Letter

Evaluation of a series of BACE 1 inhibitors containing novel het-eroaryl-fused-piperazine amidine warheads

Daniel Oehlrich, Aldo Peschiulli, Gary Tresadern, Michiel Van Gool, Juan Antonio Vega, Ana Isabel de Lucas, Sergio A. Alonso de Diego, Hana Propkopcova, Nigel Austin, Sven Van Brandt, Michel Surkyn, Michel De Cleyn, Ann Vos, Frederik J.R. Rombouts, Gregor J. Macdonald, Diederik Moechars, Harrie J.M. Gijsen, and Andrés A. Trabanco

ACS Med. Chem. Lett., **Just Accepted Manuscript** • DOI: 10.1021/acsmedchemlett.9b00181 • Publication Date (Web): 02 Jul 2019

Evaluation of a series of BACE 1 inhibitors containing novel heteroaryl-fused-piperazine amidine warheads

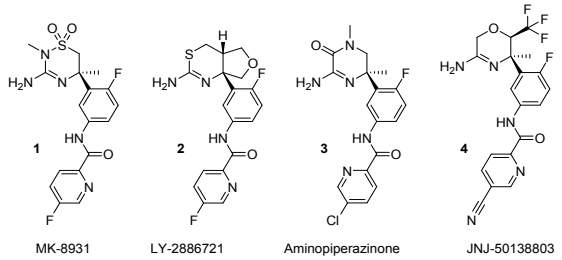
Daniel Oehlrich,*† Aldo Peschiulli,† Gary Tresadern,# Michiel Van Gool,‡ Juan Antonio Vega,‡ Ana Isabel De Lucas,‡ Sergio A. Alonso de Diego,‡ Hana Prokopcova,† Nigel Austin,# Sven Van Brandt,† Michel Surkyn,† Michel De Cleyn,† Ann Vos,# Frederik J. R. Rombouts,† Gregor Macdonald,† Dieder Moechars,§ Harrie J.M. Gijsen,† and Andrés A. Trabanco *‡.

†Medicinal chemistry; #Discovery Sciences, §Neuroscience biology, Janssen Research & Development, Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340, Beerse, Belgium.
‡Discovery Sciences Medicinal Chemistry, Janssen Research & Development, Janssen−Cilag S.A., C/Jarama 75A, 45007, Toledo, Spain.
KEYWORDS: BACE inhibitor, β-secretase 1, Alzheimer's disease, cyclic sulfamidates, pKa.

ABSTRACT: Despite several years of research, only a handful of BACE 1 inhibitors have entered clinical trials as potential therapeutics against Alzheimer's disease. The intrinsic basic nature of low MW, amidine-containing BACE 1 inhibitors makes them far from optimal as CNS drugs. Herein we present a set of novel heteroaryl-fused piperazine amidine inhibitors designed to lower the basicity of the key, enzyme binding, amidine functionality. This study resulted in the identification of highly potent ($IC_{50} \leq 10$ nM), permeable lead compounds with a reduced propensity to suffer from Pgp-mediated efflux.

Genetics has suggested β-secretase 1 (BACE 1) as a key target to attenuate the formation of amyloid beta (Aβ) in Alzheimer's disease (AD).[1] Since the discovery of BACE 1 in 1999,[2] many potent BACE 1 inhibitors have been described,[3] but relatively few successfully combine in vitro potency and the necessary pharmacokinetic (PK) properties/parameters to achieve in vivo efficacy. The amidine class of BACE 1 inhibitors have become the main focus.[3,4] and contain a protonated amidine warhead necessary for an optimal interaction with the aspartyl catalytic dyad of BACE 1.[3] The high intrinsic basicity of amidine heterocycles presents multiple liabilities for their development, including high Pgp efflux and hERG inhibition, and low permeability. Several compounds have unfortunately failed clinical trials, including MK-8931 (**1**)[5] and LY-2886721 (**2**) (Figure 1).[6]

Figure 1. Representative BACE 1 inhibitors.



Our interest in amidine BACE 1 inhibitors can be traced back to early reports of aminodihydroquinazolines.[7] Since then we have been dedicated to identifying novel warheads with adequate substitution to control the pKa of the guanidine/amidine and as such balance the liabilities with in vivo efficacy. Thus, the challenge of BACE 1 inhibition is revealed: liabilities are inherent to the amidine, yet it is crucial for key binding motifs. To be capable of identifying BACE 1 clinical candidates, there is a need to efficiently synthesize and evaluate many novel but synthetically challenging amidine containing heterocycles. Here we describe a convergent approach to meet this goal.

We have previously described our approach to assess new candidate warheads with computational approaches leading to series of cyclic amidines,[8], acylguanidines,[9], and aminopiperazinones such as **3** (Figure 1).[10] The aminopiperazinones suffered from poor brain penetration which halted their progress. However, they served as inspiration for further research, such as a series of aminomorpholine containing BACE 1 inhibitors. The morpholine series, exemplified by JNJ-50138803, (**4**) (Figure 1) was also supported by *in-silico* work, and their optimization has been described.[11,12] The initial prototypes, displayed high pKa values ($9 < pKa < 9.6$) and a strong Pgp-mediated efflux liability.[11] To attenuate the issues attributed to the elevated pKa, the amidine warhead was carefully modified with fluorine containing electron withdrawing substituents at position C-2 of the morpholine ring. This optimization focused on achieving a pKa within the range of 6.5-7.5. The evolution of the series ultimately culminated with the identification of **4** (Figure 1), a recently disclosed clinical candidate.[12]

Besides the exploration of the morpholine series, here we described a parallel effort using bioisosteric replacements of the amide in the aminopiperazinone warhead (**3**). We focused on replacing this amide functional group by nitrogen-containing

fused five membered heteroaromatic rings, where a bridge head nitrogen was maintained.

