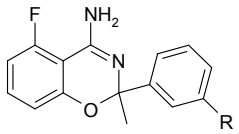


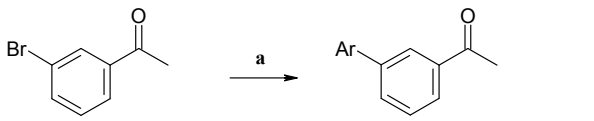
10a R=5-pyrimidyl
10b R=3-pyridyl

One strategy that was in common for the different syntheses described below was that the Suzuki-Miyaura reaction was preferably used in the last step to create multiple compounds from a common intermediate. However, some amidines with high pKa values demanded high catalyst loading to achieve complete conversion, probably due to catalyst poisoning. In this case, the Suzuki-Miyaura reaction was performed on the lactam instead.

In the case of cores containing a thioether or a base-sensitive amidine or lactam, the Suzuki-Miyaura reaction was run as the first step. Coupling of the acetophenone **1** with three various boronic acids (Scheme 2), gave three intermediates (**11a-c**) that were commonly used in several syntheses described below. The *meta*-bromide present in all starting materials was substituted for an aryl ring using the corresponding boronic acid, a source of palladium(II), a base and the

reaction was performed in an aqueous solvent at an elevated temperature. This method is referred to as standard Suzuki-Miyaura conditions in the following text.

Scheme 2. Synthesis of compounds **11a-c**[a]



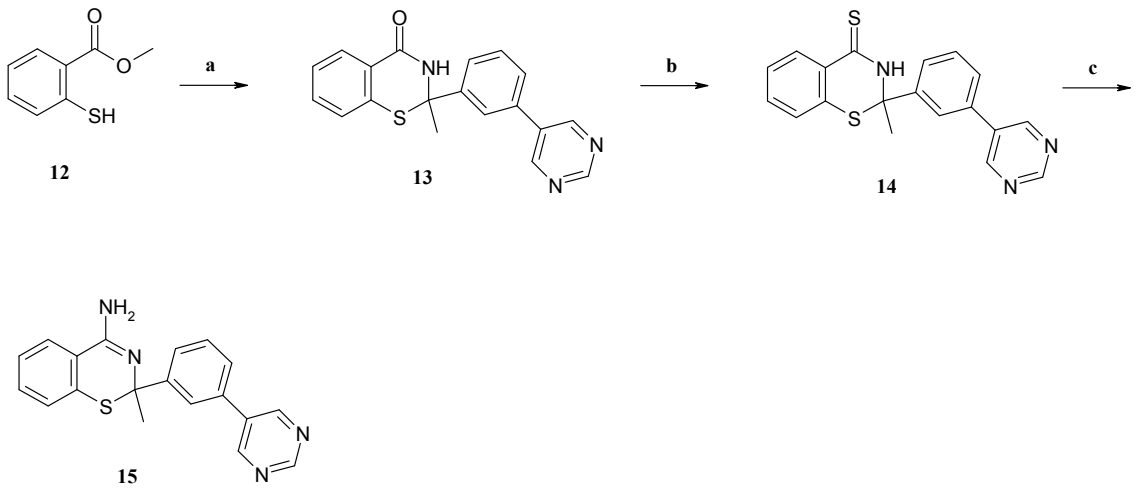
1

11a Ar=5-pyrimidyl
11b Ar=3-methoxyphenyl
11c Ar=3-pyridyl

[a]Reagents and conditions: (a) Standard Suzuki-Miyaura conditions using the corresponding boronic acid, a source of palladium(II) and a base. The reaction was run in an aqueous solvent mixture at 80 °C.

The *endo*-cyclic oxygen of the 2*H*-benzo[e][1,3]oxazin-4-amine core was replaced with sulfur to generate a 2*H*-benzo[e][1,3]thiazin-4-amine (**15**) (core type **A-3** in Figure 3). The synthesis is described in detail in Scheme 3. The synthesis started from methyl 2-mercaptobenzoate (**12**) which was condensed with compound **11a** and ammonia to form the lactam **13**. The lactam was then converted to the cyclic amidine **15** in two steps via the thiolactam **14**.

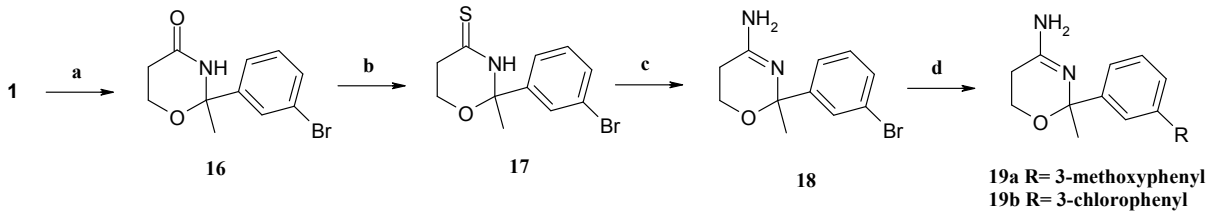
Scheme 3. Synthesis of compound **15**[a]



[a]Reagents and conditions: (a) **11a**, NH_3 (7 M in MeOH), 150 °C. (b) P_2S_5, Pyridine, 120 °C. (c) NH_3 (7 M in MeOH), 90 °C.

6-Membered monocyclic amidines (core sub-type B and B-ref). The synthesis of compounds **19a-b** started with the condensation of compound **1** with 3-hydroxypropanamide under acidic conditions,[42] continuously distilling off the formed water using a Dean-Stark trap to form the lactam **16**. The lactam was then converted to the cyclic amidine **18** in two steps via the thiolactam **17**. In the last step, the bromide (**18**) was coupled with an aryl ring using standard Suzuki-Miyaura conditions to obtain compounds **19a-b** (Scheme 4).

Scheme 4. Synthesis of core sub-type B-1[a]

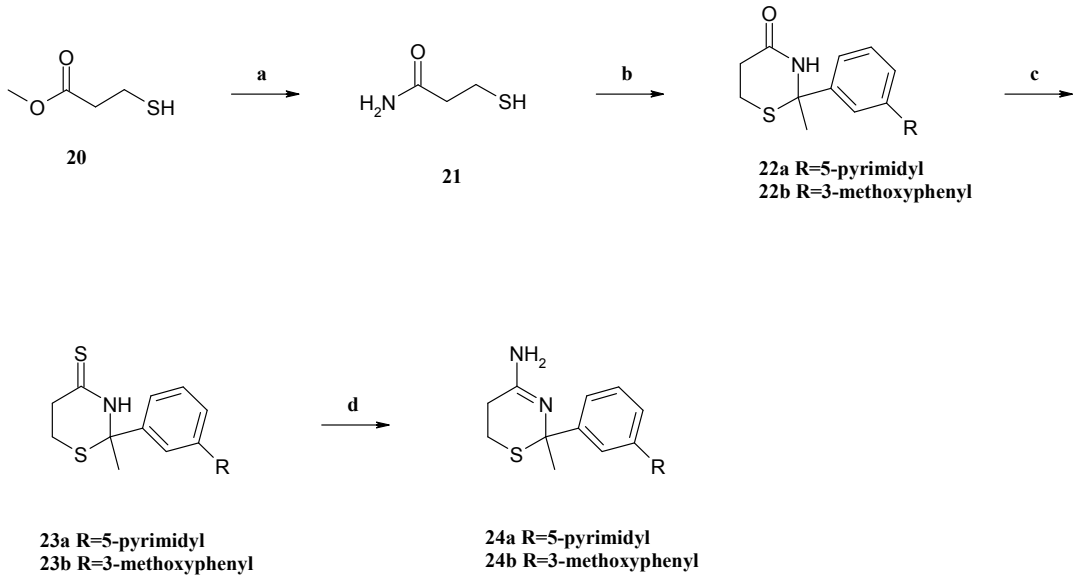


[a]Reagents and conditions: (a) 3-Hydroxypropanamide, pTSA, toluene, reflux, Dean-Stark . (b) P_2S_5, HMDO, 1,2-dichloroethane, 110 °C. (c) NH_3 (7 M in MeOH), 40 °C. (d) 3-

methoxyphenylboronic acid or 3-chlorophenylboronic acid, 1,1'-bis(diphenylphosphino)ferrocene-palladium(II)dichloride dichloromethane complex, cesium carbonate, DME:H_2O:EtOH (6:3:1), 120 °C.

To make the corresponding sulfur analogs, methyl 3-mercaptopropanoate (**20**) was converted to the amide **21** by treatment with concentrated ammonium hydroxide (Scheme 5). The amide was then refluxed in toluene together with either compound **11a** or **11b** under acidic conditions, continuously removing the formed water by azeotrope distillation to form the lactams **22a** and **22b**, respectively. The respective lactams were then converted to the cyclic amidines **24a** and **24b** in two steps via the thiolactams **23a** and **23b**.

Scheme 5. Synthesis of core sub-type B-2[a]

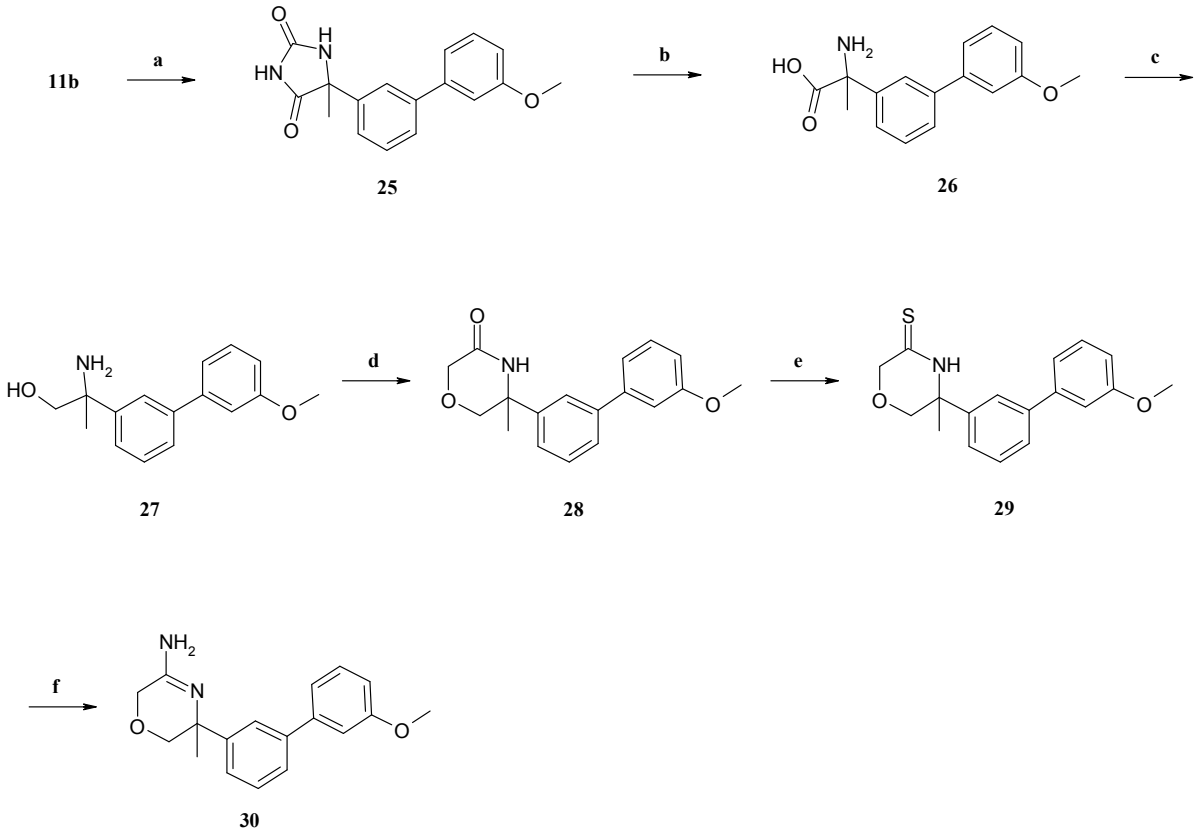


[a]Reagents and conditions: (a) Ammonium hydroxide 25%, rt. (b) **11a** or **11b**, pTSA, toluene, reflux, Dean-Stark . (c) P_2S_5, HMDO, 1,2-dichloroethane, 110 °C. (d) NH_3 (7 M in MeOH), rt.

Moving the oxygen and sulfur one position further away from the carbon holding the R1-group changed the synthetic route completely. The synthesis of compound **30** started with ketone **11b**

which was condensed by heating with potassium cyanide and ammonium carbonate in ethanol to form the hydantoin **25** (Scheme 6).[41] The hydantoin was then hydrolyzed by barium hydroxide at high temperature to give the amino acid **26**.[41] The amino acid **26** was reduced with BH_3 (generated *in situ* from sodium borohydride and iodine) in tetrahydrofuran to form the amino alcohol **27**.[43] The lactam **28** was formed by coupling the amine **27** with chloroacetyl chloride and diisopropylethylamine in tetrahydrofuran and subsequent ring closure with aqueous potassium hydroxide.[39] The lactam **28** was then converted to the cyclic amidine **30** in two steps via the thiolactam **29**.

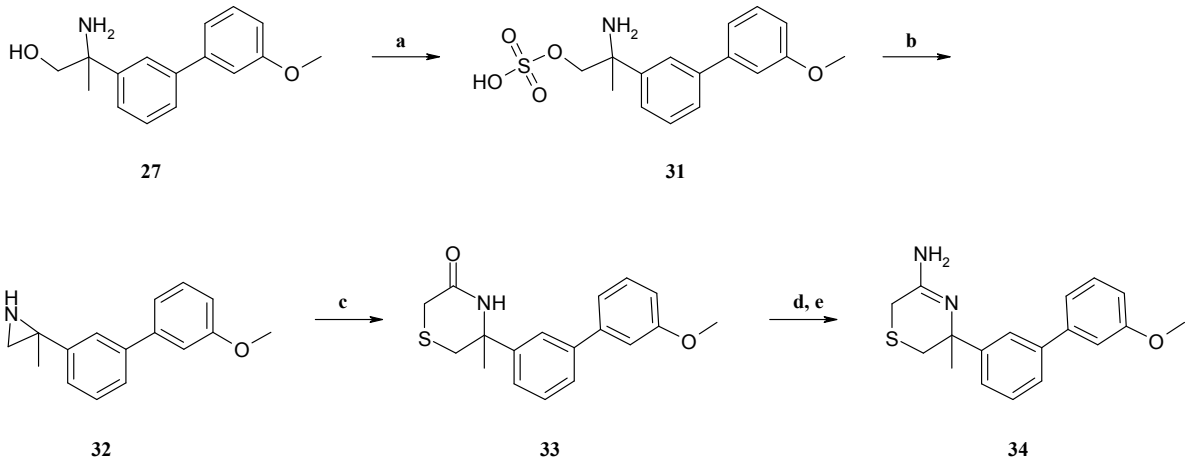
Scheme 6. Synthesis of compound **30**[a]



[a]Reagents and conditions: (a) KCN, $(NH_4)_2CO_3$, EtOH, 70 °C. (b) $Ba(OH)_2$, water, 150 °C. (c) $NaBH_4$, I_2, THF, 0 °C-reflux. (d) 1. Chloroacetyl chloride, DIPEA, THF, 0 °C-rt, 2. KOH, water, rt-60 °C. (e) P_2S_5, HMDO, 100 °C. (f) NH_3 (7 M in MeOH), 40 °C.

The corresponding sulfur analog (core type **B-1** in Figure 3) followed a similar route. The synthesis of example **34** is described in detail in Scheme 7. Compound **27** was treated with chlorosulfonic acid in diethyl ether to form compound **31**. Residual reflux in a two-phase system of aqueous sodium hydroxide and toluene afforded the aziridine **32** by a Wenker reaction.[44] Treatment with methyl 2-mercaptoacetate gave a ring expansion to the six-member lactam **33**. Finally, the lactam **33** was converted to the cyclic amidine **34** in two steps.

Scheme 7. Synthesis of compound **34**[a]

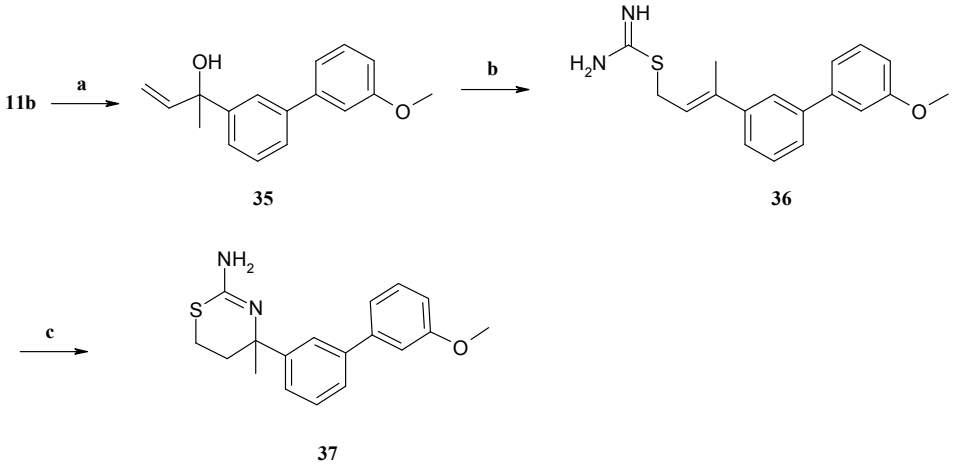


[a]Reagents and conditions: (a) Chlorosulfonic acid, Et_2O, rt. (b) NaOH (aq), toluene, reflux. (c) Methyl 2-mercaptoacetate, DIPEA, DMF, 60 °C. (d) P_2S_5, HMDO, 100 °C, (e) NH_3 (7 M in MeOH), 50 °C.

Compound **37**, based on an already known core structure[23] was synthesized to facilitate a match pair analysis (**B-ref**). The synthesis of compound **37** is outlined in Scheme 8. Acetophenone **11b** was treated with vinylmagnesium bromide in 2-methyl tetrahydrofuran at -

78°C to give the tertiary alcohol **35**. The alcohol was treated with thiourea under acidic conditions to give compound **36** which was set up for an acidic ring closure to give the final compound **37**.[45]

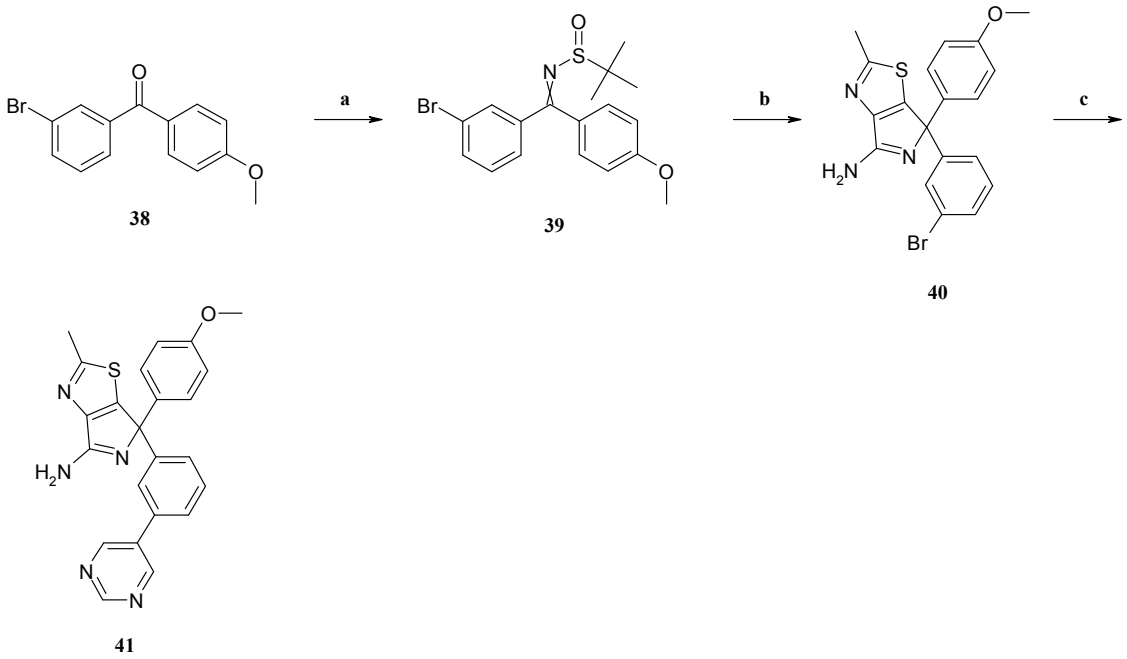
Scheme 8. Synthesis of compound **37**[a]



[a]Reagents and conditions: (a) Vinylmagnesium bromide, 2-Me-THF, -78 °C-rt. (b) Thiourea, HCl, AcOH, 45 °C. (c) $MeSO_3H$, TFA, rt.

