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BMCL Digest

The evolution of amidine-based brain penetrant BACE1 inhibitors

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ABSTRACT

Beta site amyloid precursor protein cleaving enzyme 1 (BACE1) inhibitors hold great potential as disease modifying anti-Alzheimer's drugs. This digest provides an overview of the amidine containing class of BACE1 inhibitors, of which multiple examples are now progressing through clinical trials. The various structural modifications highlight the struggle to combine potency with the optimal properties for a brain penetrant BACE1 inhibitor, and illustrate the crowded competitive landscape. This overview concludes with a summary of potential issues including substrate and target selectivity and a synopsis of the status of the current and past clinical assets.

Alzheimer's disease (AD) has clearly identified itself as an insidious disease, which chronically ravages the cognitive capabilities of the sufferer, resulting in gradual decline in cognition, memory and changes in behavior which inevitably lead to death. With the demographic shift towards an aging population, resulting in an increased number of patients, this disease is fast becoming a serious economic and social burden.

AD is characterized by the existence of two pathological features namely amyloid plaques and neurofibrillary tangles. The observation of these features has led to the development of the amyloid cascade hypothesis.[1] Two decades have passed since this hypothesis was initially postulated and during this time it has been analyzed and modified.[2,3] The amyloid cascade hypothesis is based on a culmination of genetic and histopathological data, which suggest that AD is a direct consequence of the presence of amyloid-β (Aβ) aggregates in the brain.[4] Initially, the Aβ plaques were believed to be the only toxic component, but this hypothesis was later refined to incorporate a dual effect: The Aβ oligomers are believed to cause damage to the neurons, particularly, the neuronal synapses. This neurotoxicity then leads to a cascade of events including tau hyperphosphorylation and tau neurofibrillary tangle formation.[5]

To date, the amyloid cascade hypothesis remains polemic as it has not been fully validated,[3] but still represents a widely supported theory, substantiated by genetic evidence from the various mutations of amyloid precursor protein (APP). Up until now, about 30 mutations of APP have been identified; of these, 25 are pathogenic, resulting in a higher incidence of early onset AD (EOAD).[6] Recently, the first AD protective mutation of APP, the A673T mutant, was identified.[7] The mutation of alanine-673 to threonine, leads to an APP analog that is a 50% less efficient substrate for BACE1, than wild type APP. This mutation is responsible for not only increasing the chance of reaching 85 years of age without suffering from AD by 50%, but for also slowing the natural cognitive decline in the elderly. Interestingly, due to the fact that APP is quite a poor substrate for BACE1, most amino-acid mutations close to the cleavage site of APP result in faster proteolysis and increased rate of disease progression. This finding has further reinforced the amyloid cascade hypothesis and suggests that BACE1 maybe the therapeutic target of choice when developing disease modifying anti-Alzheimer's drugs.[7–9]

BACE1, a 501 amino acid integral membrane aspartyl protease was discovered in 1999,[10] and is the rate limiting enzyme for the formation of Aβ.[11] BACE2,[12] identified shortly after BACE1, shares 64% sequence homology with BACE1 and has been shown to be expressed primarily in the periphery, while BACE1 is mainly expressed in the neurons. BACE1 is predominantly active at pH 4.5–5.5 within the acidic environment of the endosomes.

In 1999, Sinha et al. at Elan Pharmaceuticals successfully purified BACE1 from human brain using a substrate analog which exhibited a statine residue as a transition state analog. Further to that, they also successfully cloned the BACE1.[13] The first X-ray crystal structure of a complex between recombinant BACE1 and the OM99-2 inhibitor was achieved a few years later by Ghosh and Tang.[14] This initial work established BACE1 as a target and the initial X-ray crystal structure aided subsequent medicinal chemistry investigations.