To validate our approach, we initially synthesized the imidazolopiperazine warhead (**5**) with identical decoration to the reported aminopiperazinone (Table 1). Similar potency in both the BACE 1 cellular and enzymatic assays was observed giving an immediate indication this could offer a promising avenue for deeper exploration. Therefore, an efficient and versatile synthetic route based on the ring opening of a cyclic sulfamidate intermediate (**8**) was developed, enabling the synthesis of the bicyclic warheads **X1 – X8**, shown in Figure 2.

Table 1. BACE 1 cellular data for aminopiperazinone **3** and imidazolopiperazine **5**.

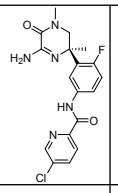
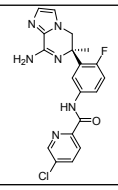
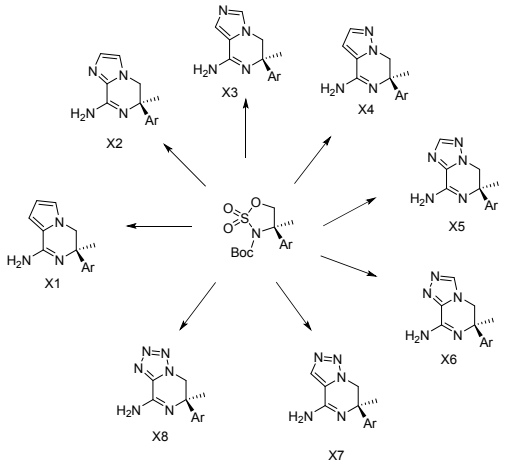
		
Compd #	**3**	**5**
BACE1 enz pIC_{50}	7.63	7.46
hAβ42 pIC_{50}	7.55	7.98

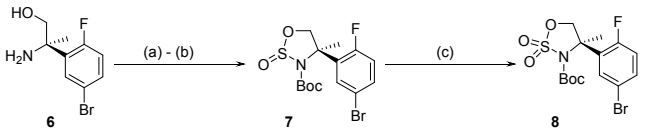
Figure 2. Proposed aminopiperazinone bisosteric replacements **X1**-**X8** from common sulfamidate intermediate.



Chemistry: Cyclic sulfamidates have emerged as important and versatile intermediates in organic synthesis because of their established ability to undergo highly selective reactions with O-, S-, N-, C-, P- and F-based nucleophiles.[13] We reasoned that nitrogen-containing heteroaromatics could be suitable nucleophiles to ring open adequately substituted sulfamidates leading to intermediates which could then be further elaborated to yield the targeted warheads (Figure 2).

Key cyclic sulfamidate **8** was by Boc protection of the β-amino alcohol **6**,[11] followed by cyclisation with thionyl chloride in acetonitrile at -40 ºC afforded the corresponding sulfamidite **7** in 93% yield over 2 steps. This was then oxidized to the target sulfamidate **8** using $NaIO_4$ and a catalytic amount of $RuCl_3$ in acetonitrile/water with excellent yield (Scheme 1).[14]

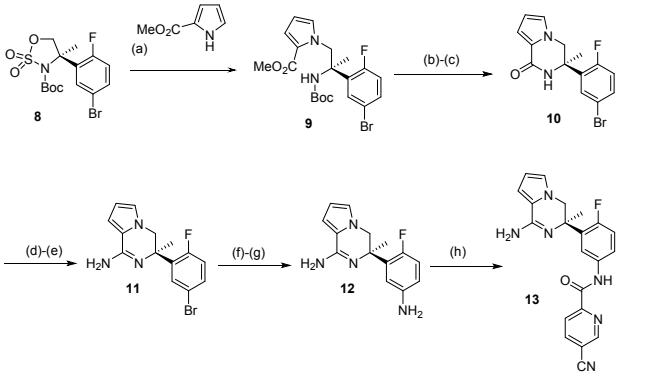
Scheme 1. Synthesis of cyclic sulfamidate **8**.



Reagents and conditions: (a) $(Boc)_2O$, $NaHCO_3$ aq, THF, rt, 2.5 d; (b) $SOCl_2$, MeCN, 2 h, rt (93% yield over 2 steps); (c) $RuCl_3$, $NaIO_4$, CAN, MeCN/H_2O, 2 h, rt; (95% yield).

With sulfamidate **8** in hand, we studied its nucleophile induced ring opening reaction with several nitrogen-containing heteroaromatics. Sulfamidate **8** was successfully ring opened using methyl 1H-pyrrole-2-carboxylate in the presence of cesium carbonate in acetonitrile, to afford **9** in 84% yield (scheme 2). The resulting intermediate **9** was then deprotected and cyclized by sequential treatment with HCl in dioxane, followed by sodium methoxide to afford lactam **10**, in 22% yield for the two steps. Lactam **10** was then elaborated to the targeted warhead by initial alkylation to form an imino-ether intermediate, and subsequent reaction with ammonia in EtOH to form the desired amidine **11**, in 33% yield over the two transformations. While there are many techniques to convert arylbromides to the corresponding anilines, and alternatives are described *vide infra*, on this occasion we used a Büchwald coupling using the benzophenone imine; which was subsequently hydrolyzed to the corresponding aniline **12**. Subsequent chemo-selective coupling of the carboxylic acid was mediated by 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM).[15] This reagent is designed to activate the carboxylic acid and concomitantly protonate the most basic center, subsequently promoting the chemo-selective amide formation on the least basic center, giving **13**, in 50% yield.[15]