5-membered bicyclic amidines (core sub-type C). The synthesis of fused thiazole **41**, an analog of one of the reference compounds (Supporting information), is described in detail in Scheme 9. In the first step, the commercially available compound **38** was converted to sulfinimide **39**, a stabilized imine, using 2-methyl-2-propanesulfinamide and titanium(IV) ethoxide which was heated to reflux in 2-methyl tetrahydrofuran. The sulfinimide **39** was then reacted with the C5-anion of 2-methyl-1,3-thiazole-4-carbonitrile.[40] Upon removal of the protecting group of the amine with hydrochloric acid, compound **40** was formed by spontaneous ring closure. Finally, the bromide (**40**) was coupled with an aryl ring using standard Suzuki-Miyaura conditions to yield compound **41**.

Scheme 9. Synthesis of compound **41**[a]

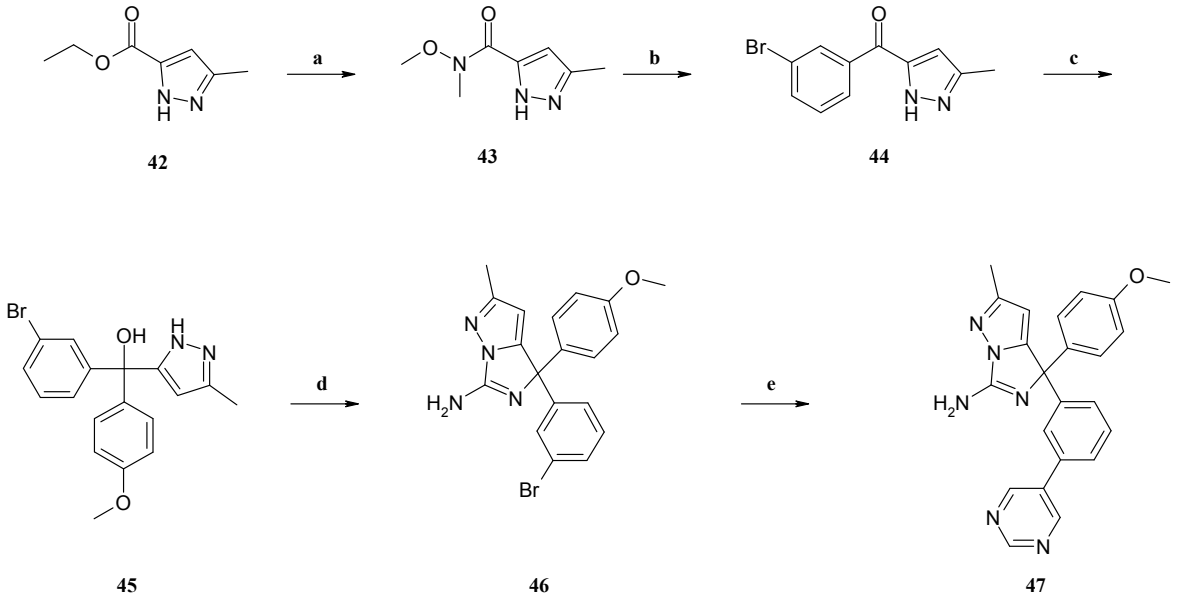


[a]Reagents and conditions: (a) 2-Methyl-2-propanesulfinamide, titanium(IV) ethoxide, 2-Me-THF, reflux. (b) 1. 2-Methyl-1,3-thiazole-4-carbonitrile, 2,2,6,6-tetramethylpiperidineyl-magnesium chloride lithium chloride (1 M in toluene/THF), THF, -25 °C-rt. 2. HCl (1 M in Et_2O), MeOH, rt. (c) Pyrimidine-5-ylboronic acid, [1,1'-bis(diphenylphosphino)ferrocene]-palladium(II) chloride, K_2CO_3, DME:H_2O:EtOH (6:3:1), 100 °C.

A related structure having a fused pyrazole in the bicyclic core was also proposed. Having one of its nitrogens at the point of fusion making it a cyclic guanidine, made the synthesis somewhat different. The synthesis is described in detail in Scheme 10. Starting with ethyl 3-methyl-1*H*-pyrazole-5-carboxylate (**42**), the ester was converted to the Weinreb amide **43** using isopropylmagnesium chloride. The amide was then converted to the ketone **44** by reaction with the mono-anion formed from 1,3-dibromobenzene and *n*-butyllithium at -78 °C in 2-methyl tetrahydrofuran. The ketone **44** was reacted with 4-methoxyphenylmagnesium bromide with copper(I) iodide as catalyst to form the tertiary alcohol **45**. Heating the alcohol with thiourea in

acetic acid formed the cyclic guanidine **46**. Finally, the bromide was replaced with an aryl ring using standard Suzuki-Miyaura conditions to yield compound **47**.

Scheme 10. Synthesis of compound **47**[a]

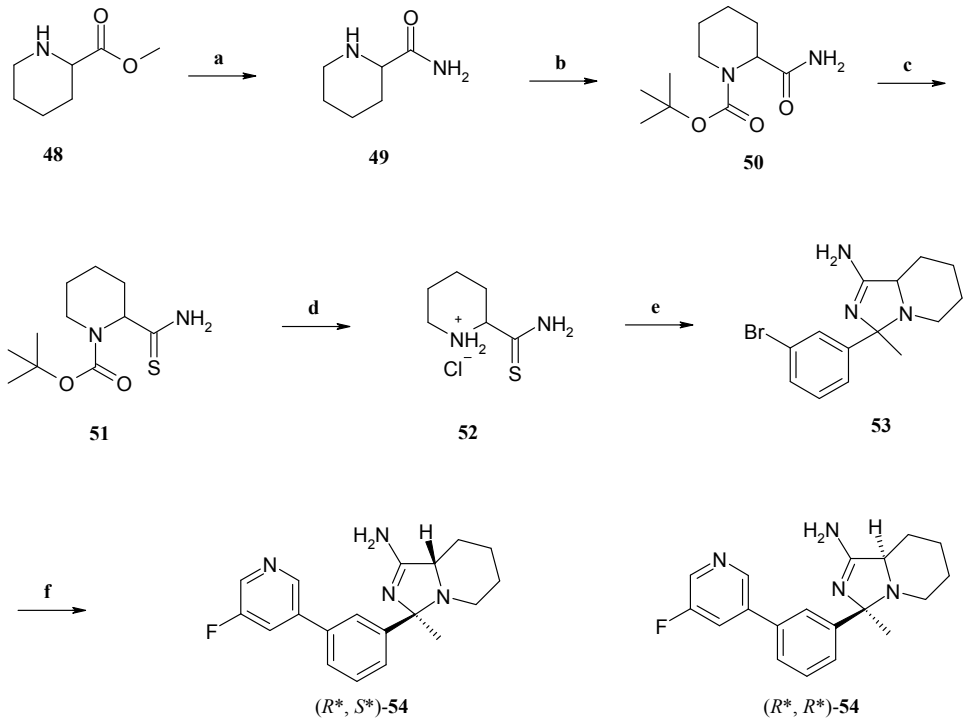


[a]Reagents and conditions: (a) *N*,*O*-Dimethylhydroxylamine hydrochloride, iso-propylmagnesium chloride (2 M in THF), 2-Me-THF, -30 °C; (b) 1,3-Dibromobenzene, *n*-Butyllithium (1.6 M in hexanes), 2-Me-THF, -78 to -30 °C; (c) 4-Methoxyphenylmagnesium bromide (1 M in THF), Copper(I) iodide, 2-Me-THF, 0 °C-rt; (d) Thiourea, acetic acid, 100 °C; (e) Pyrimidine-5-boronic acid, Bis(triphenylphosphine)palladium(II)chloride, Sodium carbonate (1 M aq), DME:H_2O (2:1), 80 °C.

The synthesis of compound **54**, also bicyclic but where the fused ring is aliphatic is outlined in Scheme 11. The amide **49** was first prepared by treating ester **48** with ammonia in methanol at elevated temperature. The piperidine was Boc-protected to yield compound **50**. The thioamide was then prepared by treating compound **50** with phosphorus pentasulfide and hexamethyldisiloxane. The piperidine was deprotected under acidic condition to obtain **52**, which was then treated with 3'-bromoacetophenone (**1**) and ammonia to obtain the racemic

bicyclic amidine **53.** The final step was then a standard Suzuki-Miyaura reaction to obtain diastereomers (*R*,S**)-**54** and (*R*,R**)-**54** after separation.

Scheme 11. Synthesis of compounds (*R*,S*)*-**54** and *(R*,R*)*-**54**[a]

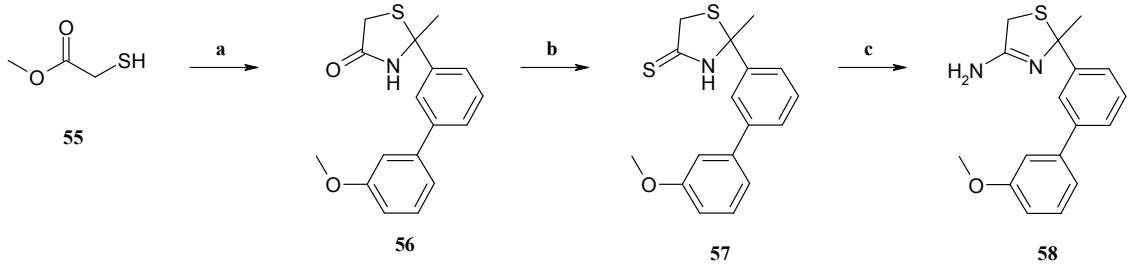


[a]Reagents and conditions: a) 7 M NH_3 in MeOH, 90 °C, 3 days, 99 %; b) DIPEA, THF:DMF (1:1), di-*tert*-butyl dicarbonate, rt, on, quant.; c) P_2S_5, HMDO, THF, 110 °C, 30 min, 71 %; d) 5 M HCl in iPrOH, MeOH, rt on, 67 %; e) 3'-bromoacetophenone (**1**), 7 M NH_3 in MeOH, 50 °C, on, 29 %; f) 5-fluoropyridin-3-ylboronic acid, cat. $(PPh_3)_2PdCl_2$, sat. $NaHCO_3$, DME:H_2O (2:1), 80 °C, 1.5 h, (*3R*,8aS**) 15 % and (*3R*,8aR**) 23 %.

5-membered monocyclic amidines (core sub-type D). In an effort to investigate smaller cores, monocyclic 5-membered cores were synthesized. The synthesis of structure **58** is

described in detail in Scheme 12. In the first step, compound **11b** was reacted with methyl 2-mercaptoacetate (**55**) using ammonia and microwave heating to form the lactam **56**. The lactam was then converted to the cyclic amidine **58** in two steps via the thiolactam **57**.

Scheme 12. Synthesis of compound **58**[a]

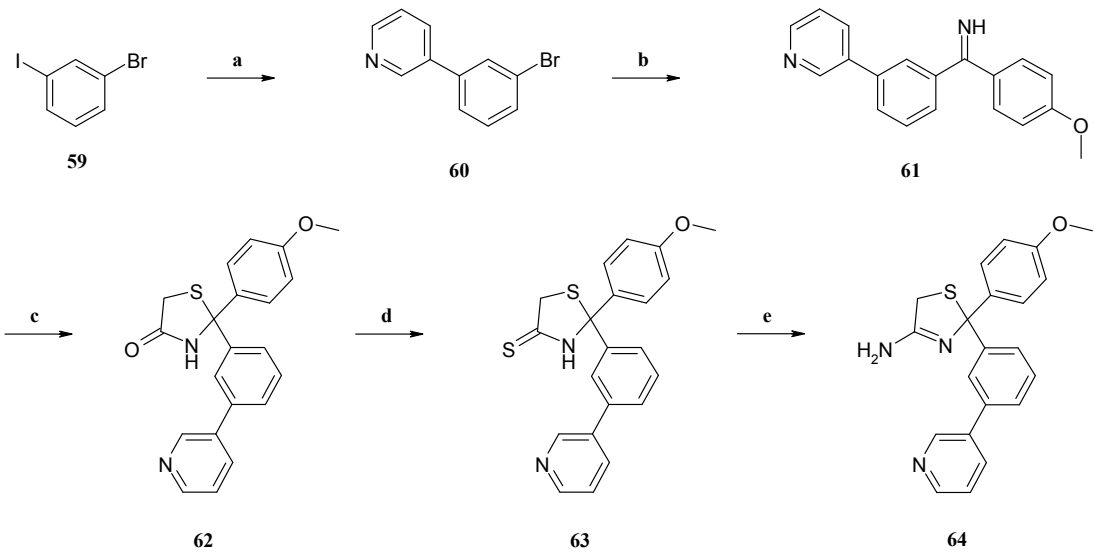


55 56 57 58

[a]Reagents and conditions: (a) **11b**, NH_3 (7 M in MeOH), 150 °C. (b) P_2S_5, Pyridine, 120 °C. (c) NH_3 (7 M in MeOH), 60 °C.

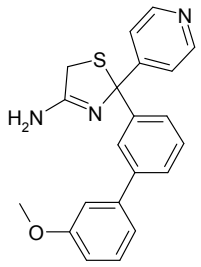
However, this route had its limitations and only worked for acetophenones in a satisfactory manner. Trying to use more hindered ketones like substituted benzophenones gave little or no product. The problem was solved by using the preformed imine prior to the cyclization reaction[46] and running the reaction without solvent. This method was used in the synthesis of compound **64** as outlined in Scheme 13. Starting with 1-bromo-3-iodobenzene (**59**) and pyridine-3-ylboronic acid under standard Suzuki-Miyaura conditions afforded the biaryl **60.** The bromide (**60**) was converted to the corresponding anion using *n*-butyllithium at -78 °C in diethyl ether and was reacted with 4-methoxybenzonitrile to form imine **61**. The imine (**61**) was stirred neat with methyl 2-mercaptoacetate and dimethylamine hydrochloride at room temperature to form the lactam **62**, which was then converted to the cyclic amidine **64** in two steps via the thiolactam **63**.

Scheme 13. Synthesis of compound **64**[a]



[a]Reagents and conditions: (a) Pyridine-3-ylboronic acid, bis(triphenylphosphine)palladium(II) chloride, Sodium carbonate (1 M aq), DME:H_2O (2:1), 80 °C. (b) *n*-BuLi, 4-methoxybenzonitrile, Et_2O, -78 °C-rt. (c) methyl 2-mercaptoacetate, dimethylamine hydrochloride, rt. (d) P_2S_5, Pyridine, 120 °C. (e) NH_3 (7 M in MeOH), 60 °C.

Compound **65** was prepared following the same reaction sequence as described in Scheme 13, using 3-bromo benzonitrile, 3-methoxy phenylboronic acid and 4-bromo pyridine.

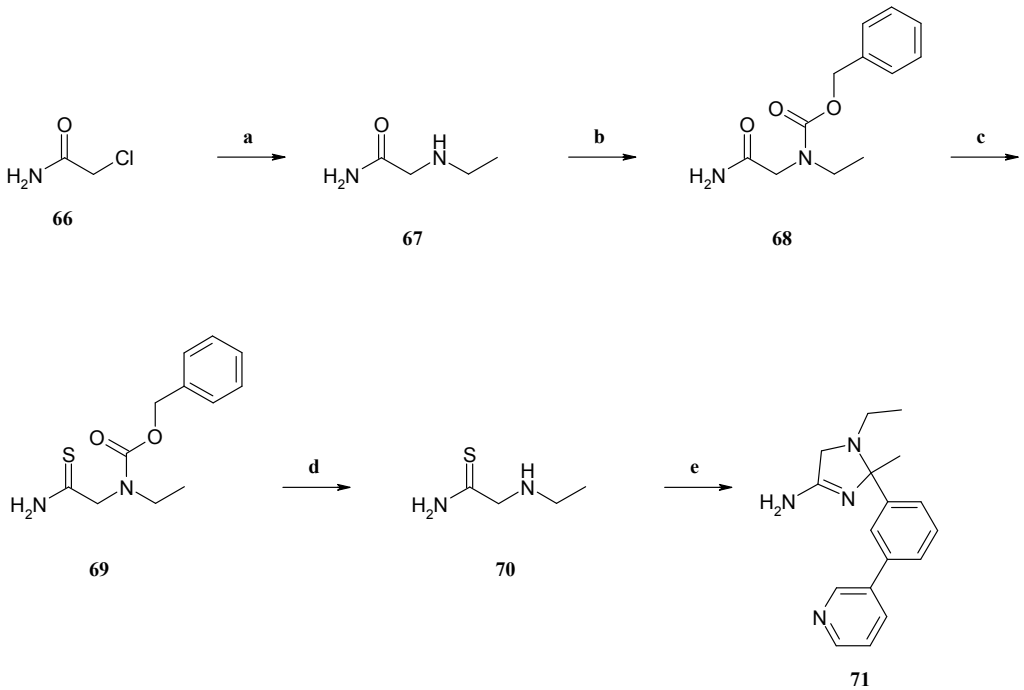


65

To synthesize the corresponding nitrogen analog 2,5-dihydro-1*H*-imidazol-4-amine, a monocyclic analog of **54**, new chemistry was developed. The synthesis of compound **71** is described in detail in Scheme 14. 2-Chloroacetamide (**66**) was reacted with ethylamine to make

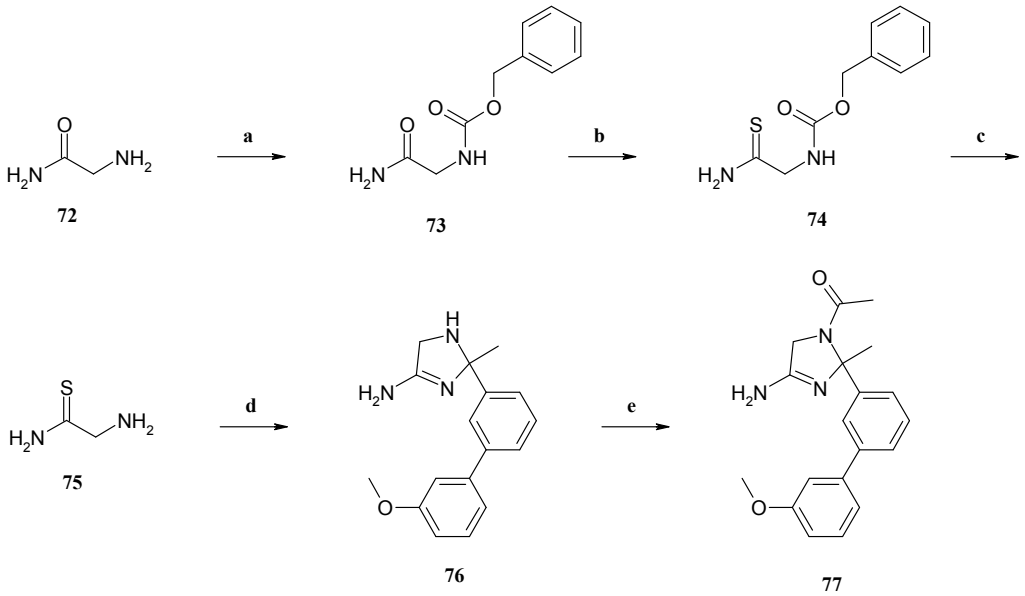
amine **67**. It was then protected as the benzyl carbamate **68** and the amide converted to the thioamide **69** by reaction with phosphorus pentasulfide in dioxane by microwave heating. The protecting group was removed with hydrogen bromide in acetic acid and intermediate **70** precipitated in diethyl ether. Compound **70** was reacted with compound **11c** in methanol with 7 M ammonia to give the cyclic amidine **71**. This core suffered from instability. To improve stability, the non-amidine nitrogen was acylated. The synthesis the acylated analog of **71**, compound **77** is outlined in Scheme 15. In the same fashion as for the tertiary amine **71**, the secondary amine **76** could be made from the aminothioacetamide **75**, generated from compound **74**, and ketone **11b**. The amine could then be acylated with acetic anhydride. Residual treatment with ammonia removed all acylation on the amidine itself to give the final compound **77**.

Scheme 14. Synthesis of compound **71**[a]



[a]Reagents and conditions: (a) Ethylamine (70% in water), rt. (b) $NaHCO_3$ (aq), benzyl chloroformate, dioxane, 0 °C-rt (c) P_2S_5, dioxane, 95 °C. (d) HBr (33% in AcOH), rt. (e) **11c**, NH_3 (7 M in MeOH), 60 °C.