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During the last two decades many potent BACE1 inhibitors have been described but few have successfully displayed balanced in vitro potency and the necessary PK properties/parameters to achieve in vivo efficacy. The initial examples of BACE1 inhibitors, like most inhibitors of aspartate proteases, originated from substrate and transition state analog-based design. This development resulted in large polar compounds with high total polar surface area (TPSA), exhibiting many rotatable bonds and numerous hydrogen bond donor (HBD) and hydrogen bond acceptors (HBA), making them prohibitive for permeability especially through the blood brain barrier (BBB), a prerequisite for CNS active compounds.[15] Further to the physiochemical properties that prevented cell penetration, many classes of substrate and transition state derived inhibitors exhibited permeability glycoprotein (Pgp) efflux activity, adding to the reduced central penetration/exposure.[16] The identification, research progress and development of BACE1 inhibitors has been discussed in several recent reviews.[17–19]

Due to the large size of the catalytic active site, the search for non-peptidomimetic BACE1 inhibitors and their optimization has proved to be very challenging. Among the non-peptidomimetic scaffolds, amidine- or guanidine-containing heterocycles were identified early on via fragment based screening to form an ideal hydrogen-bonding network with the catalytic aspartyl dyad of BACE. In 2005, patent applications from both Schering–Plough and Wyeth disclosed 5,5-disubstituted aminohydantoins, exemplified by generic structure **1** (Fig. 1).[20,21] In addition to the interaction with the catalytic aspartates, the 5,5-disubstituted sp^3 carbon provided for an optimal vector into the P1 and P2′ pockets, as apparent from co-crystal structures with BACE1.[22,23] The combination of amidine containing warheads with optimal substitution vectors has led to a series of BACE1 inhibitor with high ligand efficiency.

Since 2005, many companies have elaborated and expanded on this motif, overcoming the obstacles towards safe and centrally efficacious BACE1 inhibitors. During the rest of this digest we will concentrate our efforts on describing the development of this most promising generation of BACE1 inhibitors, which fall under the scope of the general structure shown in Figure 2. Multiple compounds from this class have now advanced into the clinic, and we will conclude with an overview of the known status of these studies.

Since BACE1 is active at a pH of between 4.5 and 5.5 and resides within the acidic intracellular compartments· BACE1 inhibitors not only need to reach the site of action, but they also need to be optimally protonated to efficiently inhibit the enzyme. Many groups have identified the importance of pK_a when designing potent cell penetrant BACE1 inhibitors.[24,25]

The amidine containing subclasses of BACE1 inhibitors were identified using both HTS and fragment based drug discovery. The hits from Eli Lilly (**3** & **4**), AstraZeneca (**5**) and Schering–Plough (**2**) were all discovered by means of fragment screens using different techniques to optimally identify the weakly active

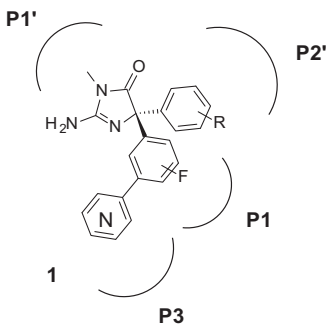


Figure 1.

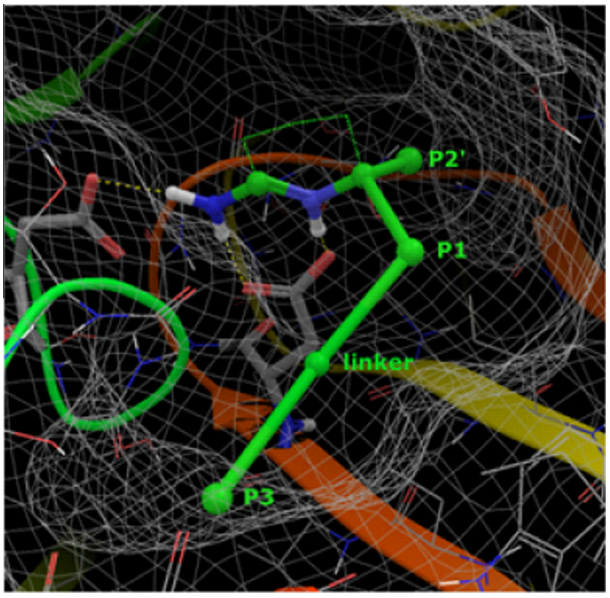


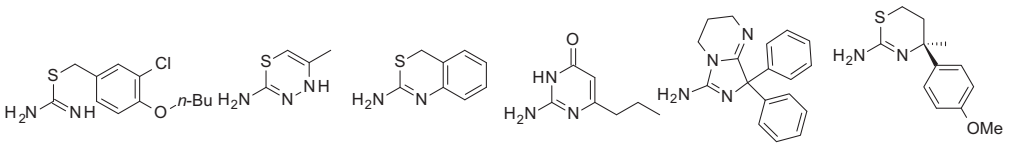
Figure 2.