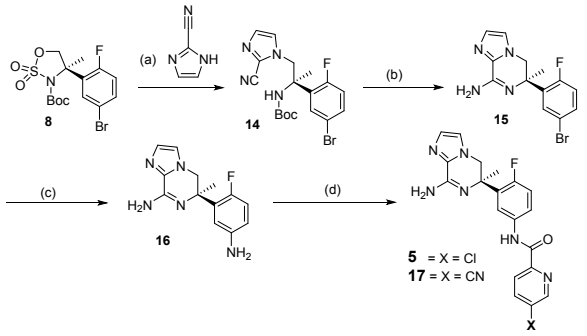
Scheme 2. Synthesis of pyrrolopiperazine BACE 1 inhibitor **13**.[16]



Reagents and conditions: (a) Cs_2CO_3, MeCN, 130 °C MW, 30 min, (84% yield); (b) HCl (4 N in 1,4-dioxane), rt, 18 h; (c) MeONa, MeOH, 60 °C, 16 h, (22% yield over 2 steps); (d) OMe_3BF_4, DCM, rt, 4 d; (e) NH_4Cl, NH_3 (2 M in MeOH), EtOH, 80 °C, 48 h (33% yield from **10**); (f) $Pd_2(dba)_3$, BINAP, Na^tBuO, benzophenone-imine, 100 °C, 2 h; (g) HCl (6 N in iPrOH) 1 h, rt; (h) 5-cyanopyridine-2-carboxylic acid, DMTMM, MeOH, 0 °C to rt, 3 h, (50% yield from **11**).

The regioisomeric imidazolepiperazine warheads **5**, **17** and **21** were also accessed using cyclic sulfamidate **8**. Reaction of 2-cyanoimidazole with **8** in the presence of DBU afforded **14** in 97% yield Scheme 3). HCl promoted cleavage of the Boc group in **14** occurred with concomitant cyclisation to amidine **15**, in 71% yield. The aryl bromide was then converted to aniline **16** using sodium azide and copper iodide in a solution of dimethylene ethylene diamine and DMSO in 75% yield. The regioselective amide formation was again achieved using DMTMM to afford the final products **5** and **17** in 45% and 57% yield, respectively.

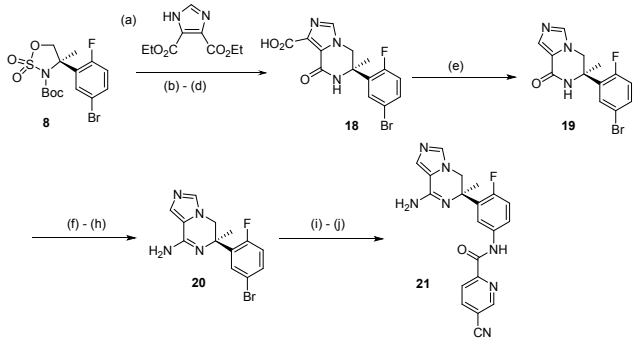
Scheme 3. Synthesis of imidazolopiperazine BACE 1 inhibitors **5** and **17**.



Reagents and conditions: (a) DBU, ACN, 90 °C, 16 h, (97% yield); (b) HCl (4 N in 1,4-dioxane), 70 °C, 4 h, (71% yield); (c) NaN_3, DMEDA, CuI, Na_2CO_3, DMSO, 110 °C, 24 h, (75% yield); (d) (**5**) 5-Chloro-pyridine-2-carboxylic acid or 5-cyanopyridine-2-carboxylic acid, DMTMM, MeOH, 0 °C to rt, 24 h, (45-57% yield).

For the preparation of **21**, due to the lack of symmetry and difference in reactivity, ethylimidazole-4-carboxylate could not be used to open the cyclic sulfamidate. Instead, reaction of **8** with symmetrical diethyl-1H-imidazole-4,5-dicarboxylate yielded the expected product in 88% yield (Scheme 4). Subsequent Boc deprotection, followed by sodium methoxide mediated intramolecular cyclisation afforded lactam **18** (55% yield over 3 steps). Decarboxylation by treatment with NaOH and heating in DMSO led to **19**. Treatment of **19** with phosphorus pentasulfide to the thio-lactam and subsequent S-methylation followed by treatment with ammonia yielded amidine **20**. Target compound **21** was obtained from **20** following analogous conditions to those described in Schemes 2 and 3.[17]

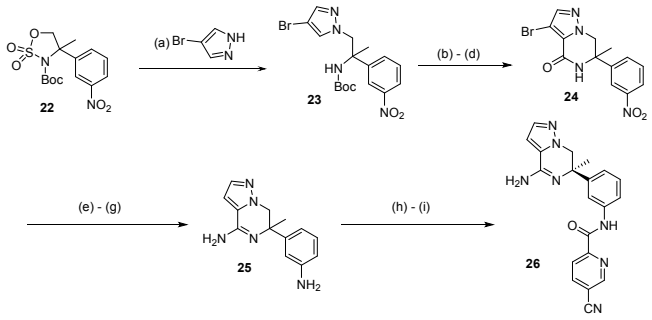
Scheme 4. Synthesis of imidazolopiperazine BACE 1 inhibitor **21**.