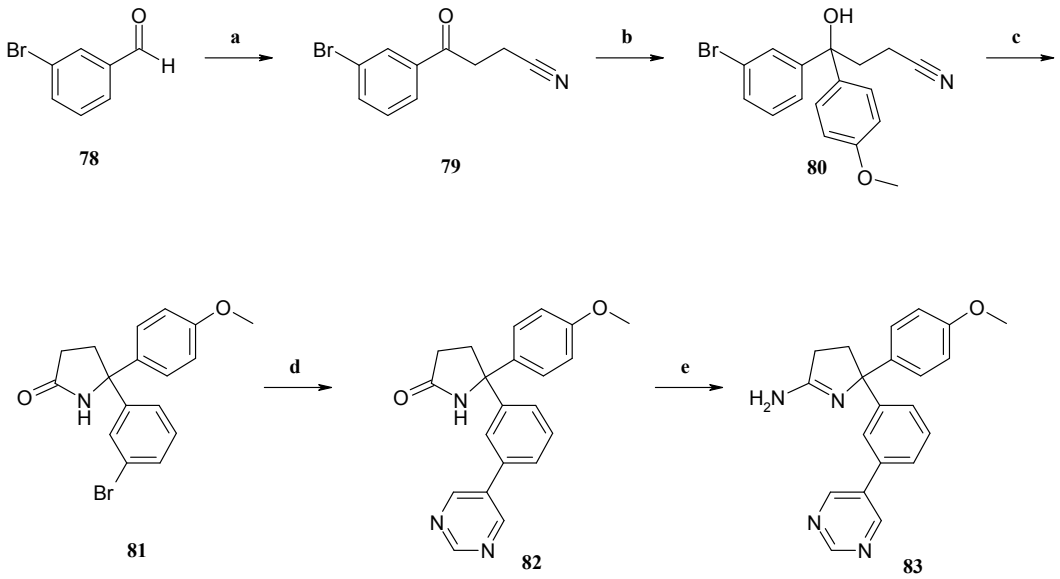
Scheme 15. Synthesis of compound **77**[a]



[a]Reagents and conditions: (a) $NaHCO_3$ (aq), benzyl chloroformate, dioxane, 0 °C-rt (b) P_2S_5, dioxane, 95 °C. (c) HBr (33% in AcOH), rt. (d) bromide **11b**, NH_3 (7 M in MeOH), rt. (e) 1. Ac_2O, TEA. 2. NH_3 (7 M in MeOH).

To afford a cyclic amidine core without an additional heteroatom in the ring (**D-4**) compound **83** was synthesized, as described in detail in Scheme 16. 3-Bromobenzaldehyde (**78**) was reacted in a Stetter reaction[47] with sodium cyanide and acrylonitrile. The formed ketone **79** was reacted with the anion prepared from 1-bromo-4-methoxybenzene and *n*-butyllithium to form the tertiary alcohol **80**. This was now set up for an intramolecular Ritter reaction,[48] which was performed in methanesulfonic acid to form the lactam **81**. The bromide (**81**) was reacted with a boronic acid using standard Suzuki-Miyaura conditions to form the lactam **82** which was finally converted to the cyclic amidine **83** in two steps.

Scheme 16. Synthesis of compound **83**[a]

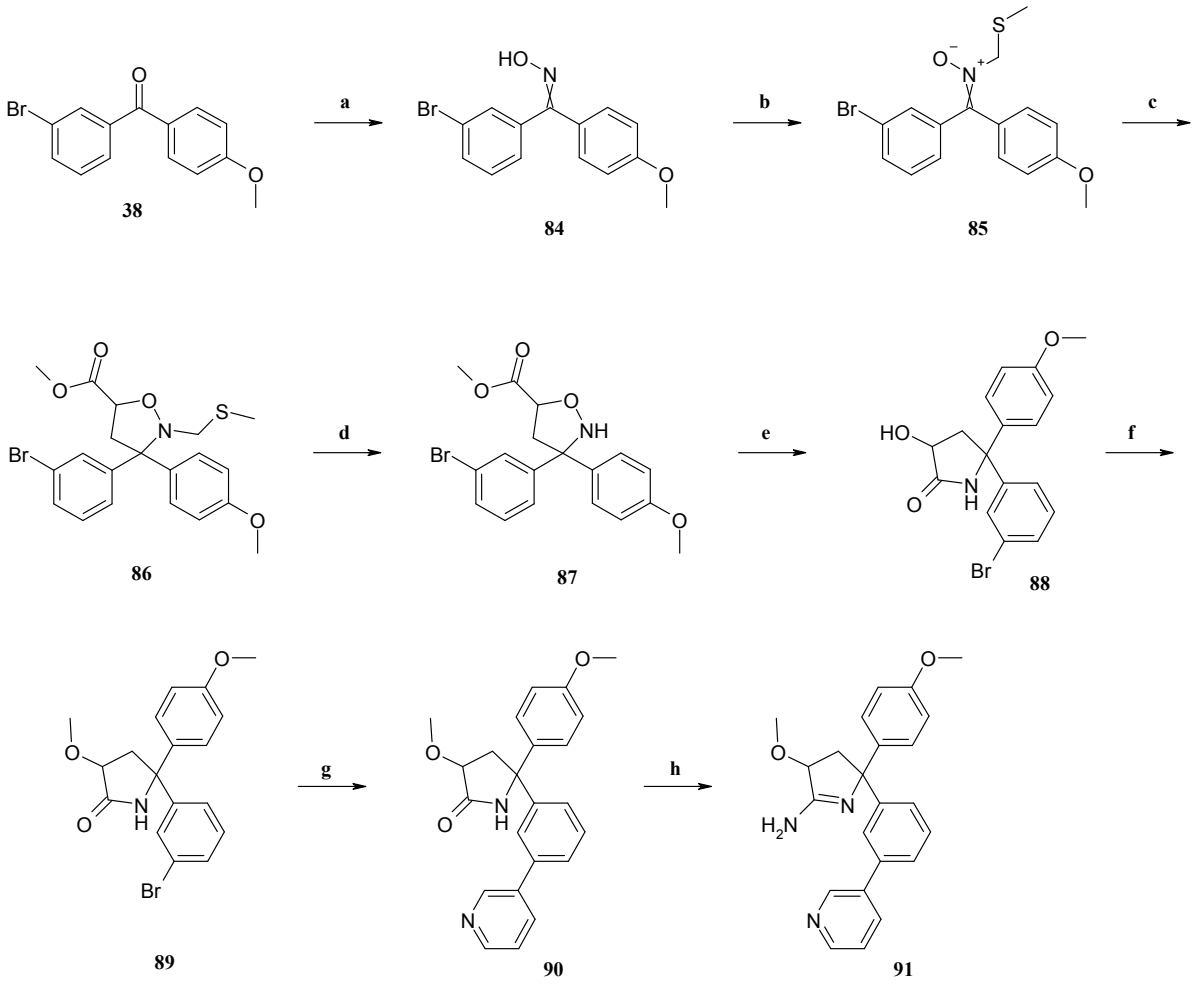


[a]Reagents and conditions: (a) NaCN, acrylonitrile, MeCN, rt. (b) 1-Bromo-4-methoxybenzene, *n*-BuLi, THF, -78 ^{o}C-5 °C. (c) MsOH, 60 °C. (d) Pyrimidine-5-ylboronic acid, bis(triphenylphosphine)palladium(II) chloride, Sodium carbonate (1 M aq), DME:H_2O (2:1), 80 °C. (e) 1. P_2S_5, Pyridine, 120 °C, 2. NH_3 (7 M in MeOH), *t*-BuOOH (70%), rt.

To make the similar core with a methoxy group in the alpha-position another synthetic route was developed. The synthesis of compound **91** is outlined in Scheme 17. Compound **38** was first converted to the oxime **84** with hydroxylamine hydrochloride. In order to activate the oxime, the nitrogen was alkylated with a methylthiomethyl group, which also acted as a protecting group, to form compound **85**. Reaction with methyl acrylate in a [3+2] cycloaddition gave the isoxazolidine **86**. The protecting group was removed with sulfuric acid to form intermediate **87** which, upon treatment with molybdenum hexacarbonyl, afforded the reductive rearrangement to the alpha hydroxy lactam **88**. The hydroxy group was alkylated with methyl iodide in the presence of silver oxide to give the alpha methoxy lactam **89**. The bromide was reacted with a

boronic acid using standard Suzuki-Miyaura conditions to obtain **90** which was finally converted to the cyclic amidine **91** in two steps. The diastereomers of **91** were separated by chiral SFC.

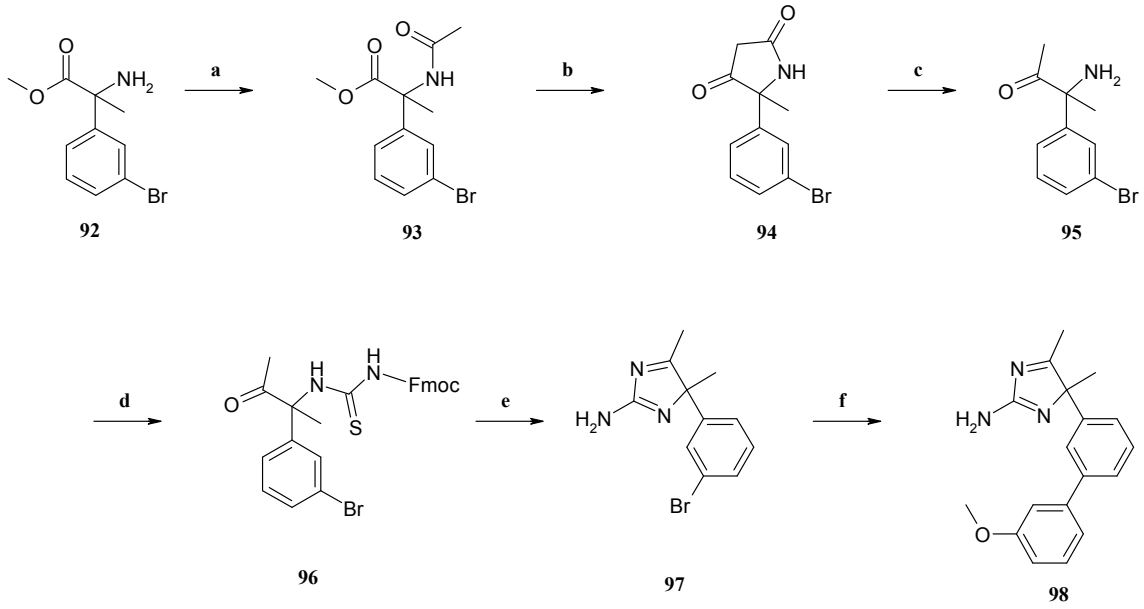
Scheme 17. Synthesis of compound **91**[a]



[a]Reagents and conditions: (a) Hydroxylamine hydrochloride, pyridine, MeOH, 50 °C. (b) DCC, DMSO, TFA, benzene, 0 °C-rt. (c) methyl acrylate, 150 °C. (d) H_2SO_4, MeOH, 60 °C. (e) $Mo(CO)_6$, MeCN, water, reflux. (f) MeI, Ag_2O, MeCN, 40 °C. (g) Pyridin-3-ylboronic acid, tetrakis(triphenylphosphine)palladium, K_2CO_3, dioxane, water, 90 °C. (h) 1. P_2S_5, pyridine, 115 °C, 2. NH_3 (7 M in MeOH), *t*-BuOOH (70% in water), rt.

To make the 5-membered cyclic guanidine core, **D-6**, the synthesis started from methyl 2-amino-2-(3-bromophenyl)propanoate (**92**), which was acylated with acetic anhydride to form intermediate **93** which was ring-closed in a Dieckmann condensation to afford the β-keto lactam **94**[49] (Scheme 18). The lactam was decarboxylated with concentrated hydrochloric acid to give the amino ketone **95** which was reacted with Fmoc-isothiocyanate to give the protected thiourea **96**. The protecting group was removed with ammonia (7 M in MeOH) to afford the free thiourea which then ring-closed to the cyclic thiourea. Addition of *tert*-butyl hydroperoxide then gave the cyclic guanidine **97**. In the last step, the bromide was reacted with a boronic acid under standard Suzuki-Miyaura conditions to yield the final compound **98**. This compound was later demonstrated to be unstable under acidic and neutral conditions.

Scheme 18. Synthesis of compound **98**[a]



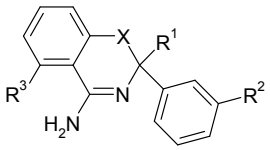
[a]Reagents and conditions: (a) Ac_2O, TEA, DCM, 0 °C-rt (b) NaH, toluene, reflux (c) conc. HCl, reflux. (d) Fmoc isothiocyanate, DCM, rt. (e) 1. NH_3 (7 M in MeOH), rt, 2. *t*-BuOOH (70%), rt. (f) 3-methoxyphenylboronic acid, bis(triphenylphosphine)palladium(II) chloride, Sodium carbonate (1 M aq), DME:H_2O (2:1), 80 °C.

RESULTS AND DISCUSSION

The reported compounds were evaluated for BACE-1 inhibition in a time response fluorescence resonance energy transfer (TR-FRET) protocol. The cell-based assay for BACE-1 inhibition used specific antibodies to monitor reduction of sAPPβ release from human neuronal-derived SH-SY5Y cells. The hERG activity in CHO cells was measured as a first indication for potential cardiotoxicity.[50] Experimental values for pKa were determined[51,52] and LogD was estimated with reversed phase liquid chromatography (eLogD).[53] Permeability in Caco-2 cells was measured and classified as good (P_{app} >25), medium (5< P_{app} <25) or poor (P_{app} <5). Susceptibility to P-gp efflux (in MDCK-MDR1 cells), was also determined, and the efflux of the compounds was classified as low (<2), medium (2-3) and high (>3). Finally, the results from both permeability measurements were combined into an "overall permeability" classification of good, medium or poor. Since both properties are important to have a chance for high brain exposure *in vivo*, the least favourable result determined the categorization.[19,20,29]

The data is divided into four tables according to the different core sub-types (Tables 1-4). An additional table, with data on previously published series, is also included in this report (Supporting information). These compounds have the same R1- and R2-substituents as the compounds synthesized here, and are used as additional references. With these substituents, we hypothesized that the differences in the *in vitro* profiles of the compounds would largely be due to the core structures, and thus the SAR discussions are focused to the cores.

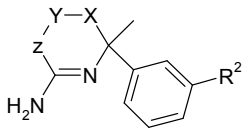
Table 1. *In vitro* properties of core sub-type A



X	R1	R2	R3	Comp	BACE-1, TR-FRET[a] IC_{50} (μM)	LE FRET	BACE-1, sAPPβ[a] IC_{50} (μM)	hERG[b] IC_{50} (μM)	eLogD[c]	pKa[d]	P_{app}[e] (10^{-6} cm/s)	Efflux class.[f]	Overall perm.[g]
O	Me	5-pyrimidyl	H	**8a**	16	0.27	1.0	5.7	1.2	8.1	31	low	G
O	Me	3-pyridyl	H	**8b**	25	0.26	0.5	2.8	1.8	8.2	17	low	M
O	Et	5-pyrimidyl	H	**9**	100	0.22	3.2	1.6	1.8	8.4	nd	nd	nd
O	Me	5-pyrimidyl	F	**10a**	32	0.24	5.0	9.0	1.6	6.9	39	low	G
O	Me	3-pyridyl	F	**10b**	32	0.24	2.5	nd	2.3	7.0	23	nd	G*
S	Me	5-pyrimidyl	H	**15**	10	0.28	0.32	6.0	1.7	8.3	39	low	G

[a]IC_{50} values are the means of at least two experiments. [b]Measured in hERG-expressing CHO cells using IonWorks technology. [c]Determined by reversed phase liquid chromatography. [d]Determined by pressure-assisted capillary electrophoresis. [e]P_{app} is the measured permeability (A to B) through Caco-2 cells. [f]MDCK-MDR1 efflux data were generated using a transwell assay. [g]G=good, M=medium, P=poor, *Efflux was not determined for this compound, so the overall permeability classification is only based on Caco-2 P_{app}. nd; not determined.

Table 2. *In vitro* properties of core sub-type B

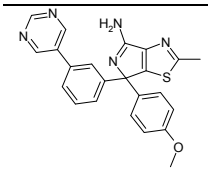
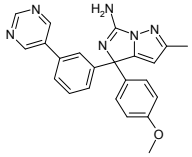
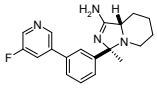
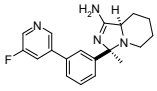


X	Y	Z	R2	Comp	BACE-1, TR-FRET[a] IC_{50} (μM)	LE FRET	BACE-1, sAPPβ[a] IC_{50} (μM)	hERG[b] IC_{50} (μM)	eLogD[c]	pKa[d]	P_{app}[e] (10^{-6} cm/s)	Efflux class.[f]	Overall perm.[g]
O	C	C	3-MeO-phenyl	**19a**	10	0.31	0.63	nd	2.5	9.3	13	low	M
O	C	C	3-chlorophenyl	**19b**	6.3	0.34	0.79	6.15	3.3	9.3	2.1	nd	P*
S	C	C	5-pyrimidyl	**24a**	1.0	0.41	0.040	>33	<0	9.1	21	low	G
S	C	C	3-MeO-phenyl	**24b**	0.63	0.38	0.063	11	2.5	9.3	5.2	nd	M*

C	O	C	3-MeO-phenyl	**30**	3.2	0.34	0.20	8.0	2.1	9.8	20	low	M
C	S	C	3-MeO-phenyl	**34**	2.0	0.35	0.32	9.0	2.2	10.5	7.3	high	P
C	C	S	3-MeO-phenyl	**37**	2.0	0.35	0.40	7.6	2.5	7.0	72	nd	G*

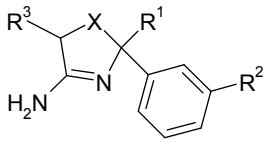
[a]IC_{50} values are the means of at least two experiments. [b]Measured in hERG-expressing CHO cells using IonWorks technology. [c]Determined by reversed phase liquid chromatography. [d]Determined by pressure-assisted capillary electrophoresis. [e]P_{app} is the measured permeability (A to B) through Caco-2 cells. [f]MDCK-MDR1 efflux data were generated using a transwell assay. [g]G=good, M=medium, P=poor, *Efflux was not determined for this compound, so the overall permeability classification is only based on Caco-2 P_{app}. nd; not determined.

Table 3. *In vitro* properties of core sub-type C

Structure	Comp	BACE-1, TR-FRET[a] IC_{50} (μM)	LE FRET	BACE-1, sAPPβ[a] IC_{50} (μM)	hERG[b] IC_{50} (μM)	eLogD[c]	pKa[d]	P_{app}[e] (10^{-6} cm/s)	Efflux class.[f]	Overall perm.[g]
	**41**	0.63	0.28	0.032	1.5	2	8.0	18	High	P
	**47**	0.32	0.29	0.79	8.6	2.3	5.4	30	Medium	M
	(*R**,*S**)-**54**	4.0	0.31	0.50[h]	nd	1.5	nd	nd	nd	nd
	(*R**,*R**)-**54**	16	0.27	0.63[h]	nd	2.0	9.2	nd	nd	nd

[a]IC_{50} values are the means of at least two experiments. [b]Measured in hERG-expressing CHO cells using IonWorks technology. [c]Determined by reversed phase liquid chromatography. [d]Determined by pressure-assisted capillary electrophoresis. [e]P_{app} is the measured permeability (A to B) through Caco-2 cells. [f]MDCK-MDR1 efflux data were generated using a transwell assay. [g]G=good, M=medium, P=poor, [h]During the assay condition, (*R**,*R**)-**54** and (*R**,*S**)-**54** epimerized. nd; not determined.

Table 4. *In vitro* properties of core sub-type D



X	R1	R2	R3	Comp	BACE-1, TR-FRET[a] IC_{50} (μM)	LE FRET	BACE-1, sAPPβ[a] IC_{50} (μM)	hERG[b] IC_{50} (μM)	eLogD[c]	pKa[d]	P_{app}[e] (10^{-6} cm/s)	Efflux Class.[f]	Overall perm.[g]
S	Me	3-MeO-phenyl	H	**58**	7.9	0.33	3.2	9.2	3.6	6.9	15	nd	M*
S	4-MeO-phenyl	3-pyridyl	H	**64**	0.79	0.32	0.40	10	2.9	6.2	19	nd	M*
S	4-pyridyl	3-MeO-phenyl	H	**65**	0.50	0.33	2.5	12	3.3	5.4	18	low	M
N-Acyl	Me	3-MeO-phenyl	H	**77**	20	0.26	nd	>33	1.4	6.5	16	low	M
C	4-MeO-Phenyl	5-pyrimidyl	H	**83**	7.9	0.27	2.0	23	0.9	10	<0.3	nd	P*
C	4-MeO-Phenyl	3-pyridyl	OMe	***rac*-91**	10	0.24	0.32	2.4	1.8	8.9	7.2	high	P*
C	4-MeO-Phenyl	3-pyridyl	OMe	**Iso1-91**	20	0.23	0.40	1.5	1.8	nd	nd	high	P
C	4-MeO-Phenyl	3-pyridyl	OMe	**Iso2-91**	40	0.21	0.79	2.5	1.8	nd	nd	high	P
C	4-MeO-Phenyl	3-pyridyl	OMe	**Iso3-91**	40	0.21	1.3	2.5	1.8	nd	nd	high	P
C	4-MeO-Phenyl	3-pyridyl	OMe	**Iso4-91**	3.2	0.27	0.079	3.2	1.8	nd	34	high	P

[a]IC_{50} values are the means of at least two experiments. [b]Measured in hERG-expressing CHO cells using IonWorks technology. [c]Determined by reversed phase liquid chromatography. [d]Determined by pressure-assisted capillary electrophoresis. [e]P_{app} is the measured permeability (A to B) through Caco-2 cells. [f]MDCK-MDR1 efflux data were generated using a transwell assay. [g]G=good, M=medium, P=poor, *Efflux was not determined for this compound, so the overall permeability classification is only based on Caco-2 P_{app}. nd; not determined.