fragments, including NMR, SPR as well as in vitro assays.[19,26,27] In contrast, Wyeth (**6**) and Roche (**7**) identified their hits through more traditional HTS campaign.[22,28] This warhead was also exploited by both Shionogi and Eli Lilly,[29] where the latter company arrived at this warhead via a fragment-based approach.[30]

AstraZeneca further refined their aminopyrimidinone BACE1 inhibitor hit **5** by screening related structures from their corporate compound collection, identifying potent analogue **8** with a phenethyl substituent (Fig. 4).[26] X-ray analysis revealed that the ethyl linker was key to optimally filling the P1/P3 pocket. In a collaborated effort with Astex Therapeutics, they identified compound **9**.[31]

Shionogi later investigated the possibility of constraining the flexible ethyl linker of **9** by introducing a cyclopropane.[32,33] Their analogues **10** and **11** exhibited an unexpected binding mode in which the cyclopropane ring forms CH-π interaction with Tyr[71] side chain of the enzyme. Nevertheless, no improvement in potency was achieved over **9** (Fig. 5).[31]

Wyeth analyzed their 8,8-disubstituted-tetrahydroimidazopyrimidin-6-amine fragment hit **6** using an X-ray crystal structure of their hit bound to BACE1. The structure revealed that the guanidine moiety forms key H-bonds with the catalytic aspartic acid residues Asp[32] and Asp[228].[22,23] The tetrahydropyrimidine ring points towards the solvent and gives no additional interactions with the enzyme. Consequently, truncation of this system resulted in the identification of the smaller 2-amino-3-methyl-5,5-diphenylhydantoin **12** with 10-fold higher potency (Fig. 6).[22] The increase in potency observed between **6** and **12** is attributed to the strength and capabilities of each compound to form the necessary H-bond with the catalytic site aspartate residues, which is rationalized by the pK_a of the system (**6**, pK_a = 5.7 vs **12**, pK_a = 7.6).[22] The initial investigation was focused towards modifications that optimally filled the P1, P3 and P2′ pockets; as a result, inhibitors **13** and **14** with more than 1000-fold increase in binding affinity over the hit **6** were identified. A key finding was the identification that *meta*-substitution of P1 phenyl ring by pyridine or pyrimidine would allow an optimal vector into the P3 pocket, with the nitrogen atoms of the heteroaromatics forming a water mediated H-bridge with Ser[229]. Since then, this substitution pattern has been applied in many R&D programs. In the P2′ pocket, substitution of the phenyl ring of **12** with *para*-methoxy or trifluoromethoxy group gave an additional increase in potency by generating a



2, Schering Plough NMR Fragment Hit IC_{50} = 200 μM K_D = 15 μM

3, Eli Lilly Fragment Hit IC_{50} = 8200 μM

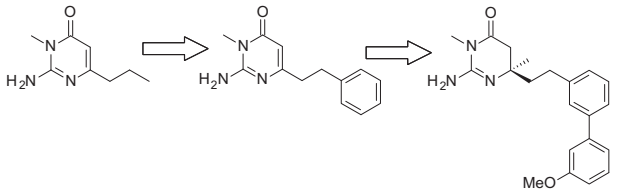
4, Eli Lilly Fragment Hit IC_{50} = 790 μM

5, AstraZeneca Fragment Hit K_D = 4450 μM

6, Wyeth HTS Hit IC_{50} = 38 μM

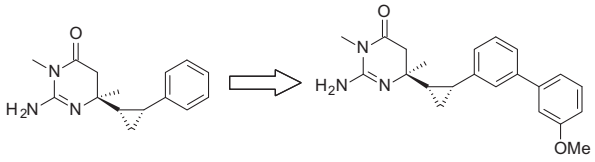
7, Roche HTS Hit IC_{50} = 41.2 μM

Figure 3.