Reagents and conditions: (a) DBU, ACN, 90 °C, 5 h, (88% yield); (b) HCl (4 M in 1,4-dioxane), 1,4-dioxane, 70°C, 15 h; (c) NaOMe, MeOH, 55 °C, 5 h, (55% yield over 3 steps); (d) 1 N NaOH, MeOH, rt, 1 h; (e) DMSO, 120 °C, 2 h (62% yield over 3 steps); (f) P_2S_5, pyridine, 110 °C, 16 h; (g) MeI, K_2CO_3, acetone, 16 h, rt; (h) NH_4Cl, NH_3 (7 M in MeOH), 80 °C, 48 h, (35% yield over 3 steps); (i) NaN_3, DMEDA, CuI, Na_2CO_3, DMSO, 110 °C, 4 h; (j) 5-cyanopyridine-2-carboxylic acid, DMTMM, MeOH, 0 °C to rt, 24 h, (33% yield over 2 steps).

Pyrazolopiperazine analog **26** could be prepared by reaction of cyclic sulfamidate **22**[18] with 4-bromopyrazole; this was achieved in good yield using cesium carbonate in dimethyl sulfoxide to yield **23** (Scheme 5). Cyclic sulfamidate **22** was synthesized in an analogous way to cyclic sulfamidate **8**, previously described in Scheme 1, albeit racemically, thus requiring that the final compound undergo chiral separation, which in the case of **26** was achieved using chiral SFC. Intermediate **23** was deprotonated with LDA and reacted with ethylchloroformate to form the lactam precursor. This was then cyclized, after TFA-mediated Boc deprotection, by treatment with KOAc in refluxing ethanol. Lactam **24** was elaborated to the amidine via generation of the thioamide and subsequent reaction with ammonia. The aromatic nitro group in **24** was reduced to the corresponding aniline using heterogeneous catalysis which went with concomitant bromide removal to afford **25** in excellent yields over three reaction steps (94%). The chemoselective amide formation was facilitated by DMTMM to afford the desired amide **26** in 10% yield after chiral separation.

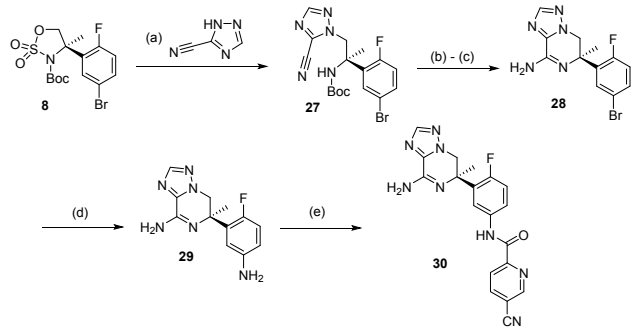
Scheme 5. Synthesis of pyrazolopiperazine BACE 1 inhibitor **26**.



Reagents and conditions: (a) Na_2CO_3, DMSO, 130 °C, 2 h, (79% yield); (b) ethylchloroformate, LDA, THF, -78 °C to -50 °C, 2 h (66% yield); (c) TFA, DCM, rt, 3 h; (d) KOAc, EtOH, reflux, 4 h, (87% yield over 2 steps); (e) P_2S_5, pyridine, 110 °C, 24 h; (f) NH_4Cl, NH_3 (7 M in MeOH), 100 °C, 16 h; (g) Zn, AcOH, EtOH, reflux, 24 h, (37% yield over 3 steps); (h) 5-cyanopyridine-2-carboxylic acid, DMTMM, MeOH, 0 °C to rt, 4 h; (i) Chiral SFC purification, (10% yield over 2 steps).

To access 1,2,4-triazolopiperazine **30**, reaction of cyclic sulfamidate **8** with 1,2,4-triazole-3-carbonitrile afforded **27** in 92% yield (Scheme 6). This was then elaborated to the final compound following a very similar sequence to that previously described in Scheme 4 for the imidazolopiperazine analog **21**. This started with formic acid-mediated Boc cleavage and subsequent intramolecular lactamization facilitated by trimethyl aluminum. Then, amination of the bromophenyl ring in **28** by treatment with sodium azide and CuI afforded **29** in 92% yield. Regioselective amide formation led to the desired BACE 1 inhibitor **30** in 52% yield.

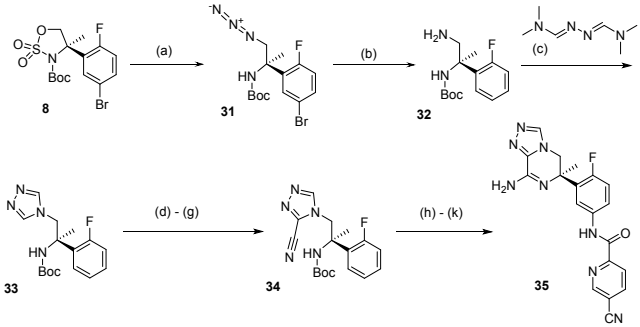
Scheme 6. Synthesis of 1,2,4-triazolopiperizine BACE 1 inhibitor **30**.



Reagents and conditions: (a) KOtBu, 60 °C, 1,4-dioxane, 16 h, (99% yield); (b) HCO_2H, rt, 2 h; (c) $AlMe_3$, toluene, 60 °C, 1 h, (53% yield over 3 steps) (d) NaN_3, DMEDA, Na_2CO_3, CuI, DMSO, 100 °C, 2 h, (50% yield); (e) 5-cyano-pyridine-2-carboxylic acid, EDCI, HCl (6 N in iPrOH), MeOH, rt, 2 h, (52% yield).