Chemical stability. During the synthesis of core structure C-3 (**54**, Scheme 11), it was realized that **54** slowly epimerized in the acidic media used when separating the diastereoisomers by reversed-phase chromatography. No visible decay of the diastereomeric mixture was seen during the acidic purification of **53** or during the basic conditions in the subsequent Suzuki-Miyaura reaction to **54**. However, treating **53** in acidic or basic media for prolonged time (more than one

day) it was apparent that the core structure was not stable, but gave 3´-bromoacetophenone and amide **49** as decomposition products. From these stability findings we believe that the epimerization occurred at the carbon holding the R1-group and not at the alpha-carbon to the amidine moiety. Data for the two stereoisomers of **54** ((*R*,S**)-**54** and (*R*,R**)-**54**) are reported in Table 3 but in the cellular assay, with a long incubation time, epimerization occurred during the experiment. Therefore, the two data points reported for the cellular assay represent in both cases a mixture of the four stereoisomers. There was no sign of decomposition under these conditions.

Due to the above mentioned stability problem, all compounds representing new cores were checked for their stability in DMSO as well as in buffer solutions at four different pH-values (1, 4.5, 7.4 and 9) over time. All of the cores were stable in DMSO and (unless discussed) the cores were stable in the buffer used in the enzymatic and cell assays (pH 4.5 and 7.4 respectively) for the time period needed to perform the experiment. The same is true for the conditions used in the other assays included in this report.

As mentioned earlier, **71** suffered from instability problem showing reversibility back to the acetophenone **11c** even under the conditions used in the enzymatic assay. For this reason, no data for compound **71** is reported here. By preparing **77**, which was predicted to be more stable due to the electron withdrawing character of the acyl group, stability increased to allow data to be generated without compound decomposition. Data for compound **99** was excluded in this report since it was shown to be unstable under acidic and neutral conditions.

It was also noticed that some amidines were slowly hydrolyzed to the lactam under basic (pH 9) conditions. This was first noticed for compounds of sub-type B, already in the preparation of the compounds. Neutral conditions had to be used when purifying the compounds by preparative

HPLC. When stability was determined as described above, compounds from cores of sub-type A also showed some hydrolysis at pH 9 but at a slower pace, whereas compounds with core D-1 showed no hydrolysis. Our first hypothesis was that amidines with high pKa were more susceptible towards hydrolysis since core sub-types A and B both mainly represent compounds with high pKa (8-9) and D-1 have examples of compounds with a low pKa (5-6), but the size of the ring might also be a factor. Later findings showed that compounds from core sub-type D-4, a 5-membered core with high pKa (10) only showed small amounts of hydrolysis and C-1, a 5-membered core with high pKa (8) and a double bond in the core ring, showed no hydrolysis at all. It might be that the 5-membered cores position their R-groups so that the amidine is less accessible for hydrolysis. Also, the 5-membered cores usually have the larger R1-group which could sterically hinder or slow down hydrolysis.

Target activity and crystal structures. The reported compounds were all active in the enzymatic TR-FRET assay (Table 1-4), with potencies ranging from 0.32 μM to 100 μM. Ligand efficiencies (LEs) were between 0.25 and 0.41 and it was encouraging to see that compounds (**24b**, **41**, **47**, **54**, **64** and **65**) from five different cores (B-2, C-1, C-2, C-3 and D-1) reached potencies below 1 μM. Some compounds (**24a**, **24b** and **34**) had equal or higher LE than the reference compound **37** (Table 2). Four compounds had promising cellular potencies below 100 nM (Table 2-4, compounds **24a**, **24b**, **41** and **Iso4-91**), with **41** (32 nM) as the most potent. There was no correlation between lipophilicity and potency for these compounds.[54] Also, there was no correlation between pKa and enzymatic potency despite the important ionic and hydrogen bond interactions to the acidic aspartates (see crystal structures below).[36] However, in line with previous reports, an increase in pKa was linearly correlated to an increase in cellular

potency, up to a pKa of about 8.5 (Figure 4).[19-21,55] As reported in the permeability section below, a pKa above 8.5 is negative for cell permeability, which may be the reason for the correlation being reversed at that point.

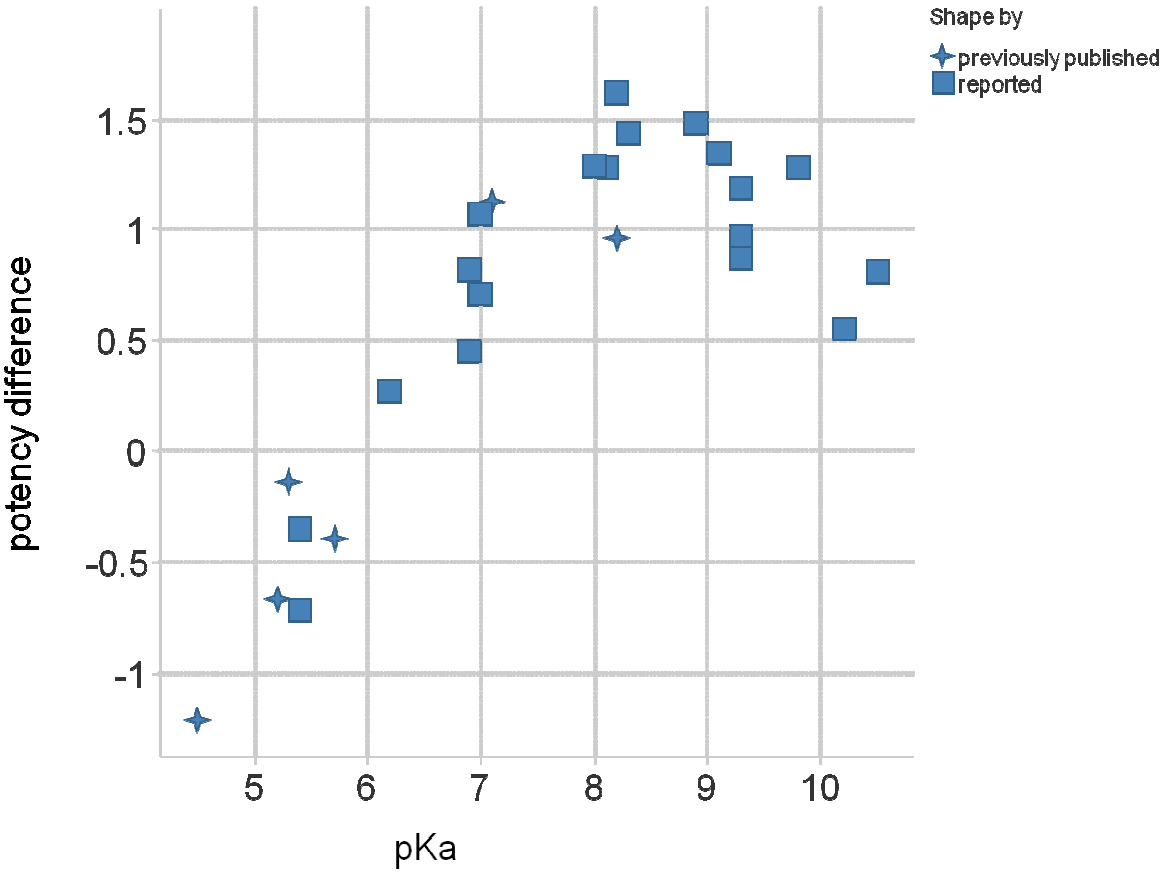


Figure 4. From a pKa of 6 up to a pKa of 8.5, there was a linear correlation between pKa and increase in potency, going from the enzymatic assay to the cell assay, where the potency difference is calculated as pIC_{50} (cellular assay) – pIC_{50} (TR-FRET). Data on previously published compounds are in agreement with these results (Supporting information, Table S2).

When the compounds were analyzed according to the four structural sub-types (A-D) as presented in Figure 5, it became clear that compounds with core sub-type B showed the highest ligand efficiencies, spanning from 0.31 up to 0.41. For compounds with 5-membered cores, LEs

were somewhat lower, as compared to the 6-membered analogues. However, compounds with the highest enzymatic potencies were found among core sub-type C. This higher specific activity could be explained by the extra hydrogen bond interaction to trp76, provided by the larger R1-substituent (see further discussion on crystal structures below).

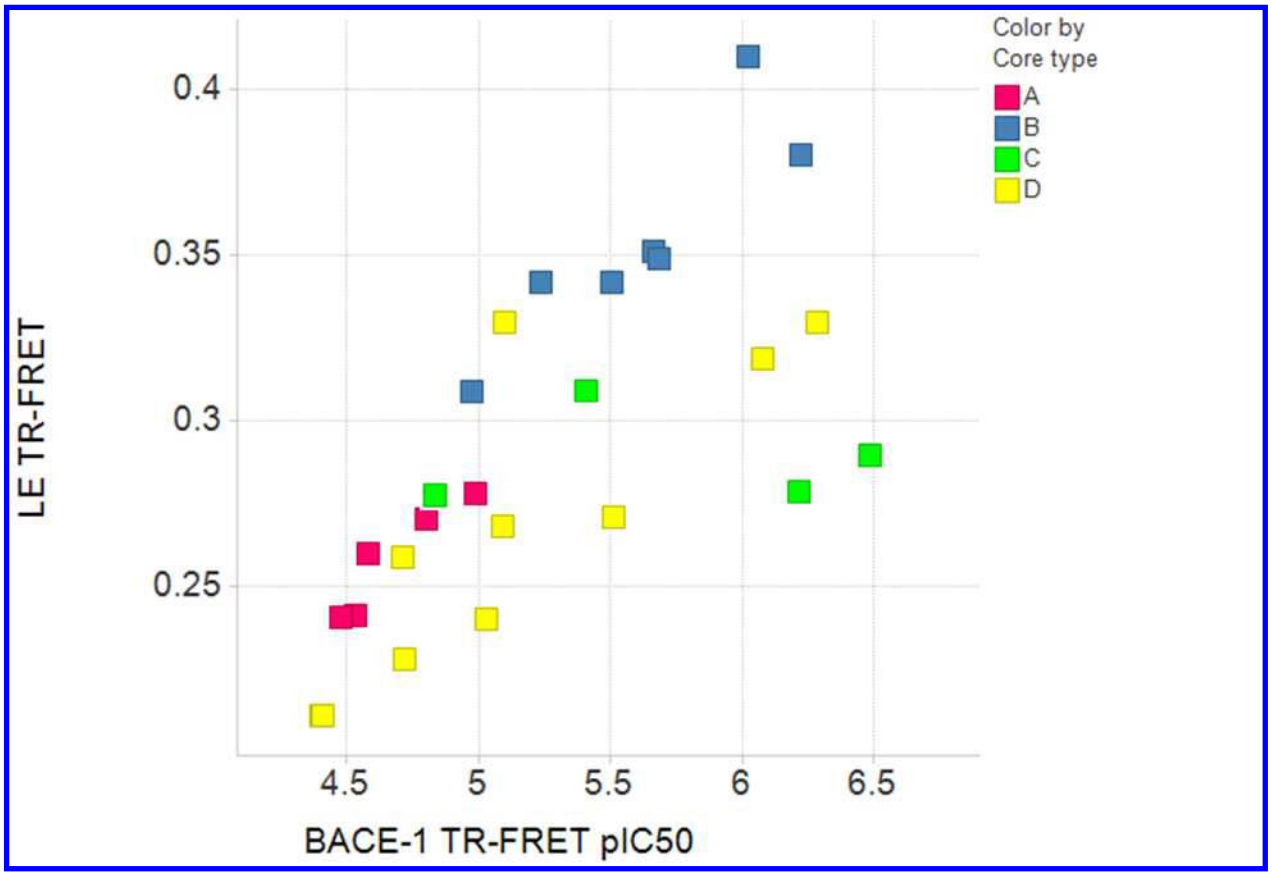


Figure 5. Compounds with monocyclic 6-membered cores had the highest LEs, while compounds with 5-membered cores reached the highest enzymatic activities.

To verify that these inhibitors bind as expected in the active site, crystal structures were determined for four compounds, in complex with BACE-1. All compounds were soaked as racemates, and only one of the enantiomers was visible in the electron density. Indeed, all

compounds bind similarly, via the key interactions to the catalytic aspartic acids, and the constant phenyl and R2 aryl well defined in the S1 and S3 sub-pockets of the active site (Figure 6a-d)

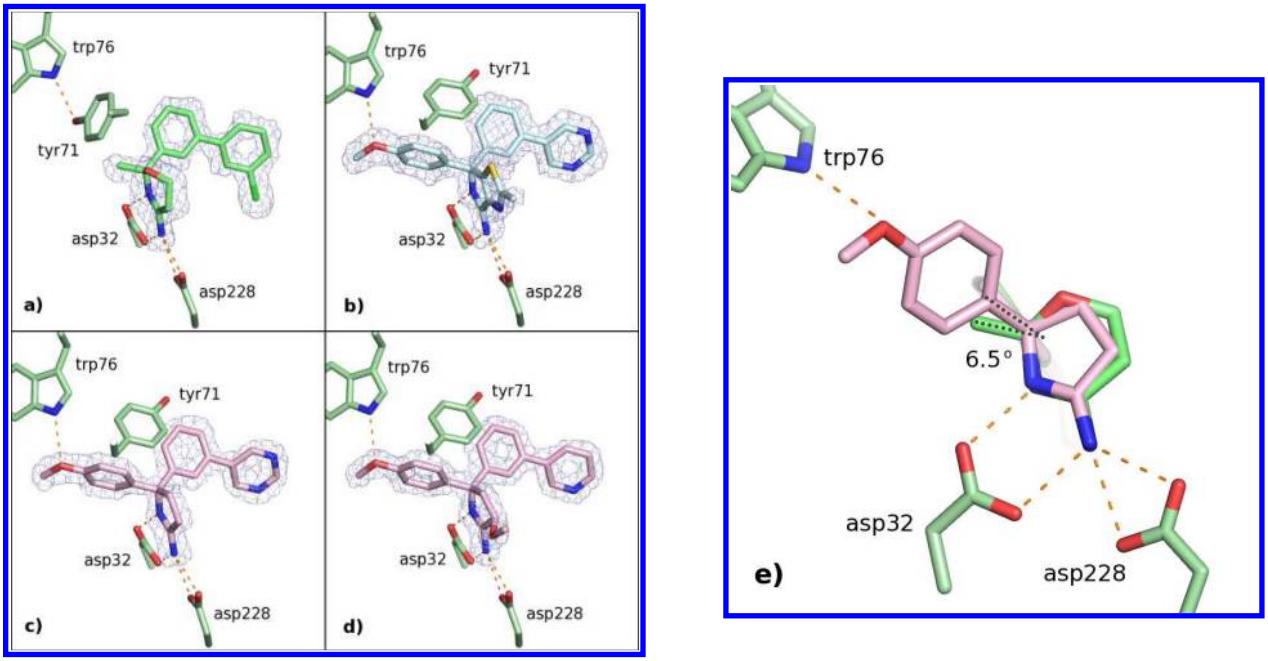


Figure 6. Crystal structures representing four of the cyclic amidine cores, in complex with BACE-1. a) Compound **19b**, 1.6 Å resolution; b) Compound **41**, 1.6 Å resolution; c) Compound **83**, 1.8 Å resolution; d) Compound **91**, 1.5 Å resolution. e) Superimposition of a 5- and 6-membered core showing the angle difference for the R1-substituent.

The compounds with 5-membered cores all bind to a protein conformation that is similar to the apo-structure.[56] In this conformation, the so called "flap residues" (P70-Y71-T72-Q73-G74) is in an open position, allowing the R1-phenyl with a methoxy-group in the *para*-position to accept a favorable hydrogen bond from the indole of trp76 (Figure 6b, c and d). In the crystal structures of **41** (Figure 6b), the additional ring of the bicyclic core is in close contact (3.6 Å) with the

aromatic ring of tyr71 (b). This interaction is not seen for the compounds with monocyclic cores (**83**, **91**, Figure 6b-c). All of this is in accordance with previously reported structures.[19-21,29]

The representative of the 6-membered cores (**19b**, Figure 6a) is bound to a different protein conformation, with the flap in closed position, and with an internal hydrogen bond between the hydroxyl of tyr71 and the indole of trp76. This protein conformation is also seen in structures of substrate analogues,[57,58] and in previously reported structures of 6-membered cyclic guanidines.[13,23,24,59]

In previous reports, the hydrogen bond with trp76 has been important for affinity of inhibitors with 5-membered cores.[22] To our knowledge, no inhibitors with 6-membered cores have been reported, with aryls in R1 making a direct hydrogen bond to Trp76. When the crystal structures of the compounds representing the 5-membered cores were overlaid, and compared to **19b**, the attachment point of R1 is in slightly different positions for these core types. The angular difference is approximately 6 degrees, making it unfavorable for aryls in R1 on 6-membered cores to reach the hydrogen bond interaction to trp76 (Figure 6e). In the literature, other ways are reported to decorate 6-membered cores that provide the interaction to trp76.[27]

Another interesting feature displayed by a crystal structure was found in the core of **91**, soaked without separating the stereoisomers. It has two stereogenic centers, but the electron density was clearly defined for only one of the stereoisomers in the crystal structure (Figure 6d): The methoxy group in the core, just above one of the catalytic aspartates, is pointing towards the prim-side of the active site. TR-FRET data on the separated stereoisomers show a big difference in activity between the most potent isomer (**Iso4-91**, 3.2 μM) and the second best (**Iso1-91**, 20 μM) (Table 4). This high potency difference from a substituent in a solvent accessible area was

surprising. However, in a previously reported in-house series, other examples indicating the importance of this region for affinity have been observed. When an isopropyl group was exchanged for a methyl in this area, the activity increased one log-unit. (Supporting information, Table S2).

hERG results and discussion. We were pleased to find that most of the compounds reported in Tables 1-4 had reduced hERG activity compared to the references (Supporting information). Compound **24a** showed no hERG activity at the highest concentration tested (33μM). However, most of the compounds showed some inhibition of the hERG channel, with activities ranging from 1 to 20 μM, which could be explained by their inherent structural features, a basic center surrounded by aromatic and lipophilic substituents[30b,60] (Table 1-4).

Analyzing the data, it could be seen that our initial hypothesis, i.e. to reduce hERG activity by reducing lipophilicity and pKa, was verified on a qualitative basis. Thus, an enrichment of highly hERG active compounds was observed in the area with high pKa and elogD (Figure 7). Since these results were generated with a cell based assay,[50] permeability of the compounds might affect the activity values. We hypothesize that the less hERG active compounds in the area of pKa above 9 might be due to a lower permeability of these compounds (see permeability section below).

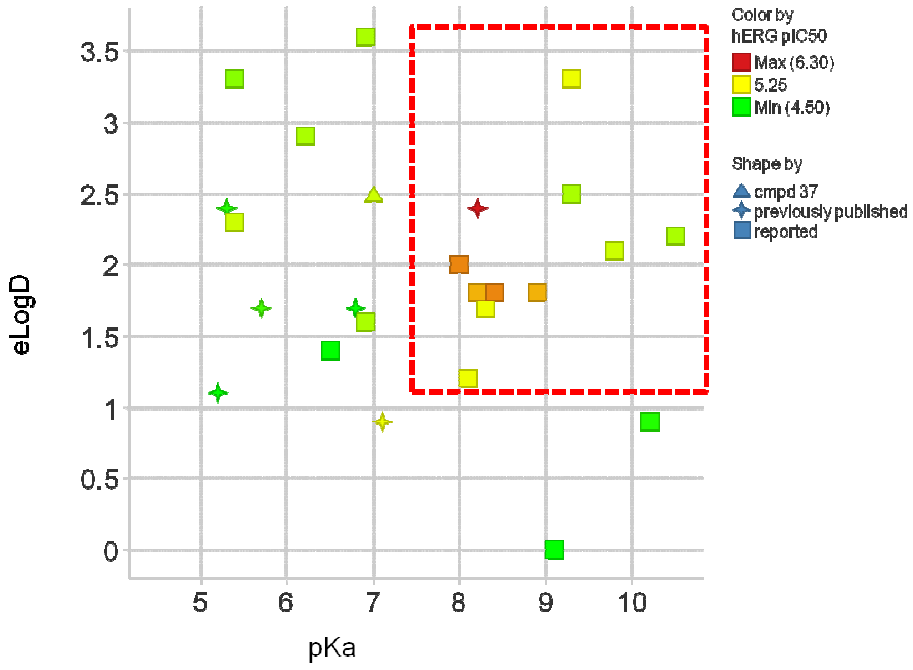


Figure 7. An enrichment of highly hERG active compounds was observed in the area with high pKa and ElogD.

Several compounds with bicyclic core structures displayed high hERG activities (**41**, **8a**, **9**, **10a** and **15**) which was supposed to largely be due to high pKa and lipophilicity of the cores. Compounds representing core type C-2 (**47**) were proposed to have a decreased risk of being hERG active, owing to the intrinsically low pKa and lipophilicity of this core. To further improve the chance of reduced effect on the hERG channel, this core was decorated with a polar R2-substituent. Despite these efforts, compound **47** was active on the hERG channel with an IC_{50} value of 8.6 µM (Table 3). With this result in hand, core size was hypothesized to be an important factor for hERG activity. Another example of the importance of core size for hERG activity was seen for compounds based on cores of sub-type A. Addition of a fluorine atom to the core structure reduced the pKa by one log unit, but only resulted in a marginal lowering of the hERG activity (Table 1, **8a** as compared to **10a**).