5, BACE1 IC_{50} = 2490 μM **8**, BACE1 IC_{50} = 220 μM **9**, BACE1 IC_{50} = 80 nM Cell Aβ40 IC_{50} = 470 nM

Figure 4.

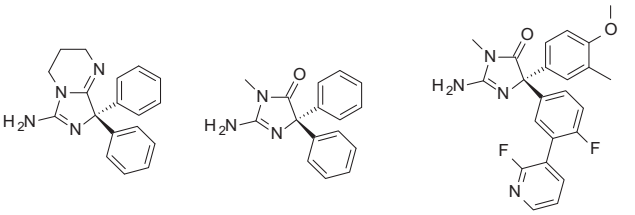


10, BACE1 IC_{50} = 157 μM **11**, BACE1 IC_{50} = 4.6 μM

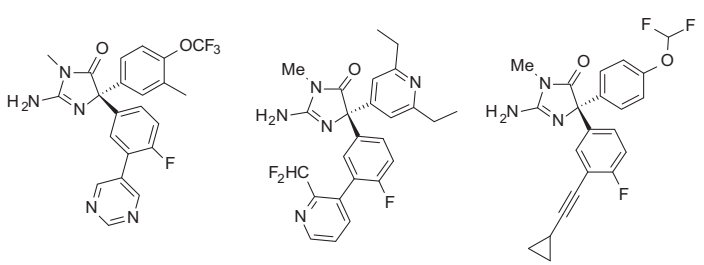
Figure 5.

hydrogen-bond interaction with the Trp[67] residue of the BACE1 enzyme. The ability of the compound **13** to reduce Aβ in vivo was evaluated and its lack of efficacy was attributed to its low brain exposure.[22] To improve brain permeability and in vivo efficacy of this aminohydantoin series, several physicochemical parameters (size, polarity, metabolic stability, Pgp affinity) were studied by Wyeth, resulting in compound **16**.[34] Selectivity of BACE1 over BACE2, was also explored by further exploration of the P2′ pocket, where the Pro[70] of BACE1 is replaced by Lys[86] in BACE2. The observed changes in selectivity were attributed to the differences in amino-acid as well as the changes in dynamics of the flap between the two enzymes.[22] One of the most selective compounds, **15** is shown in Figure 6, displaying a 670-fold preference for BACE1 versus BACE2 and even higher selectivity for cathepsin D, pepsin and renin (Fig. 6).[35]

Array BioPharma and Genentech followed a structure-based design approach when developing their potent and brain penetrant aminohydantoin inhibitors. They conformationally constrained the system using spirocyclic aminohydantoins, identifying BACE1 inhibitor **17** with an IC_{50} of 48 nM and cellular activity of 27 nM (Fig. 7).[36,37] This was an analogous exploration to that previously performed by Wyeth, exemplified by **20**.[38] Although compound **17** was able to cross the BBB, it suffered from very high Pgp mediated efflux. Further optimization of BACE1 potency was achieved by expanding the molecule into the P2′ pocket which resulted in



6, BACE1 IC_{50} = 38 μM **12**, BACE1 IC_{50} = 3.4 μM **13**, BACE1 IC_{50} = 10 nM

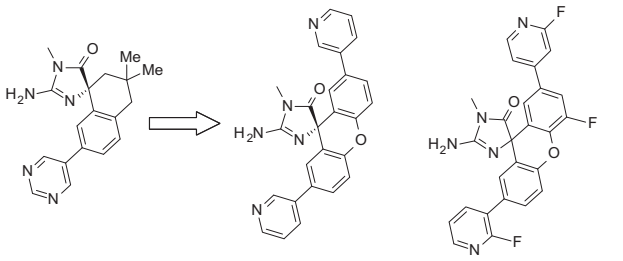


14, BACE1 IC_{50} = 20 nM

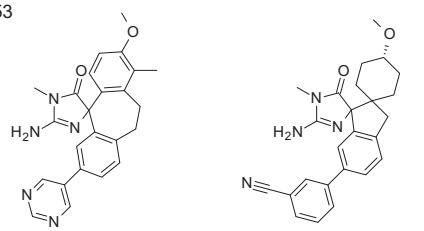
15, BACE1 IC_{50} = 10 nM
Cell EC_{50} = 130 nM
BACE 2 IC_{50} = 6.7 μM
Cathepsin D IC_{50} = 54 μM

16, BACE1 IC_{50} = 20 nM
Cell EC_{50} = 70 nM
BACE 2 IC_{50} = 120 nM
Cathepsin D IC_{50} = 1500 nM

Figure 6.