1,3,4-Triazolopiperazine **35** was accessed via ring opening of cyclic sulfamidate **8** with sodium azide to afford alkylazide **31**, which, after subsequent hydrogenation, afforded diamine **32**, with concomitant and undesired bromide removal (Scheme 7). Intermediate **32** was then treated with N,N'-bis(dimethylaminomethylene)hydrazine in the presence of a catalytic amount of p-TSA to afford the desired 1,3,4-triazole **33**. The introduction of a nitrile functional group in the heteroaromatic ring was achieved in a 4-step sequential sequence. First, a hydroxymethyl substituent was introduced with formaldehyde in the presence of triethylamine; this primary alcohol was then selectively oxidized to the corresponding aldehyde using manganese dioxide. Finally, aldehyde condensation with hydroxylamine followed by dehydration of the intermediate oxime using oxalyl chloride afforded the desired nitrile containing intermediate **34**. Boc deprotection of **34** under acidic conditions and concomitant cyclisation led to the expected cyclic amidine intermediate. Nitration of the aromatic ring under standard conditions, followed by hydrogenation of the nitro group resulted in the corresponding aniline intermediate. Final amide coupling with 5-cyanoyridine-2-carboxylic acid afforded BACE 1 inhibitor **35** in 58% yield from nitrile containing intermediate **34**.

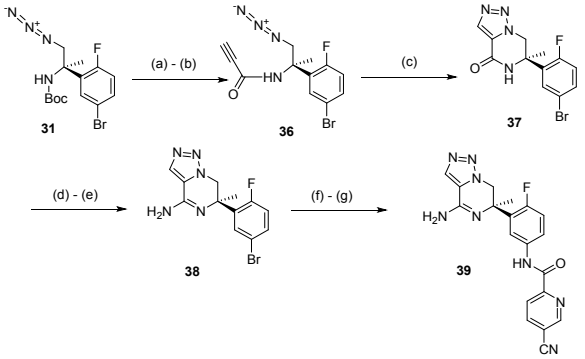
Scheme 7. Synthesis of 1,3,4-triazolo-piperizine BACE 1 inhibitor **35**.



Reagents and conditions: (a) NaN_3, DMF, 80 °C, 2 h; (quantitative); (b) H_2, Pd/C, TEA, MeOH, rt, 16 h; (87% yield) (c) p-TSA, toluene, 130 °C, 16 h, (47% yield) (d) CH_2O, TEA, 100 °C, 2 h; (e) MnO_2, $CHCl_3$, rt, 1 h; (f) $NH_2OH{\cdot}HCl$, NaOAc, EtOH, 80 °C, 1.5 h; (g) CO_2Cl_2, TEA, EtOAc, 0 °C to rt, 2 h, (31% yield over 4 steps); (h) HCO_2H, 50 °C, 1 h; (i) HNO_3, H_2SO_4 -10 °C, 30 min; (j) H_2, Pd/C, MeOH, rt, 4 h; (k) 5-cyanopyridine-2-carboxylic acid, EDCI, HCl (6 N in iPrOH), MeOH, rt, 16 h, (58% yield over 4 steps).

The 1,2,3-triazolopiperazine analogue **39** was prepared from intermediate **31** as outlined in Scheme 8. Boc deprotection of the amine group in **31** followed by coupling with propiolic acid led to amide **36**; the resulting intermediate was double cyclized under thermal conditions to afford **37**. Lactam **37** was elaborated to amidine **38** following a similar procedure as described in Scheme 5. Subsequent chemoselective amide coupling with 5-cyano-pyridine-2-carboxylic acid led to BACE 1 inhibitor **39**.[19]

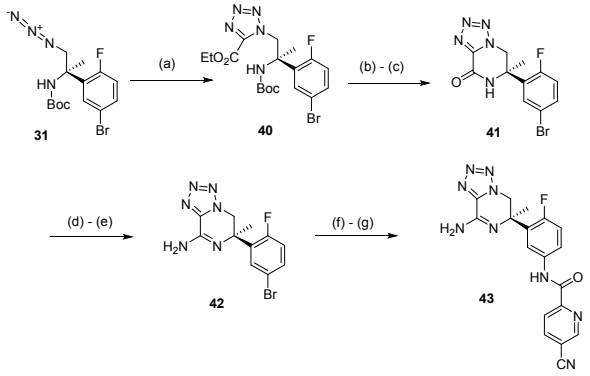
Scheme 8. Synthesis of 1,2,3-triazolo-piperizine BACE 1 inhibitor **39**.



Reagents and conditions: (a) HCl (4 N in 1,4-dioxane), rt, 24 h; (b) propiolic acid, DCC, DCM, 0 °C, 2 h; (c) toluene, 70 °C, 18 h; (78% yield over 3 steps); (d) P_2S_5, 1,4-dioxane, 95 °C, 2 h; (e) NH_3 (7 M in MeOH) 75 °C, 2 h; (f) NaN_3, DMEDA, Na_2CO_3, CuI, DMSO, 110 °C, 5 h; (g) 5-cyanopyridine-2-carboxylic acid, EDCI, HCl (6 N in iPrOH), MeOH, rt, 3 h, (39% yield over 4 steps).

Compound **43** containing a fused tetrazole ring was again generated from intermediate **31**, as shown in Scheme 9. Intermediate **31** initially underwent a 1,3-dipolar cyclisation with ethyl cyanoformate to afford tetrazole **40**. Acid-mediated Boc deprotection, followed by cyclisation gave lactam **41**. Lactam **41** was converted to the corresponding amidine **42**, as described in Schemes 5 and 7. Bromide **42** was converted to the aniline, which underwent chemoselective amide formation to yield BACE 1 inhibitor **43**.

Scheme 9. Synthesis of tetrazolo-piperizine BACE 1 inhibitor **43**.