Permeability results and discussion. Except for the reference compound **37**, only compounds of sub-type A were classified as having a high overall permeability *in vitro* (Table 1). However, previous publications have shown that a medium overall permeability *in vitro* at an early stage of development can, after further lead optimization, be developed to reach reasonable *in vivo* exposure levels in rodent brain.[20,21] When permeability of all compounds in this report was compared to the presumed important physicochemical parameters pKa, lipophilicity (elogD) and size (MW),[28b,61] pKa stood out as the most important parameter. All compounds with high overall permeability were found below a pKa of 8.5 (Figure 8). This was in accordance with medicinal chemistry principles, as well as results on similar, previously reported BACE-1 inhibitors, where a lowering of pKa had been beneficial for increasing permeability.[19,20] For these compounds, there were no clear correlations for MW or elogD to permeability. However, these parameters were not varied to the same extent as pKa, and all compounds had a lipophilicicy and size within a range known to generally ensure high permeability (Figure 8).

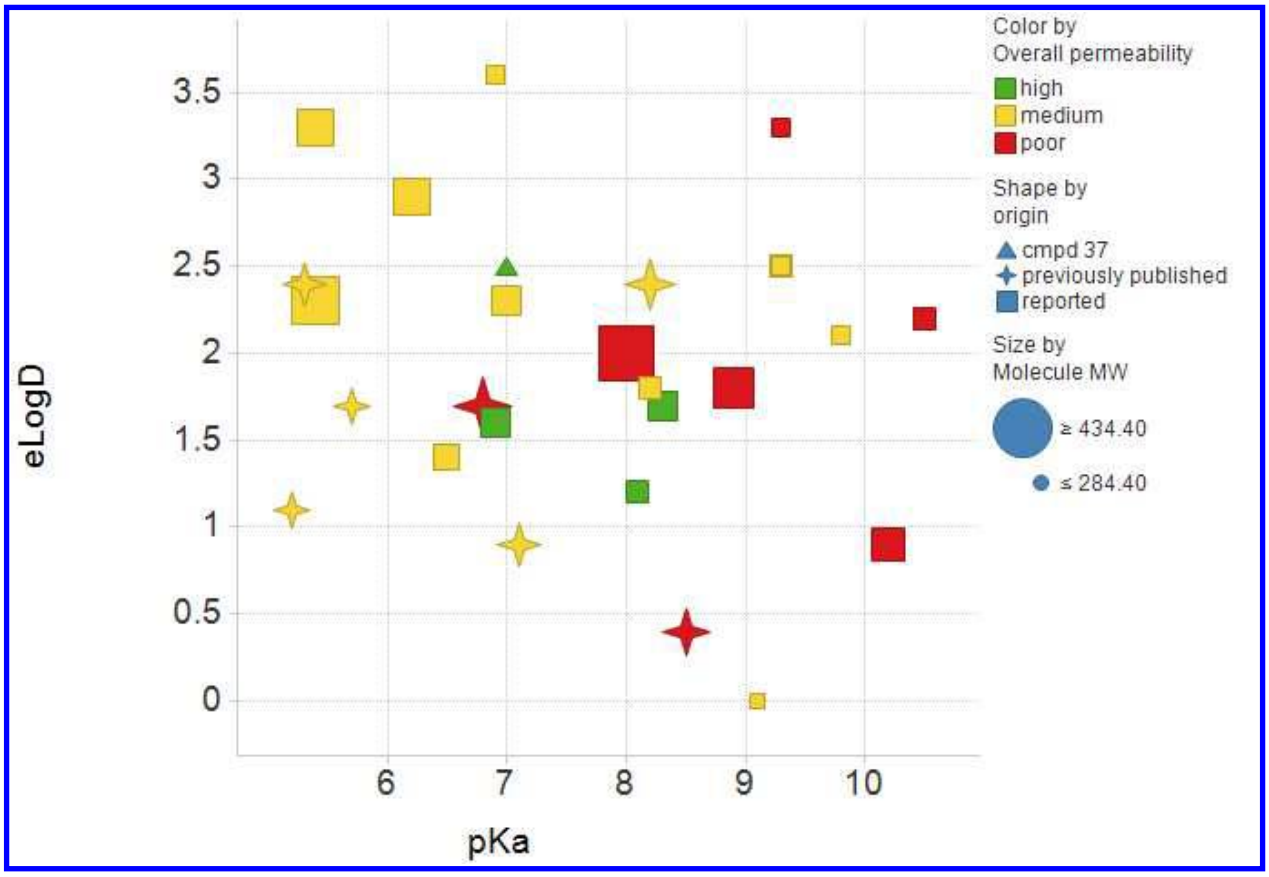


Figure 8. Overview of all compounds in this report, overall permeability was compared to MW, pKa and elogD .

When the compounds were analyzed on core sub-type level, pKa stood out again as the most important parameter affecting permeability. Compounds of core sub-type A were the only ones with generally high overall permeability, and all of these compounds had a pKa below 8. Related compounds with monocyclic 6-membered cores (sub-type B) all had lower MW (<320) but still displayed a low to medium passive permeability in Caco-2 cells, probably due to the generally high pKa (>8.6). The sub-type B reference compound **37** was different, with a much higher passive permeability in Caco-2 cells (Table 2) and also a lower pKa of 7. P-gp efflux also appeared to be related to pKa for compounds of sub-type B: compound **34** had the highest pKa in the family and also displayed the highest efflux (Table 2).

The importance of pKa for permeability was also observed in the limited data generated for compounds with bi-cyclic 5-membered cores (sub-type C); the most basic compound (**41**) was classified as poorly permeable. Compound **41** had a higher P-gp efflux than the less basic (and less lipophilic) **47**, which was classified as having medium overall permeability (Table 3).

For compounds with monocyclic 5-membered cores (sub-type D), a connection between high pKa and poor permeability was again hypothesized from the data, as illustrated by the most basic compounds **83** and ***rac*-91** (Table 4). From the separation of the stereoisomers of **91**, it was shown that P-gp efflux was equally high for all stereoisomers, indicating that the 3D-structure was of less importance (Table 4).

However, there were also exceptions from the trend that low pKa and high lipophilicity is favorable for permeability: compounds of core type D-1 were expected to have a higher overall permeability than medium. All compounds in this series had a low pKa (5.4-6.9) and were quite lipophilic (elogD = 2.9-3.6). However, not even the smallest compound in this series (**58**) exhibited a high permeability (Table 4). This can be compared to the structurally similar compounds of core type B-2, which showed a similar permeability profile, but with a much higher pKa of 9.1, and an elogD of 0 (Table 2, compound **24a**).

***In vivo* brain exposure.** To determine the brain exposure in mice and investigate whether the *in vitro* measurements of permeability could be used to predict brain exposure, four compounds (**24a**, **37**, **41** and **64**) were administered intravenously or per orally in mice at a doses of 30-50 μmol/kg. Concentrations in brain and plasma were measured after 1.5 h and the total and free brain plasma concentration ratios were calculated. (Table 5)

Table 5. *In vivo* brain exposure of four compounds.

Compound	Route of	C_{brain}/C_{plasma}	$f_{u,brain}$	$f_{u,plasma}$	$C_{u,brain}/C_{u,plasma}$	P-gp

	administration[a]					Efflux
24a	iv	1.4	0.074	0.89	0.27	low
37	po	8.9	0.005	0.07	0.64	nd
41	po	0.2	0.02	0.1	0.04	high
64	iv	0.9	0.01	0.007	1.3	low[b]

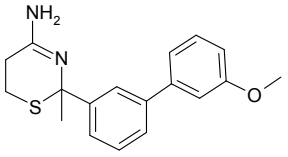
[a]iv (intravenous), po (per oral). [b]P-gp efflux was estimated from the value of the similar compound **65** (Table 1).

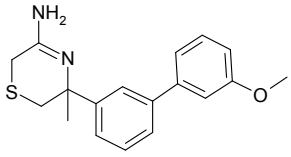
Both compounds **24a** and **64** penetrated the blood brain barrier well and showed a high CNS localization as could be predicted by the low efflux in the MDCK-MDR1 P-gp assay. The compounds had a high total brain/plasma ratio (1.4 and 0.9 respectively), and both **24a** and **64** reached reasonable to good free brain/plasma ratios (0.27 and 1.3 respectively). The relatively low brain/plasma ratio for compound **41** (0.2) was in accordance with the results from the MDCK-MDR1 P-gp assay where the compound was shown to be a P-gp substrate. Since compound **37** was only used as a reference, it was not tested for P-gp efflux, but CNS localization was high with a free brain/plasma ratio calculated to 0.64.

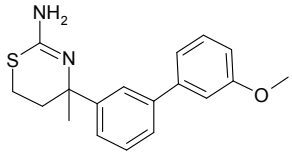
Combined profile discussion. The cores of sub-type A had the best permeability profile, but were still seen as less promising, due to poor LEs and cellular potencies and high hERG activities. Compounds of sub-type B were more promising, showing very high LEs and cellular potencies. Of these, core type B-2 had the overall most optimal *in vitro* profile, with high target activity in cell, low hERG activity and medium overall permeability. Some compounds of this core type showed better results than the clinical candidate references, when the main parameters were compared. In addition, the *in vivo* brain exposure of compound **24a** also showed high potential.

Compounds of sub-type C were highly active on target, but were regarded as less promising due to hERG issues. The sub-type D family contained the second best core type: D-1, with promising permeability *in vitro* and *in vivo*, together with reasonable target activity. Core type D-5 was exciting from an activity perspective, with surprisingly high activity differences for the separated stereoisomers. However, this core type did not exhibit a favorable permeability profile.

With these novel compounds, we have shown that the properties of the cores have a significant effect on the *in vitro* profiles of the test compounds and that small structural changes of the cores can potentially lead to large unpredictable variations, especially for target activity. For instance, when compounds **24b**, **34** and **37** were compared, differing only in the position of the sulfur in the core ring, it was interesting to see that the ElogD values were almost identical whereas the pKa values varied a lot. Despite the difference in pKa, compounds **34** and **37** had similar activities both in the enzymatic and the cell assay, whereas compound **24b** was more than three times more potent in the enzymatic assay and more than five times more potent than compound **34** in the cell assay. Permeability also differed: compound **37** had almost ten times better permeability than compound **34**. The hERG activity on the other hand was similar for all three compounds (Figure 10).







	24b	**34**	**37**
Exp. pKa:	9.1*	10.5	7.0
ElogD:	2.5	2.2	2.5
BACE-1 TR-FRET IC50 (μM):	0.63	2.0	2.0
BACE-1 sAPPbeta IC50 (μM):	0.063	0.32	0.40
hERG Mean IC50 (μM):	11	9.0	7.6
Papp (10^{-6} cm/s):	5.2	7.3	72

Figure 10. Matched pair analysis for compounds **24b**, **34** and **37**, all belonging to core sub-type B and containing one sulfur atom in the core structure. *Experimental pKa was measured for compound **24a.**

In summary, we have verified that the core structures are important for the overall profile of the test compounds. In line with previous reports, increasing pKa has a positive effect on cellular potency, up to a pKa of about 8.5. However, this needs to be balanced with the negative effect of a high pKa when addressing permeability and hERG issues: for these compounds an upper pKa limit of 8 could be seen to achieve high permeability. To increase the chances of reducing hERG activity, pKa should be even lower. Another way to reduce hERG activity for these compounds was to reduce lipophilicity.

CONCLUSION

This work describes our effort towards improved BACE-1 inhibitors by refining the amidine-containing core. The iterative design of the inhibitors was aided by predictive chemistry models and target affinity, permeability and hERG activity were addressed in a multi-parameter optimization fashion. In total thirteen new cores were designed to individually capture learnings and cover the desired physicochemical space. New synthetic pathways were developed, requiring a major synthetic effort and knowledge was generated that can be used for further optimization of other core structures. Compounds representing these cores were synthesized with matched pair substituents and evaluated on results from *in vitro* and *in vivo* experiments. Four compounds having a cellular potency under 100 nM were found, representing cores from three different sub-

types. Many of the new compounds showed a promising hERG profile, and two compounds showed a high CNS localization in vivo. With this exercise, we show that small changes in the core structures may give very different *in vitro* profiles of the test compounds. Thus, we conclude that it is important to focus on optimization of the core early in the lead generation phase, since the selection of the core is an important step, defining the framework for future lead optimization. This lead generation strategy may be suitable for other drug-design projects.

EXPERIMENTAL SECTION

Chemistry. All solvents used were commercially available and were used without further purification. Reactions were typically run using anhydrous solvents under an inert atmosphere of nitrogen or argon. Starting materials used were available from commercial sources, or prepared as described in supplementary materials. Room temperature refers to 20-25 °C. Microwave heating was performed in a Biotage® Initiator Microwave Synthesiser at the indicated temperature in the recommended microwave tubes.

^{1}H NMR spectra were recorded in the indicated deuterated solvent at 400 MHz and the spectra were obtained unless stated otherwise, using a Bruker av400 NMR spectrometer equipped with a 3 mm flow injection SEI ^{1}H/D-^{13}C probe head with Z-gradients, using a BEST 215 liquid handler for sample injection, or using a Bruker DPX400 NMR spectrometer equipped with a 4-nucleus probe head (^{19}F) with Z-gradients. 500 MHz spectra were recorded using a Bruker 500MHz Avance III NMR spectrometer. Chemical shifts are given in ppm down- and upfield from TMS. Resonance multiplicities are denoted s, d, t, q, m and br for singlet, doublet, triplet, quartet, multiplet, and broad respectively.

Preparative HPLC was performed on a Waters Auto purification HPLC-UV system with a diode array detector using a Waters XTerra MS C_8 column (19x300 mm, 7 μm) and a linear gradient of mobile phase B was applied. Mobile phase A: 0.1 M ammonium acetate in water/acetonitrile (95:5) and mobile phase B: acetonitrile. Flow rate: 20 mL/min. Flash chromatography was performed using Merck Silica gel 60 (0.040-0.063 mm), or employing a Combi Flash® Companion™ system using RediSep™ normal-phase flash columns. Column chromatography was performed using Merck Silica gel 60 (0.040-0.063 mm).

LC-MS analyses were performed on an LC-MS consisting of a Waters sample manager 2777C, a Waters 1525μ binary pump, a Waters 1500 column oven, a Waters ZQ single quadrupole mass spectrometer, a Waters PDA 2996 diode array detector and a Sedex 85 ELS detector. The mass spectrometer was equipped with an electrospray ion source (ES) operated in positive and negative ion mode. For separation a linear gradient was applied starting at 100 % 0.1 % NH_3 in MilliQ ending at 100 % methanol. The column used was an XBridge C18, 3.0 x 50 mm, 5 μm which was run at a flow rate of 2 ml/min. Or on a LC-MS system consisting of a Waters Alliance 2795 HPLC, a Waters PDA 2996 diode array detector, a Sedex 85 ELS detector and a ZQ single quadrupole mass spectrometer. The mass spectrometer was equipped with an electrospray ion source (ES) operated in positive and negative ion mode. Separation was performed on an XBridge C18, 3.0 x 50 mm, 3.5 μm run at a flow rate of 1 ml/min. A linear gradient was applied starting at 100 % 0.1 % NH_3 in MilliQ ending at 100 % methanol.

Purity analyses were performed on an Agilent HP1100 system consisting of a G1322A Micro Vacuum Degasser, a G1312A Binary Pump, a G1367 A Well-Plate Autosampler, a G1316A Thermostatted Column Compartment, a G1315C Diode Array Detector and a 6120, G1978B mass spectrometer. The mass spectrometer was configured with an atmospheric pressure

chemical ionization (APCI) ion source operated in positive and negative ion mode. The column used was an XBridge C18 3.0 x 100, 3 μm run at a flow rate of 1.0 ml/min. A linear gradient was used for both the blank and the sample, starting at 100 % 10 mM NH_4OAc in 5 % CH_3CN and ending at 95 % CH_3CN. The blank run was subtracted from the sample run. All tested compounds were purified to >95% purity as determined by reversed phase HPLC, if nothing else is stated.

SFC Purity analysis was run on a SFC Berger Analytix system with Agilent 1100 PDA detector. Column; Chiralpak AD-H 5μm 4.6 x 250 mm. The column temperature was set to 50 °C. An isocratic condition of 20-30% (2-propanol + 0.1% DEA) and 70-80% CO_2 was applied at flow rate 3.0 mL/min. The PDA was scanned from 190-600 nm and 220 nm was extracted for purity determination.

SFC Preparative chromatography was run on a SFC Berger Multigram II system with a Knauer K-2501 UV detector. Column; Chiralpak AD-H 5 μm 21.2 x 250 mm. The column temperature was set to 35 °C. An isocratic condition of 20-30% (2-propanol + 0.1% DEA) and 70-80% CO_2 was applied at flow rate 50.0 mL/min. The UV detector scanned at 220 nm. The UV signal determined the fraction collection.

2-Methyl-2-(3-(pyrimidin-5-yl)phenyl)-2*H*-benzo[e][1,3]oxazin-4-amine (8a). 1-(3-bromophenyl)ethanone (**1**) (15 mL, 0.11 mol), 2-hydroxybenzamide (**2**) (10 g, 73 mmol), and *p*-toluenesulfonic acid monohydrate (1.4 g, 7.3 mmol) were taken up in toluene (50 mL), fitted with a Dean-Stark trap and heated to reflux for 16 h. The reaction mixture was cooled to 0 °C for 30 min. The precipitate was collected by suction filtration, washed with toluene and dried to yield 22 g of **3**. A portion of this material (1 g) and phosphorus pentasulfide (1 g, 2.3 mmol) was dissolved in pyridine (7.5 mL). The reaction was heated to 120 °C for 1 h and then cooled to rt.

The reaction mixture was diluted with 1 M HCl. The mixture was extracted with DCM. The combined organic phases were washed with brine, dried over $MgSO_4$, filtered and the solvent evaporated to yield 1.4 g of **4** (MS (ES+) *m/z* 334, 336 $[M+H]^+$). A portion of this material (0.25 g) was dissolved in ammonia (7 M in MeOH) (4.3 mL, 30 mmol). *tert*-Butyl hydroperoxide (1.5 mL, 11 mmol) was added and the reaction was stirred at rt for 2 h. The solvents and excess reagents were evaporated. The product was purified by RP-HPLC to yield 23.4 mg of **5** (MS (ES+) *m/z* 317, 319 $[M+H]^+$). This material was taken up in DME (2 mL) and water (1 mL). Pyrimidin-5-ylboronic acid (18 mg, 0.15 mmol) and bis(triphenylphosphine)palladium(II) chloride (5.2 mg, 7.4 μmol) and sodium carbonate (1 M) (0.18 mL, 0.18 mmol) were added and the reaction was heated to 80 °C for 2 h. The product was purified by RP-HPLC to yield 12 mg (6%) of the title compound over several steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.91 (s, 3 H) 4.30 (br. s., 2 H) 6.91 (td, *J*=7.58, 1.01 Hz, 1 H) 7.01 (dd, *J*=8.21, 0.88 Hz, 1 H) 7.27 (dd, *J*=7.83, 1.52 Hz, 1 H) 7.37 (ddd, *J*=8.15, 7.39, 1.64 Hz, 1 H) 7.40 - 7.47 (m, 2 H) 7.68 - 7.71 (m, 1 H) 7.79 - 7.82 (m, 1 H) 8.91 (s, 2 H) 9.19 (s, 1 H). MS (ES+) *m/z* 317 $[M+H]^+$.

2-Methyl-2-(3-(pyridin-3-yl)phenyl)-2*H*-benzo[e][1,3]oxazin-4-amine (8b). A portion of **7** (5 g) was dissolved in $POCl_3$ (8.8 mL, 94 mmol). PCl_5 (0.66 g, 3.1 mmol) was added and the reaction was stirred at 50 °C for 3 h. Excess reagent was evaporated. The residue **6** (MS (ES+) *m/z* 338 $[M+H]^+$) was taken up in DMF (50 mL). DIPEA (14 mL, 79 mmol) and *O*-methylhydroxylamine (6.6 g, 79 mmol) were added and the reaction was stirred at 90 °C for 16 h. The solvent was evaporated and the residue poured into brine. The mixture was extracted with diethyl ether. The combined organic phases were dried over $MgSO_4$, filtered and the solvents evaporated. The product was purified by column chromatography using EtOAc 0% to 50% in heptane to yield 6 g of **7** (MS (ES+) *m/z* 347, 349 $[M+H]^+$). This material was dissolved in acetic

acid (50 mL). Zinc (3 g, 46 mmol) was added and the reaction was stirred at rt for 1 h. The reaction mixture was filtered and the filtrate reduced. The product was purified by column chromatography using MeOH 0% to 10% in DCM to yield 2.3 g of **5** (MS (ES+) *m/z* 317, 319 $[M+H]^+$). A portion of this material (100 mg) was taken up in DME (2 mL) and water (1 mL). Pyridin-3-ylboronic acid (47 mg, 0.38 mmol), bis(triphenylphosphine)-palladium(II) chloride (22 mg, 0.03 mmol) and sodium carbonate (1 M) (0.79 mL, 0.79 mmol) were added and the reaction was heated to 80 °C for 2 h. The product was purified by acidic RP-HPLC, followed by column chromatography using EtOAc 0% to 100% in heptane, followed by basic RP-HPLC to yield 7.7 mg (3%) of the title compound over several steps. ^{1}H NMR (400 MHz, MeOD) δ ppm 1.87 (s, 3 H) 6.90 (td, *J*=7.64, 1.14 Hz, 1 H) 6.96 - 7.02 (m, 1 H) 7.32 - 7.41 (m, 2 H) 7.43 - 7.52 (m, 3 H) 7.57 (dt, *J*=7.83, 1.52 Hz, 1 H) 7.75 (t, *J*=1.77 Hz, 1 H) 7.96 - 8.02 (m, 1 H) 8.50 (dd, *J*=4.93, 1.39 Hz, 1 H) 8.69 (d, *J*=1.77 Hz, 1 H). MS (ES+) *m/z* 316 $[M+H]^+$.