17, BACE1 IC_{50} = 48 nM **18**, BACE1 IC_{50} = 0.3 nM **19**, BACE1 IC_{50} = 0.2 nM
Cell EC_{50} - 27 nM
PgP efflux = 53



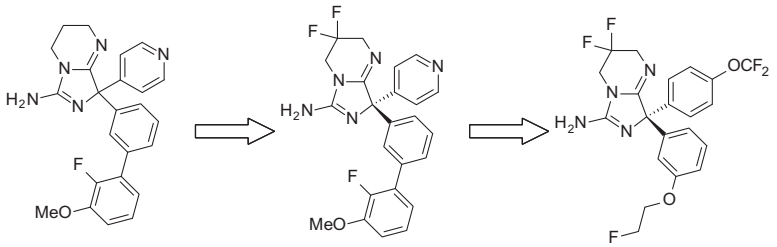
20, BACE1 IC_{50} = 77 nM **21**, BACE IC_{50} = 4 nM

Figure 7.

the identification of novel inhibitors **18** with sub-nanomolar affinity for BACE1 (Fig. 7).[39,40] Similar spiro-2-aminohydantoins and their six-membered analogues 2-amino-dihydropyrimidinones were also published and investigated by Amgen, exemplified by **19**.[41] Vitae Pharmaceuticals described several analogous series of double spiro systems, represented by **21**.[42]

Interestingly, AstraZeneca followed a scaffold hopping protocol from dihydroisocytosine to aminohydantoin which finally identified a series of bicyclic aminoimidazoles similar to the HTS hit previously described by Wyeth (**6**) (Fig. 3).[22,43] Aminoimidazole **22** (Fig. 8) displayed low permeability particularly through the BBB which was attributed to the high basicity of the amino-imidazole warhead (calculated pK_a >8). It was rationalized that lowering the pK_a would be beneficial for permeability, as at the physiological pH, the molecule is highly protonated and as such not membrane permeable. Modification of the pK_a was achieved by adding electron-withdrawing groups, such as fluorines; this resulted in the identification of **23** with a pK_a of 5.3. This modification resulted in improved permeability; however the compounds still suffered from a high Pgp efflux liability. Further modifications resulted in the identification of **24** with reasonable permeability and efflux in vitro. The in vitro result however did not correlate to that observed in vivo. To understand this discrepancy, **24** was co-administered with the Pgp inhibitor elacridar, which highlighted the still severe efflux liability of the compound.[43,44]

In addition to the amino-imidazole series, AstraZeneca evaluated amino-isoindoles as BACE1 inhibitors.[43] Introduction of a fluorine atom *ortho* to the amidine was key in achieving an optimal pK_a, permeability and efflux, (Fig. 9) (25–26). It was rationalized that, in addition to a lower pK_a, the fluorine atom forms a weak internal H-bond with the exocyclic nitrogen of the amidine, which is beneficial for both permeability and Pgp efflux. Nevertheless,

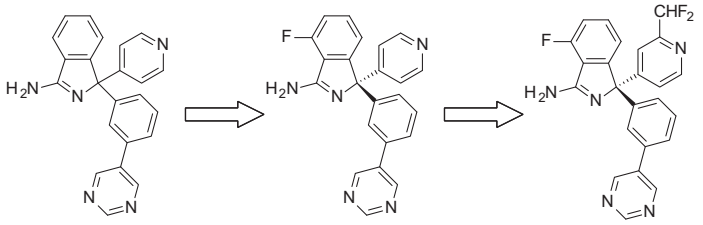


22, BACE1 IC_{50} = 90nM
Caco-2 = 1.10^{-6} cm/s
PSA = 68 Å

23, BACE1 IC_{50} = 20 nM
Cell pIC_{50} = 7.51
pKa = 5.3
Caco-2 = $6.7.10^{-6}$ cm/s
Pgp efflux = 8.1

24, BACE1 IC_{50} = 32 nM
Cell pIC_{50} = 8.10
pKa = 6.5
Caco-2 = 8.10^{-6} cm/s
Pgp efflux = 0.8

Figure 8.