Reagents and conditions: (a) Ethylcyanoformate, 120 °C, 20 h; (b) TFA, DCM, rt, 2 h ; (c) K_2CO_3, EtOH, reflux, 20 h, (75% yield over 3 steps); (d) P_2S_5, 1,4-dioxane, 95 °C, 2 h; (e) NH_3, (7 M in MeOH), 70 °C, 18 h; (f) NaN_3, DMEDA, Na_2CO_3, CuI, DMSO, 110 °C, 2 h; (g) 5-cyanopyridine-2-carboxylic acid, EDCI, HCl (6 N, iProH), MeOH, rt, 2h, (5% yield over 4 steps).

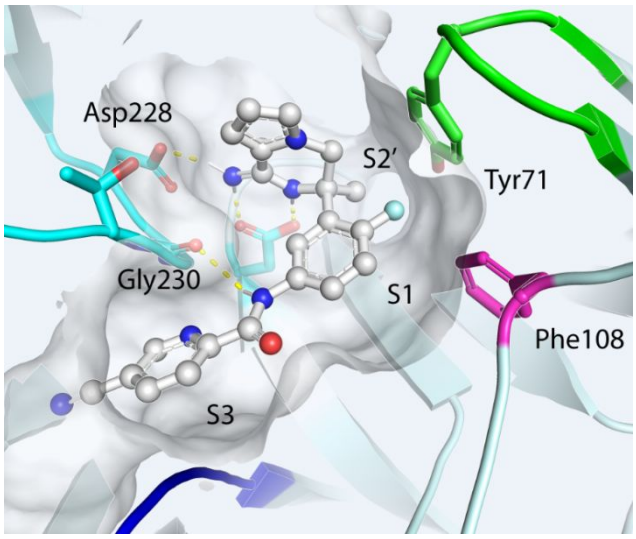
The resulting eight bicyclic heteroaryl-fused aminopiperazine BACE 1 inhibitors **13**, **17**, **21**, **26**, **30**, **35**, **39**, **43** provide an excellent opportunity for the side-by-side comparison of the effect of the fused heterocycle. They were profiled for their BACE 1 and 2 inhibitory activity in both our enzymatic assay and also cellular BACE1 assay, Table 2. Cathepsin D was not measured for this series of compound as it known that the expansion to the S3 pocket via the amide linked heteroaromatic removes the Cathepsin D liability.[20]

Most compounds showed high cellular potency in the range of pIC_{50} 7.7-8.9. The low activity observed for the tetrazole derivative **43** (hAβ42 cell pIC_{50} = 7.01) was attributed to the low basicity of the amidine function (pKa = 5.1). In general, we have observed a dependency for BACE 1 cellular potency with pKa. This is rationalized by the BACE 1 cellular location in the endosomes, an acidic compartment that favors basic compound distribution.[21]

Our working hypothesis is that the optimal pKa for a BACE 1 inhibitor should be in the range of 6.5-8.5. Higher pKa´s are tolerated and, in some cases, result in increased cellular potency. As demonstrated with compounds **13** and **43**, compound **13** has a pKa of 10.3 and this is translated into a cellular potency of pIC_{50} of 8.88, which compound **43** has the lowest pKa of 5.1 and has a cellular potency of pIC_{50} of 7.1. However, the higher basicity can also be responsible for off-target related complications, such as hERG and Pgp-efflux liabilities, this is not always the case and Pgp liability is not resultant on one factor and multiple properties of the molecule can affect this value. The measured pKa for compounds **13** (pKa = 10.3) and **21** (pKa = 9.0) was found to be higher than the optimal range. This high basicity could contribute to the high Pgp efflux observed for both compounds (**13**, AB+/AB- = 4.6 and **21**, AB+/AB- = 5.3), but a clear outlier is compound **39** that has an ideal pKa and suffers from significant Pgp efflux. We did see in this series of compounds a clear correlation between hERG and pKa, with compound **13** with the highest pKa having a significant hERG liability and compound **43** not showing any hERG inhibition. Therefore, further work around these warhead modifications should aim at lowering the high pKa of the amidine function and modulating the vitro and vivo profile.

The crystal structure of **13** with BACE 1 was solved at 2 Å resolution (PDB code 6OD6, Figure 3). The result confirmed the binding mode to be similar to those seen for analogous amidine containing BACE 1 inhibitors.[11,22] This includes the key interactions between the protonated amidine and the catalytic dyad (Asp32 and Asp228). The quaternary center is optimal for the orientation of the aryl ring and the methyl group, allowing the methyl to protrude into the S2' pocket without clashing with the protein. The quaternary center favorably positions the F-aryl ring in an axial orientation to occupy the S1 pocket. The amide linker then optimally orientates the pyridine ring in the S3 pocket of BACE 1, while generating a favorable H-bond interaction with Gly230. Most of the compounds have a similar enzymatic activity with pIC_{50} approx. 8-8.5, with examples **17** and **39** showing the biggest difference: pIC_{50} 7.54 and 8.70 respectively. Comparing pairs **13** and **17**, **21** and **35**, **21** and **35**, **26** and **30**, **39** and **43**, all demonstrate that incorporation of a nitrogen at position 2' led to a loss of activity. The crystal structure suggests that this nitrogen points towards the oxygen of Thr231, and is therefore electrostatically unfavorable explaining the lower activity. The rest of the fused cycle is solvent exposed. Although not attempted here, additional substituents to the fused bicycle may allow further modulation of the potency by forming additional interactions with the receptor.

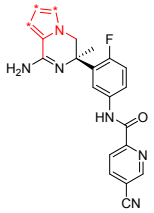
Figure 3. Crystal structure of **13** in BACE 1 showing key interactions.



Compounds **17**, **26**, **30**, **35** and **39** possess pKas in an almost optimal range between 6.4-8.5 and show good to excellent intrinsic permeability.