2-Ethyl-2-(3-(pyrimidin-5-yl)phenyl)-2*H*-benzo[e][1,3]oxazin-4-amine (9). 2-(3-Bromophenyl)-2-ethyl-2*H*-benzo[e][1,3]oxazin-4(3*H*)-one (4 g, 12 mmol) was dissolved in $POCl_3$ (6.7 mL, 72 mmol). PCl_5 (0.5 g, 2.4 mmol) was added and the reaction was stirred at 50 °C for 3 h. Excess reagent was evaporated. The residue 2-(3-bromophenyl)-4-chloro-2-ethyl-2*H*-benzo[e][1,3]oxazine was taken up in DMF (50 mL). DIPEA (11 mL, 60 mmol) and O-methylhydroxylamine (5 g, 60 mmol) was added and the reaction was stirred at 90 °C for 16 h. The solvent was evaporated and the residue poured into brine. The mixture was extracted with diethyl ether. The combined organic phases were dried over $MgSO_4$, filtered and the solvents evaporated. The product was purified by column chromatography using EtOAc 0% to 30% in heptane to yield 2 g of *N*-(2-(3-bromophenyl)-2-ethyl-2*H*-benzo[e][1,3]oxazin-4-yl)-O-methylhydroxylamine (MS (ES+) *m/z* 361, 363 $[M+H]^+$). This material was dissolved in acetic

acid (20 mL). Zinc (1 g, 15 mmol) was added and the reaction was stirred at rt for 1 h. The reaction mixture was filtered and the filtrate reduced. The product was purified by column chromatography using MeOH 0% to 10% in DCM to yield 1.5 g of 2-(3-bromophenyl)-2-ethyl-2H-benzo[e][1,3]oxazin-4-amine (MS (ES+) *m/z* 331, 333 $[M+H]^+$). A portion of this material (100 mg) was taken up in DME (2 mL) and water (1 mL). Pyrimidin-5-ylboronic acid (45 mg, 0.36 mmol), bis(triphenylphosphine)palladium(II) chloride (21 mg, 0.03 mmol) and sodium carbonate (1 M) (0.76 mL, 0.76 mmol) were added and the reaction was heated to 80 °C for 2 h. The reaction mixture was extracted with DCM. The combined organic phases were evaporated. The product was purified by RP-HPLC to yield 29 mg (11%) of the title compound at 93% purity, over several steps. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.90 (t, *J*=7.33 Hz, 3 H) 1.92 - 2.12 (m, 2 H) 6.49 (s, 2 H) 6.85 (td, *J*=7.52, 1.14 Hz, 1 H) 7.01 (dd, *J*=8.21, 0.88 Hz, 1 H) 7.28 - 7.35 (m, 1 H) 7.40 - 7.46 (m, 1 H) 7.52 (dd, *J*=7.83, 1.26 Hz, 1 H) 7.53 - 7.58 (m, 1 H) 7.58 - 7.65 (m, 1 H) 7.80 (t, *J*=1.64 Hz, 1 H) 9.03 (s, 2 H) 9.19 (s, 1 H). MS (ES+) *m/z* 331 $[M+H]^+$.

5-Fluoro-2-methyl-2-(3-(pyrimidin-5-yl)phenyl)-2*H*-benzo[e][1,3]oxazin-4-amine (10a). 2-Fluoro-6-hydroxybenzamide (**24**) (5 g), 1 (6.4 mL, 48 mmol) and *p*-toluenesulfonic acid monohydrate (6.7 g, 35 mmol) were taken up in toluene (50 mL), fitted with a Dean-Stark trap and heated to reflux for 16 h. The reaction mixture was shaken with 1 M LiOH. The phases were separated. The aqueous phase was extracted with DCM. The combined organic phases were washed with brine, dried over $MgSO_4$, filtered and the solvents evaporated. The product was purified by column chromatography using EtOAc 0% to 100% heptane to yield 2.2 g of 2-(3-bromophenyl)-5-fluoro-2-methyl-2*H*-benzo[e][1,3]oxazin-4(3*H*)-one (MS (ES+) *m/z* 336, 338 $[M+H]^+$). This material was dissolved in $POCl_3$ (7.2 mL, 77 mmol). PCl_5 (0.53 g, 2.6 mmol) was added and the reaction was stirred at 50 °C for 3 h. Excess reagent was evaporated. The residue

2-(3-bromophenyl)-4-chloro-5-fluoro-2-methyl-2*H*-benzo[e][1,3]oxazine was taken up in DMF (20 mL). DIPEA (5.6 mL, 32 mmol) and *O*-methylhydroxylamine (2.8 g, 32 mmol) were added and the reaction was stirred at 90 °C for 16 h. The solvent was evaporated and the residue poured into brine. The mixture was extracted with DCM. The combined organic phases were dried over $MgSO_4$, filtered and the solvents evaporated. The product was purified by column chromatography using EtOAc 0% to 50% in heptane to yield 0.28 g of *N*-(2-(3-bromophenyl)-5-fluoro-2-methyl-2*H*-benzo[e][1,3]oxazin-4-yl)-*O*-methylhydroxylamine (MS (ES+) *m/z* 365, 367 $[M+H]^+$). This material was dissolved in acetic acid (3 mL). zinc (0.34 g, 5.20 mmol) was added and the reaction was stirred at rt for 3 h. The reaction mixture was filtered and the filtrate reduced. The residue was taken up in DCM and washed with 1 M LiOH, brine, dried over $MgSO_4$, filtered and the solvent evaporated to yield 170 mg of 2-(3-bromophenyl)-5-fluoro-2-methyl-2*H*-benzo[e][1,3]oxazin-4-amine (MS (ES+) *m/z* 335, 337 $[M+H]^+$). A portion of this material (85 mg) was taken up in DME (2 mL) and water (1 mL). Pyrimidin-5-ylboronic acid (35 mg, 0.28 mmol), bis(triphenylphosphine)palladium(II) chloride (18 mg, 0.03 mmol) and sodium carbonate (1 M) (0.63 mL, 0.63 mmol) were added and the reaction was heated to 80 °C for 2 h. The product was purified by RP-HPLC to yield 30 mg (0.4%) of the title compound over several steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.92 (s, 3 H) 5.45 (br. s., 2 H) 6.65 (dd, *J*=11.12, 8.59 Hz, 1 H) 6.85 (d, *J*=8.08 Hz, 1 H) 7.29 - 7.36 (m, 1 H) 7.43 - 7.50 (m, 2 H) 7.67 - 7.72 (m, 1 H) 7.79 (s, 1 H) 8.92 (s, 2 H) 9.21 (s, 1 H). MS (ES+) *m/z* 335 $[M+H]^+$.

5-Fluoro-2-methyl-2-(3-(pyridin-3-yl)phenyl)-2*H*-benzo[e][1,3]oxazin-4-amine (10b). A portion of 2-(3-bromophenyl)-5-fluoro-2-methyl-2*H*-benzo[e][1,3]oxazin-4-amine (85 mg) was taken up in DME (2 mL) and water (1 mL). Pyridin-3-ylboronic acid (34 mg, 0.28 mmol), bis(triphenylphosphine)palladium(II) chloride (18 mg, 0.03 mmol) and sodium carbonate (1 M)

(0.63 mL, 0.63 mmol) was added and the reaction was heated to 80 °C for 2 h. The product was purified by RP-HPLC to yield 41 mg (0.6%) of the title compound at 93% purity, over several steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.88 (s, 3 H) 5.28 (br. s., 2 H) 6.59 (dd, *J*=11.24, 8.46 Hz, 1 H) 6.81 (d, *J*=8.08 Hz, 1 H) 7.22 - 7.48 (m, 4 H) 7.60 (dt, *J*=7.52, 1.55 Hz, 1 H) 7.77 (t, *J*=1.77 Hz, 1 H) 7.82 (dt, *J*=7.96, 1.83 Hz, 1 H) 8.56 (dd, *J*=4.80, 1.52 Hz, 1 H) 8.79 (d, *J*=2.27 Hz, 1 H). MS (ES+) *m/z* 334 $[M+H]^+$.

2-Methyl-2-(3-(pyrimidin-5-yl)phenyl)-2*H*-benzo[e][1,3]thiazin-4-amine (15). Compound **11a** (340 mg, 1.7 mmol) and methyl 2-mercaptobenzoate (1.2 mL, 8.6 mmol) were taken up in ammonia (7 M in MeOH) (2 mL, 14 mmol). The reaction was heated to 150 °C for 3 h by microwave heating and the solvent was evaporated. The product was purified by column chromatography using EtOAc 0% to 100% in heptane to yield 0.16 g of **13** (MS (ES+) *m/z* 334 $[M+H]^+$). This material and phosphorus pentasulfide (0.16 g, 0.35 mmol) were taken up in pyridine (1 mL). The reaction was heated to 120 °C for 1 h and then cooled to rt. Ammonia (7 M in MeOH) (2.5 mL, 18 mmol) was added and the reaction was stirred at 90 °C for 16 h. The product was purified by RP-HPLC followed by column chromatography using MeOH (with 7 M NH_3) 0% to 10% in DCM to yield 7.2 mg (1.3%) of the title compound over several steps. ^{1}H NMR (400 MHz, MeOD) δ ppm 1.93 (s, 3 H) 7.14 - 7.22 (m, 1 H) 7.26 - 7.36 (m, 2 H) 7.38 - 7.44 (m, 1 H) 7.47 - 7.54 (m, 1 H) 7.65 - 7.75 (m, 2 H) 7.88 (t, *J*=1.89 Hz, 1 H) 8.97 (s, 2 H) 9.12 (s, 1 H). MS (ES+) *m/z* 333 $[M+H]^+$.

2-(3'-Methoxybiphenyl-3-yl)-2-methyl-5,6-dihydro-2*H*-1,3-oxazin-4-amine (19a). A mixture of compound **18** (45 mg, 0.17 mmol), 3-methoxyphenylboronic acid (51 mg, 0.33 mmol), 1,1'-bis(diphenylphosphino)ferrocene-palladium(II)dichloride dichloromethane complex (20 mg, 0.03 mmol) and cesium carbonate (0.16 g, 0.50 mmol) in 1,2-

dimethoxyethane:water:ethanol (6:3:1, 5 mL) was heated at 120 °C for 20 min by microwave heating. When cooled to ambient temperature the mixture was concentrated and the residue partitioned between dichloromethane-water (4:1, 15 mL). The organic layer was dried over Na_2SO_4, filtered, concentrated and the product purified by RP-HPLC to yield 3.4 mg (5%) of the title compound as the acetate salt. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm (s, 3 H) 2.22 - 2.32 (m, 1 H) 2.77 (ddd, *J*=17.50, 10.56, 7.25 Hz, 1 H) 3.69 (td, *J*=11.35, 3.78 Hz, 1 H) 3.88 (s, 3 H) 3.93 (dd, *J*=11.35, 7.25 Hz, 1 H) 6.91 (dd, *J*=8.20, 2.21 Hz, 1 H) 7.16 (s, 1 H) 7.22 (d, *J*=7.57 Hz, 1 H) 7.33 - 7.40 (m, 1 H) 7.42 - 7.50 (m, 2 H) 7.55 (d, *J*=7.25 Hz, 1 H) 7.75 (s, 1 H). MS (ES+) *m/z* 297 $[M+H]^+$.

2-(3'-Chlorobiphenyl-3-yl)-2-methyl-5,6-dihydro-2*H*-1,3-oxazin-4-amine (19b). A mixture of **18** (45 mg, 0.17 mmol), 3-chlorophenylboronic acid (52 mg, 0.33 mmol), 1,1'-bis(diphenylphosphino)ferrocene-palladium(II)dichloride dichloromethane complex (14 mg, 0.02 mmol) and cesium carbonate (0.16 g, 0.50 mmol) in 1,2-dimethoxyethane:water:ethanol (6:3:1, 5 mL) was heated at 120 °C for 20 min by microwave heating. When cooled to ambient temperature the mixture was concentrated and the residue partitioned between dichloromethane-water (4:1, 15 mL). The organic layer was dried over Na_2SO_4, filtered, concentrated and the product purified by RP-HPLC to yield 4.5 mg (8%) of the title compound as the acetate salt. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.80 (s, 3 H) 2.34 (d, *J*=16.39 Hz, 1 H) 2.81 (ddd, *J*=17.26, 10.32, 7.41 Hz, 1 H) 3.69 (td, *J*=11.35, 3.47 Hz, 1 H) 3.96 (dd, *J*=11.35, 6.94 Hz, 1 H) 7.30 - 7.41 (m, 2 H) 7.43 - 7.56 (m, 4 H) 7.62 (s, 1 H) 7.74 (s, 1 H). MS (ES+) *m/z* 301 $[M+H]^+$.

2-Methyl-2-(3-(pyrimidin-5-yl)phenyl)-5,6-dihydro-2*H*-1,3-thiazin-4-amine (24a). Methyl 3-mercaptopropanoate (8.0 g, 67 mmol) was dissolved in ammonium hydroxide 25% (0.14 L, 0.89 mol) and the obtained solution was stirred at rt for 8 h. The mixture was concentrated, co-

evaporated with toluene and dried at reduced pressure and over P_2O_5 to yield 7 g of **21**. A portion of this material (315 mg), **11a** (495 mg, 2.5 mmol) and pTSA (43 mg, 0.22 mmol) in toluene (25 mL) was refluxed for 8 h using a Dean-Stark trap. The mixture was concentrated at reduced pressure. The product was purified by column chromatography using EtOAc 60% to 100% in heptane to yield 225 mg of **22a** (MS (ES+) *m/z* 286 $[M+H]^+$). This material, phosphorus pentasulfide (175 mg, 0.79 mmol) and hexamethyldisiloxane (0.5 mL, 2.4 mmol) were taken up in dry 1,2-dichloroethane (2 mL) and heated to 110 °C for 1 h by microwave heating. The mixture was filtered through a pad of silica gel, eluted with EtOAc and concentrated at reduced pressure. The product was purified by column chromatography using EtOAc 0% to 50% in heptane to yield 105 mg of **23a** (MS (ES+) *m/z* 302 $[M+H]^+$). This material was taken up in ammonia (7 M in MeOH) (21 mL, 147 mmol) in a 50 mL round bottom flask which was sealed with a septum. The reaction mixture was stirred at rt overnight. The product was purified by RP-HPLC to yield 37 mg (3%) of the title compound over several steps. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.80 (s, 3 H) 2.25 (t, *J*=6.31 Hz, 2 H) 2.42 (dt, *J*=13.64, 6.90 Hz, 1 H) 2.76 (dt, *J*=13.48, 5.56 Hz, 1 H) 5.94 (br. s., 2 H) 7.44 - 7.49 (m, 1 H) 7.52 - 7.57 (m, 1 H) 7.63 (dt, *J*=7.57, 1.42 Hz, 1 H) 7.80 (t, *J*=1.73 Hz, 1 H) 9.09 (s, 2 H) 9.20 (s, 1 H). MS (ES+) *m/z* 285 $[M+H]^+$.

2-(3'-Methoxybiphenyl-3-yl)-2-methyl-5,6-dihydro-2*H*-1,3-thiazin-4-amine (24b). A mixture of compound **21** (0.84 g), 5 (1.8 g, 8 mmol) and pTSA (0.136 g, 0.72 mmol) in toluene (25 mL) was refluxed for 8 h using a Dean-Stark trap. The reaction mixture was concentrated at reduced pressure. The product was purified by column chromatography using EtOAc 60% to 100% in heptane to yield 0.5 g of **22b**. A portion of this intermediate material (260 mg), phosphorus pentasulfide (184 mg, 0.83 mmol) and hexamethyldisiloxane (0.52 mL, 2.5 mmol)

were taken up in dry 1,2-dichloroethane (2 mL) and heated to 110 °C for 1 h by microwave heating. The mixture was filtered through a pad of silica gel, eluted with EtOAc and concentrated at reduced pressure. The product was purified with column chromatography using EtOAc 0% to 50% in heptane to yield 100 mg of **23b** (MS (ES+) *m/z* 330 $[M+H]^+$). This material was taken up in ammonia (7 M in MeOH) (20 mL, 140 mmol) in a 50 mL round bottle flask which was sealed with a septum. The reaction mixture was stirred at rt for 16 h. The product was purified by RP-HPLC to yield 5.4 mg (0.4%) of the title compound as the acetate salt, over several steps. ^{1}H NMR (500 MHz, MeOD) δ ppm 1.99 (s, 3 H) 2.54 - 2.67 (m, 1 H) 2.81 - 2.99 (m, 3 H) 3.83 (s, 3 H) 6.92 (dd, *J*=8.20, 2.21 Hz, 1 H) 7.11 (t, *J*=2.05 Hz, 1 H) 7.16 (d, *J*=8.20 Hz, 1 H) 7.35 (t, *J*=7.88 Hz, 1 H) 7.44 - 7.49 (m, 2 H) 7.52 - 7.59 (m, 1 H) 7.66 (s, 1 H). MS (ES+) *m/z* 313 $[M+H]^+$.

5-(3'-Methoxybiphenyl-3-yl)-5-methyl-5,6-dihydro-2*H*-1,4-oxazin-3-amine (30). A solution of **29** (0.25 g, 0.81 mmol) in ammonia (7 M in MeOH) (1.8 mL, 81 mmol) was stirred at 40 °C for 3 days. The product was purified by RP-HPLC to yield 95 mg (27%) of the title compound as the acetate salt. ^{1}H NMR (500 MHz, MeOD) δ ppm 1.78 (s, 3 H) 3.85 (s, 3 H) 3.91 - 4.04 (m, 2 H) 4.59 (s, 2 H) 6.94 (dd, *J*=8.20, 2.21 Hz, 1 H) 7.15 (t, *J*=2.05 Hz, 1 H) 7.19 (d, *J*=7.57 Hz, 1 H) 7.36 (t, *J*=8.04 Hz, 1 H) 7.40 - 7.45 (m, 1 H) 7.50 (t, *J*=7.72 Hz, 1 H) 7.59 (d, *J*=7.57 Hz, 1 H) 7.62 - 7.65 (m, 1 H). MS (ES+) *m/z* 297 $[M+H]^+$.

5-(3'-Methoxybiphenyl-3-yl)-5-methyl-5,6-dihydro-2*H*-1,4-thiazin-3-amine (34). A mixture of **33** (0.36 g, 1.2 mmol), phosphorus pentasulfide (0.28 g, 1.3 mmol) and hexamethyldisiloxane (0.73 mL, 3.5 mmol) in 1,2-dichloroethane (3 mL) was heated at 100 °C for 1 h. The mixture was purified by column chromatography using DCM to yield 170 mg. This material was stirred in ammonia (7 M in MeOH) (7.4 mL, 52 mmol) at 50 °C for 3 days. The

product was purified by RP-HPLC to yield 71 mg (31%) of the title compound as the acetate salt. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.86 (s, 3 H) 3.07 (d, *J*=13.87 Hz, 1 H) 3.21 (d, *J*=13.87 Hz, 1 H) 3.26 - 3.32 (m, 1 H) 3.40 - 3.46 (m, 1 H) 3.88 (s, 3 H) 6.92 (dd, *J*=8.20, 2.21 Hz, 1 H) 7.13 (t, *J*=1.89 Hz, 1 H) 7.18 (d, *J*=7.88 Hz, 1 H) 7.34 (d, *J*=7.88 Hz, 1 H) 7.36 - 7.40 (m, 1 H) 7.45 (t, *J*=7.72 Hz, 1 H) 7.50 - 7.54 (m, 1 H) 7.56 (s, 1 H). MS (ES+) *m/z* 313 $[M+H]^+$.

4-(3'-Methoxybiphenyl-3-yl)-4-methyl-5,6-dihydro-4*H*-1,3-thiazin-2-amine (37). Compound **11b** (2 g, 8.8 mmol) was dissolved in dry 2-methyltetrahydrofuran (25 mL). The mixture was cooled to -78 °C before vinylmagnesium bromide (19.5 mL, 19.5 mmol) was added. The mixture was stirred for 1 h and was then allowed to reach rt. Saturated aqueous NH_4Cl solution was added and the mixture was extracted with EtOAc. The organic layer was washed with sat $NaHCO_3$ and brine. The combined organic phases were dried over $MgSO_4$, filtered and the solvent evaporated. The product was purified by column chromatography using EtOAc 10% to 50% in heptane to yield 1.85 g of **35**. This material was dissolved in acetic acid (30 mL). Thiourea (0.61 g, 8 mmol) and HCl (8 mL, 8 mmol) (1 M in acetic acid) were added. The solution was stirred at 45 °C for 16 h. The solvent was evaporated and the product crystallized from a mixture of MeOH and diethyl ether to yield 1.47 g of **36** (MS (ES+) *m/z* 313 $[M+H]^+$). A portion of this material (0.5 g) was taken up in TFA (5 mL, 65 mmol). Methanesulfonic acid (0.23 mL, 3.5 mmol) was added drop-wise during cooling on an ice bath. The solution was stirred at rt for 1 h. The solution was concentrated under reduced pressure. The residue was dissolved in DCM and washed with sat. $NaHCO_3$. The organic phase was filtered and the solvent evaporated. The product was purified by column chromatography using MeOH (with 7 M NH_3) 0% to 6% in DCM to yield 0.2 g (21%) of the title compound over several steps. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.45 (s, 3 H) 1.75 (ddd, *J*=13.79, 10.32, 3.63 Hz, 1 H) 2.08 (ddd,

J=13.40, 6.31, 3.31 Hz, 1 H) 2.55 (ddd, *J*=12.14, 10.40, 3.63 Hz, 1 H) 2.91 (ddd, *J*=12.22, 6.70, 3.78 Hz, 1 H) 3.82 (s, 3 H) 5.84 (br. s., 2 H) 6.93 (dd, *J*=8.20, 1.89 Hz, 1 H) 7.10 - 7.15 (m, 1 H) 7.18 (d, *J*=7.88 Hz, 1 H) 7.33 - 7.42 (m, 3 H) 7.47 (dt, *J*=7.09, 1.81 Hz, 1 H) 7.57 - 7.63 (m, 1 H). MS (ES+) *m/z* 313 $[M+H]^+$.

6-(4-Methoxyphenyl)-2-methyl-6-[3-(pyrimidin-5-yl)phenyl]-6*H*-pyrrolo[3,4d][1,3]thiazol-4-amine (41). A mixture of **40** (64 mg, 0.15 mmol), pyrimidine-5-ylboronic acid (29 mg, 0.23 mmol), [1,1'-bis(diphenylphosphino)ferrocene]palladium(II) chloride (6.4 mg, 7.8 μmol), potassium carbonate (43 mg, 0.31 mmol), DME (2 mL), H_2O (1 mL) and EtOH (0.33 mL) under an argon atmosphere was heated to 100 °C for 15 minutes using a microwave reactor. The reaction mixture was filtered through diatomaceous earth, which was washed with EtOAc and then the filtrate was evaporated. Purification by RP-HPLC yielded 27 mg (43%) of the title compound. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.75 (s, 3 H), 3.69 (s, 3 H), 6.77 (br s, 2 H), 6.81 - 6.87 (m, 2 H), 7.30 - 7.35 (m, 2 H), 7.43 - 7.53 (m, 2 H), 7.60 - 7.65 (m, 1 H), 7.69 - 7.72 (m, 1 H), 9.00 (s, 2 H), 9.19 (s, 1 H); LCMS (ES+) *m/z* 414 $[M+H]^+$.

4-(4-Methoxyphenyl)-2-methyl-4-(3-(pyrimidin-5-yl)phenyl)-4*H*-imidazo[1,5-b]pyrazol-6-amine (47). To a solution of **46** (15 mg, 0.04 mmol) and pyrimidine-5-ylboronic acid (5.4 mg, 0.04 mmol) in DME (0.6 mL)/water (0.3 mL) was added sodium carbonate (1 M aq) (0.091 mL, 0.09 mmol) followed by bis(triphenylphosphine)palladium(II) chloride (2.6 mg, 3.7 μmol) and the reaction heated at 80 °C for 1h 40 min. Water and DCM were added and the phases separated. The aq phase was extracted with DCM (3x). The combined organic phases were dried over Na_2SO_4, filtered and the solvent evaporated. The mixture was purified by column chromatography using MeOH 0% to 5% in DCM to yield 11 mg (75%) of the title product. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.29 (s, 3 H) 3.69 (s, 3 H) 6.80 - 6.86 (m, 3 H) 7.17 (br. s., 2

H) 7.42 - 7.49 (m, 3 H) 7.64 (t, *J*=8.35 Hz, 2 H) 7.83 (t, *J*=1.58 Hz, 1 H) 9.05 (s, 2 H) 9.19 (s, 1 H). MS (ES+) *m/z* 397 $[M+H]^+$.

(3*R,8a*S**)-3-(3-(5-Fluoropyridin-3-yl)phenyl)-3-methyl-3,5,6,7,8,8a-hexahydroimidazo[1,5-a]pyridin-1-amine and (3*R**,8a*R**)-3-(3-(5-fluoropyridin-3-yl)phenyl)-3-methyl-3,5,6,7,8,8a-hexahydroimidazo[1,5-a]pyridin-1-amine ((3*R**,8a*S**)-54 and (3*R**,8a*R**)-54).** *Rac*-3-(3-Bromophenyl)-3-methyl-3,5,6,7,8,8a-hexahydroimidazo[1,5-a]pyridin-1-amine (**53**) (14 mg, 50 μmol), 5-fluoropyridin-3-ylboronic acid (8 mg, 50 μmol) and bis(triphenylphosphine)palladium(II) chloride (2 mg, 2 μmol) were taken up in dimethoxy ethane (0.4 mL) and water (0.2 mL). Sodium carbonate (12 mg, 0.11 mmol) was added and the reaction was heated at 80 °C for 1.5 h. The cooled solution was filtered and purified by RP-HPLC to give (3*R**,8a*S**)-3-(3-(5-fluoropyridin-3-yl)phenyl)-3-methyl-3,5,6,7,8,8a-hexahydroimidazo[1,5-a]pyridin-1-amine (2.4 mg, 16 %) and (3*R**,8a*R**)-3-(3-(5-fluoropyridin-3-yl)phenyl)-3-methyl-3,5,6,7,8,8a-hexahydroimidazo[1,5-a]pyridin-1-amine (3.4 mg, 23 %).

(3*R**,8a*S**)-**54**:

^{1}H NMR (500 MHz, methanol-*d*4) δ ppm 8.65 (t, *J*=1.6 Hz, 1 H) 8.45 (d, *J*=2.5 Hz, 1 H) 7.88 (m, 1 H) 7.59 (m, 1 H) 7.48-7.53 (m, 2 H) 7.39 (dt, *J*=7.8, 1.2 Hz, 1 H) 3.08 (m, 1 H) 2.87 (dd, *J*=11.2, 2.7 Hz, 1 H) 2.00 (dd, *J*=11.9, 2.8 Hz, 1 H) 1.80 (m, 1 H) 1.74 (s, 3 H) 1.63 (td, *J*=11.2, 2.8 Hz, 1 H) 1.36-1.57 (m, 3 H) 1.10 (qt, *J*=12.9, 4.3 Hz, 1 H); MS (ES+) *m/z* 325 $[M+H]^+$.

(3*R**,8a*R**)-**54**:

^{1}H NMR (500 MHz, methanol-*d*4) δ ppm 8.67 (t, *J*=1.5 Hz, 1 H) 8.45 (d, *J*=2.5 Hz, 1 H) 7.90 (m, 1 H) 7.85 (t, *J*=1.5 Hz, 1 H) 7.67 (m, 1 H) 7.59 (m, 1 H) 7.48 (t, *J*=7.7 Hz, 1 H) 3.34 (m, 1 H) 2.78 (m, 1 H) 2.46 (td, *J*=11.2, 2.5 Hz, 1 H) 2.09 (dd, *J*=12.0, 2.5 Hz, 1 H) 1.92 (m, 1 H) 1.63 (m, 1 H) 1.51 (s, 3 H) 1.31-1.49 (m, 3 H); MS (ES+) *m/z* 325 $[M+H]^+$.

2-(3'-Methoxybiphenyl-3-yl)-2-methyl-2,5-dihydrothiazol-4-amine (58). Compound **11b** (0.85 g, 3.76 mmol) and methyl 2-mercaptoacetate (**55**) (1.7 mL, 19 mmol) were taken up in ammonia (7 M in MeOH) (10 mL, 70 mmol). The reaction was heated to 150 °C for 3 h by microwave heating. The product was purified by column chromatography using EtOAc 0% to 100% in heptane to yield 0.32 g of **56** (MS (ES+) *m/z* 300 $[M+H]^+$). This material and phosphorus pentasulfide (0.32 g, 0.71 mmol) were taken up in pyridine (1.5 mL). The reaction was heated to 120 °C for 1 h. The reaction mixture was cooled to rt and ammonia (7 M in MeOH) (6.0 mL, 42 mmol) was added. The reaction was stirred at 60 °C for 16 h. The product was purified by RP-HPLC to yield 34 mg (3%) of the title compound over several steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.03 (s, 3 H) 3.87 (s, 3 H) 3.89 - 4.01 (m, 2 H) 4.83 (br. s., 2 H) 6.90 (ddd, *J*=8.21, 2.53, 0.88 Hz, 1 H) 7.13 - 7.16 (m, 1 H) 7.18 - 7.21 (m, 1 H) 7.33 - 7.41 (m, 2 H) 7.42 - 7.48 (m, 2 H) 7.69 (t, *J*=1.77 Hz, 1 H). MS (ES+) *m/z* 299 $[M+H]^+$.

2-(4-Methoxyphenyl)-2-(3-(pyridin-3-yl)phenyl)-2,5-dihydrothiazol-4-amine (64). 1-Bromo-3-iodobenzene (**59**) (5 g, 18 mmol), pyridin-3-ylboronic acid (2.4 g, 19 mmol) and bis(triphenylphosphine)palladium(II) chloride (0.62 g, 0.88 mmol) were taken up in DME (30 mL) and water (15 mL). Sodium carbonate (1 M) (44 mL, 44 mmol) was added and the reaction was stirred at 80 °C for 2 h. The reaction mixture was extracted with EtOAc. The combined organic phases were dried over $MgSO_4$, filtered and the solvents evaporated. The product was purified by column chromatography using EtOAc 0% to 50% in heptane to yield 2.1 g of **60** (MS (ES+) *m/z* 234, 236 $[M+H]^+$). This material was dissolved in Et_2O (40 mL) and THF (10 mL) and cooled to -78ºC. *n*-BuLi (1.6 M in hexanes) (5.7 mL, 9.1 mmol) was added and the solution stirred for 30 min. 4-Methoxybenzonitrile (1.2 g, 9.1 mmol) was added in Et_2O (20 mL) at -78ºC and the reaction was allowed to warm to room temperature over 30 min. Ammonium acetate

(0.70 g, 9.1 mmol) dissolved in MeOH (20 mL) was added. The solvents were evaporated and the residue dissolved in DCM and water. The phases were separated and the aqueous phase was extracted with DCM. The combined organic phase were washed with brine and dried over $MgSO_4$, filtered and the solvent evaporated to yield 3 g of **61** (MS (ES+) *m/z* 289 $[M+H]^+$). This material and methyl 2-mercaptoacetate (0.93 mL, 10 mmol) were mixed and stirred at rt for 16 h. Dimethylamine hydrochloride (45 mg, 0.55 mmol) was added and the reaction was stirred for 3 days. The mixture was taken up in DCM and MeOH and evaporated onto silica. The product was isolated by column chromatography using EtOAc (with 10% MeOH) 0% to 100% in heptane to yield 0.1 g of **62** (MS (ES+) *m/z* 363 $[M+H]^+$). This material and phosphorus pentasulfide (0.1 g, 0.23 mmol) were taken up in pyridine (2 mL). The reaction was stirred at 120 °C for 1 h. Ammonia (7 M in MeOH) (1.6 mL, 11 mmol) was added and the reaction was stirred at 60 °C for 16 h. The solvent was evaporated. The product was purified by RP-HPLC followed by column chromatography using EtOAc (with 10% MeOH) 0% to 100% in heptane to yield 17 mg (0.26%) of the title compound over several steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.79 (s, 3 H) 3.97 (s, 2 H) 5.29 (br. s., 2 H) 6.81 - 6.86 (m, 2 H) 7.34 (ddd, *J*=7.83, 4.80, 0.76 Hz, 1 H) 7.38 - 7.45 (m, 4 H) 7.45 - 7.51 (m, 1 H) 7.72 (t, *J*=1.64 Hz, 1 H) 7.82 - 7.89 (m, 1 H) 8.57 (dd, *J*=4.80, 1.52 Hz, 1 H) 8.82 (dd, *J*=2.40, 0.63 Hz, 1 H). MS (ES+) *m/z* 362 $[M+H]^+$.

2-(3'-Methoxybiphenyl-3-yl)-2-(pyridin-4-yl)-2,5-dihydrothiazol-4-amine (65). 3-Bromobenzonitrile (2 g, 11 mmol), 3-methoxyphenylboronic acid (1.8 g, 12 mmol) and bis(triphenylphosphine)palladium(II) chloride (0.39 g, 0.55 mmol) were taken up in DME (20 mL) and water (10 mL). Sodium carbonate (1 M) (27 mL, 27 mmol) was added and the reaction was heated to 80 °C for 2 h. The reaction mixture was extracted with DCM. The combined organic phases were dried over $MgSO_4$, filtered and the solvents evaporated. The product was

purified by column chromatography using EtOAc 0% to 100% in DCM to yield 2.1 g of 3'-methoxybiphenyl-3-carbonitrile (MS (APCI+) *m/z* 210 $[M+H]^+$). This material was dissolved in THF (40 mL) and added to a solution of 4-bromopyridine (1.6 g, 9.9 mmol), made fresh by washing a solution of 4-bromopyridinium chloride in DCM with sat. Na_2CO_3 and drying over $MgSO_4$, filtering and evaporating the solvent, dissolved in Et_2O (40 mL) and cooled to -78ºC. *n*-BuLi (1.6 M in hexanes) (6.2 mL, 10 mmol) had previously been added and the solution stirred for 30 min. The 3'-methoxybiphenyl-3-carbonitrile (2.1 g) was added at -78 °C and the reaction was stirred for 30 min before allowing it to warm to room temperature over 30 min. Ammonium acetate (0.77 g, 9.9 mmol) dissolved in MeOH (20 mL) was added. The solvents were evaporated and the residue dissolved in DCM and water. The phases were separated and the aqueous phase was extracted with DCM. The combined organic phase were washed with brine and dried over $MgSO_4$, filtered and the solvent evaporated to yield 2.6 g of (3'-methoxybiphenyl-3-yl)(pyridin-4-yl)methanimine (MS (APCI+) *m/z* 289 $[M+H]^+$). A portion of this material (1.5 g) and methyl 2-mercaptoacetate (0.47 mL, 5.3 mmol) were mixed and stirred at rt for 16 h. The mixture was taken up in DMF and MeOH. The product was isolated by RP-HPLC. The product was purified by column chromatography using EtOAc/Acetone 1:1 0% to 100% in heptane to yield 68 mg of 2-(3'-methoxybiphenyl-3-yl)-2-(pyridin-4-yl)thiazolidin-4-one (MS (ES+) *m/z* 363 $[M+H]^+$). This material and phosphorus pentasulfide (68 mg, 0.15 mmol) were taken up in pyridine (1 mL). The reaction was stirred at 120 °C for 1 h. Ammonia (7 M in MeOH) (1 mL, 7 mmol) was added and the reaction was stirred at 60 °C for 16 h. The solvents were evaporated and the product purified by RP-HPLC to yield 17 mg (0.7%) of the title compound over several steps. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.80 (s, 3 H) 3.90 (s, 2 H) 6.70 (br. s., 2 H) 6.95 (dt, *J*=8.15, 1.23 Hz, 1 H) 7.06 - 7.09 (m, 1 H) 7.12 (m, *J*=0.76 Hz, 1 H) 7.35 - 7.41 (m, 5 H)

7.51 (m, *J*=5.40, 3.50, 2.00 Hz, 1 H) 7.68 (d, *J*=1.01 Hz, 1 H) 8.42 - 8.51 (m, 2 H). MS (ES+) *m/z* 362 $[M+H]^+$.

1-Ethyl-2-methyl-2-(3-(pyridin-3-yl)phenyl)-2,5-dihydro-1*H*-imidazol-4-amine (71). Compound **68** (4.1 g, 18 mmol) and phosphorus pentasulfide (1.9 g, 4.4 mmol) were taken up in dioxane (20 mL). The reaction was stirred for 2 h at 95 °C by microwave heating. The reaction mixture was poured into 50% brine and extracted with DCM. The combined organic phases were dried over $MgSO_4$, filtered and the solvents evaporated. The product was purified by column chromatography using EtOAc 0% to 50% in DCM to yield 1.5 g of **69** (MS (ES+) *m/z* 253 $[M+H]^+$). This material was taken up in hydrobromic acid (33% in acetic acid) (15 mL, 86 mmol). The reaction was stirred at rt for 20 min. The reaction mixture was slowly added to stirring diethyl ether (75 mL). The precipitate was isolated by suction filtration to yield 1.3 g of **70** (MS (ES+) *m/z* 119 $[M+H]^+$). A portion of this material (0.3 g) was added, one third at a time to a solution of **11c** (100 mg, 0.51 mmol) in ammonia (7 M in MeOH) (2.9 mL, 20 mmol). The reaction was heated at 60 °C and the reaction was stirred for 1 h between each addition. The product was purified by column chromatography using MeOH (with 7 M NH_3) 0% to 10% in DCM to yield 17 mg (12%) of the title compound at 94% purity. ^{1}H NMR (500 MHz, MeOD) δ ppm 1.05 (t, *J*=7.09 Hz, 3 H) 1.66 (s, 3 H) 2.31 (dq, *J*=11.66, 7.25 Hz, 1 H) 2.59 (dq, *J*=11.86, 7.08 Hz, 1 H) 3.57 - 3.74 (m, 2 H) 7.46 - 7.60 (m, 4 H) 7.74 (s, 1 H) 8.09 (dt, *J*=7.88, 1.73 Hz, 1 H) 8.52 (dd, *J*=4.73, 1.26 Hz, 1 H) 8.79 (d, *J*=2.21 Hz, 1 H). MS (ES+) *m/z* 281 $[M+H]^+$.

1-(4-Amino-2-(3'-methoxybiphenyl-3-yl)-2-methyl-2,5-dihydro-1*H*-imidazol-1-yl)ethanone (77). Sodium bicarbonate (12 g, 0.14 mol) was dissolved in water (60 mL) and cooled to 0 °C. Dioxane (60 mL) was added followed by 2-aminoacetamide hydrochloride (**72**) (8 g, 72 mmol). Benzyl chloroformate (10 mL, 72 mmol) was added drop-wise. The reaction was

stirred at rt for 16 h. The formed precipitate was collected by suction filtration and washed with water to yield 6.5 g of **73**. The filtrate was concentrated and the precipitate collected by suction filtration and washed with water to yield another 5.8 g of **73**. These materials were taken up in dioxane (90 mL) and phosphorus pentasulfide (6.6 g, 15 mmol) was added. The reaction was stirred for 1 h at 95 °C. The reaction mixture was poured into 50% brine and extracted with DCM. The combined organic phases were dried over $MgSO_4$, filtered and the solvents evaporated. The product was purified by column chromatography using EtOAc 0% to 50% in DCM to yield 2.2 g of **74** (MS (ES+) *m/z* 253 $[M+H]^+$). A portion of this material (0.33 mg) was taken up in hydrobromic acid (33% in acetic acid) (3.8 mL, 22 mmol). The reaction was stirred at rt for 20 min. The precipitate was isolated by suction filtration and washed with diethyl ether to yield 0.33 mg of **75**. This material and **11b** (0.14 g, 0.63 mmol) were dissolved in ammonia (7 M in MeOH) (3.6 mL, 25 mmol) and stirred at rt for 16 h. The product was purified by RP-HPLC to yield 45 mg of **76** (MS (ES+) *m/z* 282 $[M+H]^+$). This material was dissolved in DCM (2 mL). Et_3N (0.044 mL, 0.32 mmol) was added followed by acetic anhydride (0.029 mL, 0.32 mmol). The reaction was stirred at rt for 16 h. Ammonia (7 M in MeOH) (0.9 mL, 6.3 mmol) was added and the reaction was stirred at rt for 6 days. The product was purified by column chromatography using MeOH (with 7 M NH_3) 0% to 10% in DCM to yield 30 mg (13%) of the title compound, as the acetate salt, over several steps. Rotamers present: (Major) ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.75 (s, 3 H) 2.01 (s, 3 H) 3.85 (s, 3 H) 4.42 - 4.60 (m, 1 H) 4.96 (br. s., 1 H) 5.70 - 6.02 (m, 1 H) 6.90 (dd, *J*=8.20, 2.21 Hz, 1 H) 7.06 - 7.10 (m, 1 H) 7.11 - 7.16 (m, 1 H) 7.27 - 7.30 (m, 1 H) 7.31 - 7.44 (m, 3 H) 7.50 (dd, *J*=7.72, 1.10 Hz, 1 H) 7.51 - 7.55 (m, 1 H).