Unfortunately, despite their good pKa´s, the Pgp-efflux liability measured for **35** (AB+/AB- = 4.8) and **39** (AB+/AB- = 9.8) precluded further investigation around these two chemotypes. On the other hand, compounds **17**, **26** and **30** exhibit the desired in vitro profile combining potency, with good intrinsic permeability a reduced Pgp-efflux and hERG liability and the heteroaryl-fused aminopiperazine warheads they represent have been selected for further profiling and optimization.

Table 2. Overview of BACE 1 inhibitor in vitro profile.



									
Compound #	**13**	**17**	**21**	**26**	**30**	**35**	**39**	**43**
X	F	F	F	H	F	F	F	F
BACE1 enz pIC_{50}	8.21	7.54	8.40	8.34	8.22	7.97	8.70	8.04
hAβ42 cell pIC_{50} nM	8.88	8.20	7.74	8.66	8.01	8.18	8.92	7.01
BACE2 enz pIC_{50}	7.60	6.85	7.72	7.58	7.43	7.25	8.26	7.57
hERG IC_{50} µM	0.49	4.17	3.72	1.78	11.75	>10	7.24	>50
Pgp								
AB+	11.1	17.2	1.5	15.6	24.8	5.9	11.6	22.1
AB+/AB-	4.6	2.0	5.3	3.6	2.0	4.8	9.8	1.9
pKa	10.3	8.2	9.0	8.5	6.5	6.4	7.1	5.1

See supplementary information for assay details

In summary, an efficient evaluation of multiple novel BACE 1 inhibitors has been achieved. Exploiting the reactivity of the cyclic sulfamidates we have developed a convergent synthesis to explore the replacement of the aminopiperazinone warhead by bioisosteric replacements in the form of fused heteroaromatics. Several new series of BACE 1 inhibitors have been identified, and efforts are currently focused on the optimization of properties and progression of key compounds. These BACE 1 inhibitors represent a unique opportunity, as the fused 5-membered heterocyclic ring offers additional handles for modification to enhance their overall profile.

ASSOCIATED CONTENT.

Supporting Information.

The Supporting Information is available free of charge on the ACS Publications website.

Experimental procedures for the synthesis and the analytical characterization of all intermediates and final compounds are included.

AUTHOR INFORMATION.

Corresponding Author.

* (D. O.) Phone: +3214603100. Email: doehlric@its.jnj.com

* (A. A.T.) Phone: +34925245771. Email: atrabanc@its.jnj.com

ORCID

Daniel Oehlrich: 0000-0002-3392-1952

Gary Tresadern: 0000-0002-4801-1644

Andrés A. Trabanco: 0000-0002-4225-758X

Harrie J.M. Gijsen: 0000-0001-8020-4828

Frederik J.R. Rombouts: 0000-0003-1986-0476

Author Contributions.

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

ACKNOWLEDGMENT.

We thank the analytical departments in Beerse and Toledo for helping with compound characterization and purification and the DMPK and toxicology departments for their contributions in profiling the compounds.The authors would also like to thank Claudio Meyer for reviewing the manuscript.

REFERENCES

[1] Hardy, J. A.; Higgins, G. A. Alzheimer's disease: the amyloid cascade hypothesis. *Science,* **1992**, *256*, 184–185.

[2] Sinha, S.; Anderson, J. P.; Barbour, R.; Basi, G. S.; Caccavello, R.; Davis, D.; Doan, M.; Dovey, H. F.; Frigon, N.; Hong, J.; Jacobson-Croak, K.; Jewett, N.; Keim, P.; Knops, J.; Lieberburg, I.; Power, M.; Tan, H.; Tatsuno, G.; Tung, J.; Schenk, D.; Seubert, P.; Suomensaari, S. M.; Wang, S.; Walker, D.; Zhao, J.; McConlogue, L.; Varghese, J. Purification and cloning of amyloid precursor protein beta-secretase from human brain. *Nature*, **1999**, *402*, 537–540.

[3] Oehlrich, D; Prokopcova, H; Gijsen, H. J. M. The evolution of amidine-based brain penetrant BACE 1 inhibitors. *Bioorg. Med. Chem. Lett.*; **2014**, *24*, 2033–2045.

[4] Hsiao, C-C., Rombouts, F., Gijsen, H.J.M.; New evolutions in the BACE1 inhibitor field from 2014 to 2018. . *Bioorg. Med. Chem. Lett.*; **2019**, *29*, 761–777.

[5] https://clinicaltrials.gov/ct2/show/NCT01953601.

[6] https://clinicaltrials.gov/ct2/show/NCT01561430.

[7] Baxter, E.W., Conway, K.A., Kennis, L., Bischoff, F., Mercken, M.H., De Winter, H.L., Reynolds, C.H., Tounge, B.A., Luo, C., Scott, M.K., Huang, Y., Braeken, M., Pieters, S.M.A., Berthelot, D., Masure, S., Bruinzeel, W.D., Jordan, A.D., Parker, M.H., Boyd, R.E., Qu, J., Alexander, R.S., Brennaman, D.E., Reitz, A.E.; 2-Amino-3,4-dihydroquinazolines as Inhibitors of BACE-1 (β-Site APP Cleaving Enzyme): Use of Structure Based Design to Convert a Micromolar Hit into a Nanomolar Lead. *J. Med. Chem*. **2007**, *50*, 4261−4264.

[8] Ciordia, M., Perez-Benito, L., Delgado, F., Trabanco, A., Tresadern, G.; Application of Free Energy Perturbation for the Design of BACE1 Inhibitors. *J.*

Chem. Inf. Model., **2016**, *56*, 1856-1871.
[9] Keranen, H.K., Perez-Benito, L., Ciordia, M., Delgado, F., Steinbrecher, T.B., Oehlrich, D., Van Vlimen, H.W.T., Trabanco, A., Tresadern, G. Acylguanidine Beta Secretase 1 Inhibitors: A Combined Experimental and Free Energy Perturbation Study. *J.Chem. Theory Comput*., **2017**, *13*, 1439-1453.
[10] Tresadern, G.; Delgado, F; Delgado, O; Gijsen, H.; Macdonald, G, J.; Moechars, D; Rombouts, F.; Alexander, R.; Spurlino, J.; Van Gool, M.; Vega, J.A.;Trabanco, A.A. Rational design and synthesis of aminopiperazinones as β-secretase (BACE) inhibitors. *Bioorg. Med. Chem. Lett*, **2011**, *21*, 7255–7260.
[11] Rombouts, F.; Tresadern, G.; Delgado, O,; Martínez-Lamenca, C.; Van Gool, M.; García-Molina, A.; Alonso de Diego, S.; Oehlrich, D.; Prokopcova, H.; Alonso, J.A.; Austin, N.; Borghys,H.; Van Brandt, S.; Surkyn, M.; De Cleyn, M.;Vos, A.; Alexander, R.; Macdonald, R.; Moechars, D.; Gijsen, H.; Trabanco, A.A. 1,4-Oxazine β-Secretase 1 (BACE 1) Inhibitors: From Hit Generation to Orally Bioavailable Brain Penetrant Leads. *J. Med. Chem*. **2015**, *58*, 8216−8235.
[12] Gijsen, H.; Alonso de Diego, S.; De Cleyn, M.; García-Molina, A.; Macdonald, G. J.; Martínez-Lamenca, C.; Oehlrich, D.; Prokopcova, H.; Rombouts, F.; Surkyn, M.; Trabanco, A. A.; Van Brandt, S.; Van den Bossche, D.; Van Gool, M.; Austin, N.; Borghys, H.; Dhuyvetter, D.; Moechars, D. Optimization of 1,4-oxazine β-secretase 1 (BACE 1) inhibitors toward a clinical candidate. *J. Med. Chem.*, **2018**, *61,* 5292–5303.
[13] Bower, J.F.; Rujirawanichab, J.; Gallagher, T.; *N*-Heterocycle construction *via* cyclic sulfamidates: Applications in synthesis. *Org. Biomol. Chem*., **2010**, *8*, 1505–1519.
[14] Trabanco-Suarez, A.A.; Delgado-Jimenez, F; Vega Ramiro, J.A.; Tresadern, G.J.; Gijsen, H.J.M.; Oehlrich, D. Preparation of 5,6-dihydro-imidazo[1,2-a]pyrazin-8-ylamine derivatives as beta-secretase inhibitors. WO2012085038.
[15] Kunishima, M.; Kawachi, C.; Monta, J.;Terao, K.; Iwasaki, F.; Tani, S. 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride: an efficient condensing agent leading to the formation of amides and esters. *Tetrahedron. **1999**, 55*, 13159-13170.
[16] Trabanco-Suarez, A.A.; Delgado-Jimenez, F; 3,4-Dihydro-pyrrolo[1,2-a]-1-ylamine derivatives useful as inhibitors of beta-secretase (BACE). WO2012120023.)
[17] Oehlrich, D.; Gijsen, H.J.M.; Surkyn, M.; 4-Amino-6-phenyl-5,6-dihydroimidazo[1,5-A]pyrazine derivatives as inhibitors of beta-secretase (BACE) WO2014198853.
[18] Trabanco-Suarez, A.A.; Gijsen, H.J.M.; Van Gool, M.L.M.; Vega Ramiro, J.A.; Delgado-Jimenez, F. Preparation of dihydropyrazolopyrazinylamine derivatives for use as beta-secretase inhibitors. WO2012117027.
[19] Oehlrich, D.; Gijsen, H.J.M.; 4-Amino-6-phenyl-6,7-dihydro[1,2,3]triazolo[1,5,A]pyrazine derivatives as inhibitors of beta-secretase (BACE) WO2014198854.
[20] Low, J.D., Bartberger, M.D., Chen, K., Cheng, Y., Fielden, M.R., Gore, V., Hickman, D., Liu, Q., Sickmier, E.A., Vargas, H.W., Werner, J., White, R.D., Wittington, D.A., Wood, S., Minatti, A.E.; Development of 2-aminooxazoline 3-azaxanthene β-amyloid cleaving enzyme (BACE) inhibitors with improved selectivity against Cathepsin D. *Med. Chem. Commun*.; **2017***, 8,* 1196-1206.
[21] Huse J.T.,Pijak D.S., Leslie G.J., Lee V.M., Doms R.W. Maturation and endosomal targeting of beta-site amyloid precursor protein-cleaving enzyme. The Alzheimer's disease beta-secretase. *J Biol Chem*. **2000** *275* 33729-33737.
[22] Hilpert, H.; Guba, W.; Woltering, T. J.; Wostl, W.; Pinard, E.; Mauser, H.; Mayweg, A. V.; Rogers-Evans, M.; Humm, R.; Krummenacher, D.; Muser, T.; Schnider, C.; Jacobsen, H.; Ozmen, L.; Bergadano, A.; Banner, D. W.; Hochstrasser, R.; Kuglstatter, A.; David-Pierson, P.; Fischer, H.; Polara, A.; Narquizian, R. β-Secretase (BACE 1) inhibitors with high in vivo efficacy suitable for clinical evaluation in Alzheimer's disease. *J. Med. Chem*. **2013**, *56*, 3980−3995.