(Minor) ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 2.01 (s, 3 H) 2.04 (s, 3 H) 3.84 (s, 3 H) 4.31 - 4.40 (m, 1 H) 4.96 (br. s., 1 H) 5.70 - 6.02 (m, 1 H) 6.87 (dd, *J*=7.88, 2.21 Hz, 1 H) 7.06 - 7.10 (m, 1 H) 7.11 - 7.16 (m, 1 H) 7.31 - 7.44 (m, 5 H) 7.51 - 7.55 (m, 1 H). MS (ES+) *m/z* 324 $[M+H]^+$.

2-(4-Methoxyphenyl)-2-(3-(pyrimidin-5-yl)phenyl)-3,4-dihydro-2*H*-pyrrol-5-amine (83). Compound **82** (100 mg, 0.29 mmol) was dissolved in pyridine (1 mL). Phosphorus pentasulfide (100 mg, 0.22 mmol) was added and the reaction was stirred at 120 °C for 1 h. The reaction was cooled to rt, ammonia (7 M in MeOH) (1.7 mL, 12 mmol) and *tert*-butyl hydroperoxide (70% in water) (0.6 mL, 4.3 mmol) was added. The reaction was stirred at rt for 16 h. The solvents were evaporated and the product purified by column chromatography using MeOH (with 7 M NH_3) 0% to 10% in DCM to yield 49 mg (49%) of the title compound. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 2.53 - 2.76 (m, 4 H) 3.76 (s, 3 H) 4.60 (br. s., 2 H) 6.81 (d, *J*=8.51 Hz, 2 H) 7.30 (d, *J*=8.51 Hz, 2 H) 7.35 - 7.49 (m, 3 H) 7.61 (s, 1 H) 8.90 (s, 2 H) 9.15 (s, 1 H). MS (ES+) *m/z* 345 $[M+H]^+$.

4-Methoxy-2-(4-methoxyphenyl)-2-(3-(pyridin-3-yl)phenyl)-3,4-dihydro-2*H*-pyrrol-5-amine (91). A mixture of **90** (0.25 g, 0.67 mmol) and phosphorus pentasulfide (0.24 g, 0.53 mmol) in pyridine (15 mL) was heated at 115 °C for 50 minutes. To the reaction mixture was added ammonia (7 M in MeOH) (9.6 mL, 67 mmol) and *tert*-butyl hydroperoxide (70% in water) (0.96 mL, 10 mmol). The reaction was stirred at ambient temperature for 60 h. Water and DCM were added and the layers were separated. The aqueous phase was extracted with DCM (3 x 70 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered and the solvent evaporated. The residue was purified by column chromatography using MeOH (with 7 M ammonia) 0% to 10% in DCM to yield 0.19 g (76%) of the title compound at 90% purity. ^{1}H NMR (500 MHz, MeCN-d_3) δ ppm 1.97 (d, *J*=3.78 Hz, 1 H) 2.28 (dd, *J*=12.61, 8.20 Hz, 1 H)

3.26 (dd, *J*=12.61, 6.94 Hz, 1 H) 3.34 (d, *J*=1.89 Hz, 3 H) 3.73 (d, *J*=4.73 Hz, 1 H) 4.33 (dd, *J*=8.20, 6.94 Hz, 1 H) 5.02 (br. s., 2 H) 6.77 - 6.85 (m, 2 H) 7.29 - 7.33 (m, 1 H) 7.34 - 7.50 (m, 5 H) 7.59 - 7.74 (m, 1 H) 7.92 (tt, *J*=7.68, 1.93 Hz, 1 H) 8.54 (dd, *J*=4.73, 1.58 Hz, 1 H) 8.79 (dd, *J*=7.57, 1.89 Hz, 1 H). MS (ES+) *m/z* 374 $[M+H]^+$.

The diastereomers of *rac*-**91** were separated by chiral SFC.

Isomer 1 (eluted first)

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.18 - 2.28 (m, 1 H), 3.16 - 3.24 (m, 1 H), 3.29 (s, 3 H), 3.68 (s, 3 H), 4.24 (s, 1 H), 5.96 - 6.16 (m, 2 H), 6.75 - 6.83 (m, 2 H), 7.29 - 7.41 (m, 3 H), 7.43 - 7.53 (m, 3 H), 7.76 (t, *J*=1.64 Hz, 1 H), 7.98 (d, *J*=0.76 Hz, 1 H), 8.56 (dd, *J*=4.80, 1.52 Hz, 1 H), 8.81 (dd, *J*=2.27, 0.76 Hz, 1 H). MS (ES+) *m/z* 374.2 $[M+H]^+$. *de* 100%.

Isomer 2 (eluted second)

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.23 (dd, *J*=12.63, 8.08 Hz, 1 H), 3.20 (dd, *J*=12.51, 6.95 Hz, 1 H), 3.29 (s, 3 H), 3.67 (s, 3 H), 4.24 (t, *J*=7.45 Hz, 1 H), 5.92 - 6.16 (m, 2 H), 6.74 - 6.83 (m, 2 H), 7.29 - 7.41 (m, 3 H), 7.43 - 7.53 (m, 3 H), 7.76 (t, *J*=1.64 Hz, 1 H), 7.94 - 8.03 (m, 1 H), 8.56 (dd, *J*=4.80, 1.52 Hz, 1 H), 8.81 (dd, *J*=2.27, 0.76 Hz, 1 H). MS (ES+) *m/z* 374.2 $[M+H]^+$. *de* 94.3%

Isomer 3 (eluted third)

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.22 (dd, *J*=12.76, 7.71 Hz, 1 H), 3.21 (dd, *J*=12.76, 6.95 Hz, 1 H), 3.27 (s, 3 H), 3.65 - 3.70 (m, 3 H), 4.26 (t, *J*=7.33 Hz, 1 H), 6.07 (br. s., 2 H), 6.77 - 6.83 (m, 2 H), 7.32 - 7.50 (m, 6 H), 7.65 (t, *J*=1.64 Hz, 1 H), 7.94 - 8.00 (m, 1 H), 8.55 (dd, *J*=4.67, 1.64 Hz, 1 H), 8.79 (d, *J*=1.77 Hz, 1 H). MS (ES+) *m/z* 374.2 $[M+H]^+$. *de* 100%.

Isomer 4 (eluted forth)

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.23 (dd, *J*=12.76, 7.71 Hz, 1 H), 3.22 (dd, *J*=12.88, 7.07 Hz, 1 H), 3.28 (s, 3 H), 3.69 (s, 3 H), 4.27 (t, *J*=7.33 Hz, 1 H), 5.99 - 6.14 (m, 2 H), 6.79 - 6.83 (m, 2 H), 7.33 - 7.51 (m, 6 H), 7.66 (t, *J*=1.64 Hz, 1 H), 7.96 - 8.00 (m, 1 H), 8.56 (dd, *J*=4.67, 1.64 Hz, 1 H), 8.80 (dd, *J*=2.40, 0.63 Hz, 1 H). MS (ES+) *m/z* 374.1 $[M+H]^+$. *de* 98.2%.

4-(3'-Methoxybiphenyl-3-yl)-4,5-dimethyl-4*H*-imidazol-2-amine (98). Compound **95** (0.89 g, 3.7 mmol) was taken up in DCM (10 mL). *O*-(9*H*-fluoren-9-yl)methyl carbonisothiocyanatidate (1.0 g, 3.7 mmol) was added and the reaction was stirred at rt for 2 h. The solvent was evaporated to yield intermediate **96**. Ammonia (7 M in MeOH) (32 mL, 1.5 mol) was added and the reaction was stirred at rt for 2 h. *tert*-Butyl hydroperoxide (7.5 mL, 55 mmol) was added and the reaction was stirred for 3 days. The product was isolated by RP-HPLC and purified by column chromatography using MeOH (with 7 M NH_3) 0% to 10% in DCM to yield 64 mg of **97** (MS (ES+) *m/z* 266, 268 $[M+H]^+$). This material was taken up in DME (1 mL) and water (0.5 mL). 3-Methoxyphenylboronic acid (35.8 mg, 0.24 mmol), bis(triphenylphosphine)palladium(II) chloride (15 mg, 0.02 mmol) and sodium carbonate (1 M) (0.54 mL, 0.54 mmol) were added and the reaction was heated to 80 °C for 2 h. The reaction mixture was filtered and extracted with DCM. The combined organic phases were dried over $MgSO_4$, filtered and the solvent evaporated. The product was purified by column chromatography using MeOH (with 7 M NH_3) 0% to 10% in DCM to yield 44 mg (4%) of the title compound over several steps. ^{1}H NMR (500 MHz, MeOD) δ ppm 1.71 (s, 3 H) 2.14 (s, 3 H) 3.82 (s, 3 H) 6.90 (dd, *J*=8.20, 2.21 Hz, 1 H) 7.08 (t, *J*=2.05 Hz, 1 H) 7.12 (d, *J*=7.57 Hz, 1 H) 7.26 (d, *J*=7.88 Hz, 1 H) 7.32 (t, *J*=7.88 Hz, 1 H) 7.40 (t, *J*=7.88 Hz, 1 H) 7.44 (t, *J*=1.58 Hz, 1 H) 7.50 (d, *J*=7.57 Hz, 1 H). MS (ES+) *m/z* 294 $[M+H]^+$.

BACE-1 TR-FRET assay. The soluble part of human *β*-secretase (AA1-AA460) diluted in reaction buffer (Na-acetate, chaps, triton x-100, EDTA pH4.5) was mixed with the test compound diluted in DMSO. After a pre-incubation period of 10 minutes substrate, (Europium)CEVNLDAEFK(Qsy7), was added and the reaction allowed to proceed for 15 min at RT. The reaction was stopped by addition of 7 µL Na-acetate, pH 9. The fluorescence of the product was measured on a Victor II plate reader with an excitation wavelength of 340 nm and an emission wavelength of 615 nm. The final concentration of the enzyme was 2.7 µg/mL; the final concentration of substrate was 100 nM. Reported values are means of n≥2 determinations, standard deviation up to 10%.

Cell sAPP*β* release assay. SH-SY5Y cells (human neuroblastoma cell line) were cultured in DMEM /F-12 with Glutamax, 10% FCS and 1% non-essential amino acids. The test compound was incubated with cells for 16 h at 37°C, 5% CO_2. Meso Scale Discovery (MSD) plates were used for the detection of sAPP*β* release. MSD sAPP*β* plates were blocked in 3% BSA in Tris wash buffer for 1 h at RT and washed 4 times in Tris buffer. After incubation, 20 µL of medium was transferred to the pre-blocked and washed 384 well MSD sAPP*β* microplate, incubated with shaking at RT for 2 h followed by washing 4 times in Tris buffer. 10 µL detection antibody was added (1 nM) followed by incubation with shaking in RT for 2 h followed by washing 4 times in Tris buffer. 40 µL Read Buffer was added per well and the plates were read in a SECTOR Imager. In addition, the cells incubated with test compound were further lyzed and analyzed for any cytotoxic effects of the compounds using the ViaLight™ Plus cell proliferation/cytotoxicity kit (Cambrex BioScience) according to the manufacturer's instructions. Reported values are means of n≥2 determinations, standard deviation up to 10%.

Permeability Assays. Caco-2 cells were grown for 14-21 days to achieve confluency and polarization before being used for transport experiments. For both, apical to basolateral (A–B) and basolateral to apical (B–A) transport directions, the pH was adjusted to 7.4. All compounds were investigated at a concentration of 10 μM. Buffer volumes in the 24 well plates were 0.20 mL on the apical side and 0.80 mL on the basolateral side. Samples were withdrawn after 30 or 60 minutes. The integrity of the epithelial cell monolayer was monitored by measuring the passive trans membrane diffusion of [^{14}C]mannitol. Concentrations of compounds in donor and receiver samples were analyzed by liquid chromatography tandem mass spectrometry. Liquid scintillation was used for analysis of [^{14}C]mannitol. The apparent permeability coefficient P_{app} was calculated according to $P_{app} = (dQ/dt)/(A*C0)$, where dQ/dt is the slope at 30 or 60 minutes of the graph of the cumulative amount transported vs time, A is the surface area of the membrane, and C0 is the starting concentration.

MDCK-MDR1 efflux data were generated using a transwell assay as described by LeDonne et al.[62] with minor adaptations. Cells were sown at 400,000 – 600,000 cells per well in a 96-well plate and cultured for 3-5 days. Tested at 1 μM compound concentration the efflux incubation time was 2.5 hours; this was shortened to 60 minutes when the compound concentration was 10 μM. In each format the compounds were classified as low, medium or high efflux.

Plasma protein and brain tissue binding. Brain and plasma exposure, Plasma protein and brain tissue binding were performed as previously described.[63] Compounds were added to mouse plasma to a final concentration of 10 μM. An aliquot of 180 μL was transferred to a dialysis plate with phosphate buffer on the other site and incubated for 18 h at 37 ^{o}C. Proteins were removed from aliquots (50 μL) of plasma and buffer samples, internal standard added, and analyzed with LC-MS/MS. The unbound fraction in plasma was calculated from the ratio of the

MS-area of compound in buffer divided by sum of the areas of compound in buffer and plasma. Recovery and stability in plasma were controlled for.

To determine brain tissue binding, 300 μm thick coronal rat brain slices were incubated for 5 h in 10 mL of an artificial interstitial fluid buffer containing 1 μM of compound. Following the incubation, slices were weighted and homogenized, proteins were removed and LC-MS/MS analysis was performed. The unbound fraction in brain was calculated as previously described.[64,65]

Mice brain exposure. All animal experimentations were performed in accordance with relevant guidelines and regulations provided by the Swedish Board of Agriculture. The ethical permissions were provided by an ethical board specialized in animal experimentations, by the Stockholm Norra Animal Research Ethical Board.

To study brain exposure, female C57BL/6 mice purchased from Harlan Laboratories, The Netherlands, were sacrificed 1.5 hours after an intravenous (iv) or per oral (po) dose of 30 μmol/kg (dose volume 4 mL/kg) of compounds **24a** (iv) and **37** (po) or 50 μmol/kg of **41** (po) all with 5% dimethylacetamide and 0.1 M gluconic acid or 30 μmol/kg of **64** (iv) with 0.1 M gluconic acid, 3 mice per compound respectively. Animals were anaesthetized before plasma and brain samples were collected. Plasma was isolated from blood collected by cardiac puncture into EDTA tubes and was centrifuged for 10 minutes at approximately 3000g at 4 °C within 20 minutes of sampling. Animals were then sacrificed by decapitation to collect brains.

Briefly, whole brains of mice were homogenized in 2 volumes (w/v) of Ringer solution. Aliquots of plasma (25 μL) and brain homogenate (50 μL) were precipitated with 150 μL acetonitrile containing a generic internal standard (200 nmol/L warfarin). Samples were mixed, centrifuged, appropriately diluted with mobile phase and analyzed on a LC-MS/MS system.

Brain exposure was corrected for compound exposure in plasma/blood present in the brain sample, since no brain perfusion was performed, by assuming 1.3% blood/plasma in brain. The total plasma and brain concentrations were converted to free concentrations by multiplying with the unbound fraction and the ratio of free concentration in brain and plasma Cu,br/Cu,pl were calculated.

Chemical stability assay. 100 μM compound solutions in 0.1 M phosphate buffer at pH 1, 7.4 and 9 as well in FRET buffer (pH 4.5) in presence of 5% DMSO and in pure methanol and DMSO. Samples were stored at room temperature in the dark at 37 °C and gently shaked (~300 rpm). The samples were analyzed after 24 h and 96 h (~4 days).

Protein Crystallography.

Protein expression, purification and crystallization. Human BACE, CID1328 14-453, was cloned, expressed, refolded, activated and purified according to previously published protocols.[66] The protein buffer was exchanged to 20 mM Tris pH 8.5, 150 mM NaCl and concentrated to 3.5 mg/mL. Concentrated protein was mixed 1:1 with a stock of 11% PEG6k, 100 mM Na acetate pH 5.0 at RT and crystallized using vapor diffusion techniques in combination with seeding. The crystals were soaked with 10 mM of compounds **19b**, **41**, **83**, and **91** in 10% DMSO, 18% PEG6000, 90 mM Na acetate pH 4.85, 18 mM Tris pH 8.5 and 135 mM NaCl for 24 hours and flash frozen in liquid nitrogen using 20% glycerol as a cryoprotectant.

Data collection and refinement. Data of soaked crystals were collected on a Rigaku FR-E+ SuperBright rotating anode and a HTC imaging plate to resolutions between 1.5-1.8 Å. All data were indexed and integrated with MOSFLM[67] and scaled with SCALA[68] in space group P212121, with cell dimensions of about [48,76,105], giving a Matthews coefficient of 2.2 $Å^3$ / Da with one monomer per asymmetric unit. The compound **19b**, **41**, **83** and **91** structures were

solved by rigid body refinement of a previously determined BACE-1 structure based on the published 1FKN structure[57] using Refmac5.[69] The initial models were further refined by alternative cycles of model rebuilding in Coot[70] and refinement in Refmac5 and AutoBuster.[50,68]

The four compounds were placed in the refined omit maps by Flynn.[71] Final refinement of the BACE-inhibitor complexes was performed in Refmac5 and AutoBuster. Resulting 2Fo-Fc maps of can be seen in Figures 6a-d. Full data collection and refinement statistics can be found in Supporting information, Table S1.

ABBREVIATIONS

BACE, beta-site APP-Cleaving Enzyme; AD, Alzheimer's disease; Aβ, β-amyloid; APP, amyloid-β precursor protein; cPr, cyclopropyl; sAPPβ, soluble amyloid-β precursor protein; SAR, structure-activity relationship; pTSA, 4-methylbenzenesulfonic acid ; PSA, polar surface area; ER, efflux ratio; PD, pharmacodynamic; DEA, diethylamine; TEA, triethylamine; IPA, isopropanol; iv, intravenous; P-gp, permeability glycoprotein; RP, reverse-phase; HMDO, hexamethyldisiloxane; hERG, human ether-a-go-go related gene, DMPK, Drug metabolism and pharmacokinetics; R&D, Research and development; μM, micromolar (micromoles per liter); nM, nanomolar (nanomoles per liter); PS1, Presenilin 1; Asp, aspartatic acid; CSF, Cerebral spine fluid; CNS, Central nervous system; DIPEA, diisopropyl ethylamine; TR-FRET, Time-resolved fluorescence energy transfer; CHO, Chinese hamster ovary; MDCK, Madin Darby canine kidney; nd, not determined; IC_{50}, Concentration at 50% inhibition; Perm. , Permeability; LE, Ligand efficiency; trp, tryptophan; tyr, tyrosine; f_u, Fraction unbound; LC, Liquid chromatography; ES, Electro spray; APCI, atmospheric pressure chemical ionization; SFC, Supercritical fluid chromatography; PDA, Photodiode array; mg, milligram; sat., saturated;

DMEM, Dulbeccos modified Eagle's medium; MSD, Meso Scale Discovery; BSA, Bovine Serum Albumin; Tris, 2-Amino-2-hydroxymethyl-propane-1,3-diol; PEG, Polyethyleneglycol; PDB, Protein Data Bank

ACKNOWLEDGMENT

We thank Jörg Holenz and Juliette Janson for valuable proofreading of the manuscript. We thank the Physiochemical Characterization Team and the Analytical & Purification Sciences Team at the Department of Medicinal Chemistry at AstraZeneca Södertälje for physiochemical characterization, enantiomer separations and purity determinations. We thank Elin Hagström and Johan Meuller for FRET and sAPPβ measurements. We thank Carolina Sandman and Carrie Tsoi for Caco-2 measurements, Sara Rohdin and Jenny Johansson for doing the *in vivo* experiment respectively bioanalysis and also Maria Sandberg-Ställ for the determination of free fraction in brain. In addition, we thank Anna Aagaard for crystallization and Safety Assessment screening centre at AstraZeneca Alderly Park for hERG assessments.

ASSOCIATED CONTENT

Supporting Information. Synthetic procedures and analytical data for intermediates **2-(3-bromophenyl)-2-ethyl-2*H*-benzo[e][1,3]oxazin-4(3*H*)-one, 11a, 11b, 11c, 16, 18, 26, 28, 29, 32, 33, 39, 40, 43, 44, 45, 46, 49, 50, 51, 52, 53, 67, 68, 79, 82, 84, 88, 89, 90, 95** and Table S1 and Table S2 can be found free of charge via the Internet at http://pubs.acs.org.

PDB ID Codes. New protein/ligand coordinates for **19b**, **41**, **83** and **91** have been deposited in the PDB with IDs of 4b70, 4b72, 4b77, 4b78, respectively.

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

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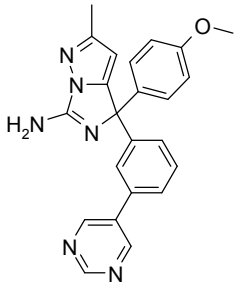
Table of Contents Graphic

Focus on finding promising cores



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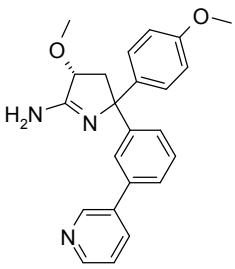

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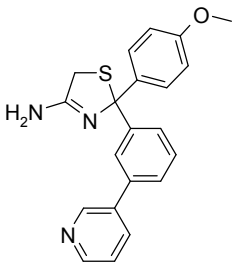

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BACE-1 cellular potency (nM)