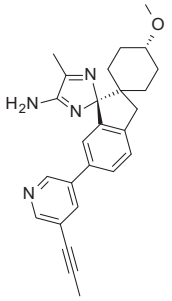


25, BACE1 IC_{50} = 500 nM
Cell IC_{50} = 90.3 nM
pKa = 8.4
Caco-2 = $3.4.10^{-6}$ cm/s
PgP efflux = 12
hERG IC_{50} = 16 μM

26, BACE1 IC_{50} = 124 nM
Cell IC_{50} = 8.25 nM
pKa = 7.2
Caco-2 = 22.10^{-6} cm/s
PgP efflux = 1.9
hERG IC_{50} = 3.1 μM

27, AZD-3839.
BACE1 IC_{50} = 35.1 nM
Cell IC_{50} = 16.7 nM
pKa = 7.1
Caco-2 = 34.10^{-6} cm/s
PgP efflux = 0.9
hERG IC_{50} = 4.8 μM

Figure 9.



28, BACE IC_{50} = 0.2 nM.

Figure 10.

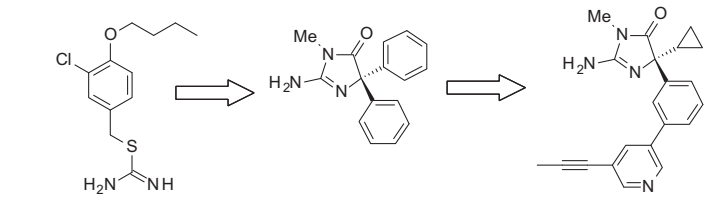
this modification increased the affinity for the hERG ion channel. Addition of a lipophilic electron-withdrawing group in the 2-position of the pyridyl ring such as difluoromethyl group, lowered the hERG liability and further fine-tuned other properties. Inhibitor **27** (**AZD-3839**), based on its preclinical profile, was progressed into phase I clinical trials (Fig. 10).[43]

Recently, AstraZeneca highlighted their interest in a class of double spiro amino-imidazoles, with the publication of a process patent describing the large scale synthesis of a camsylate salt of **28**.[45,46] This series of compounds arises from the introduction of a spiro substituent to the warhead, nearly identical to the modification previously performed by Vitae Pharmaceuticals on the hydantoin warhead **21**, (Fig. 7).[42]

Schering–Plough arrived at the same amino-hydantoin **12** as identified by Wyeth via modification of their isothiourea fragment hit **2**, obtained via NMR screening.[27] Optimization of this series identified **29**, with an enzymatic IC_{50} of 5.4 nM and a cellular IC_{50} of 82 nM, which also lowered Aβ40 in vivo (Fig. 11).[47]

It was hypothesized that molecules with slightly higher basicity than the aminohydantoins ($pK_a \sim 7$) would accumulate in the endosomes, and result in a higher cellular and thus in vivo potency.[48] To achieve this, they expanded the ring into a slightly more basic six-membered amino-dihydropyrimidinone, arriving at a series previously explored by AstraZeneca, (**9**, Fig. 4).[31,48] However, the addition of one sp^3 carbon has an effect on the shape of the ring and thus the possible vectors into the enzyme pockets. In the five-membered aminohydantoins one of the groups of the quaternary center protrudes into the P2′ pocket (cyclopropyl group in **29**). In the amino-dihydropyrimidines series this group projects towards Ile[118]. As a result, only small substituents, like methyl, are tolerated.[48] In addition, the biaryl substituent of the amino-dihydropyrimidinone needs to be in a pseudo axial conformation to occupy the P1/P3 pocket. Amino-dihydropyrimidinone **30** exhibits moderate activity towards BACE1. Subsequent modification of the biaryl ring system to optimally occupy the P1 and P3 pocket, delivered highly active analogue **31** with an oral bioavailability of 69%, good permeability, an efflux ratio of 2.4 and an average brain/plasma ratio of 3 (Fig. 11).[48,49]

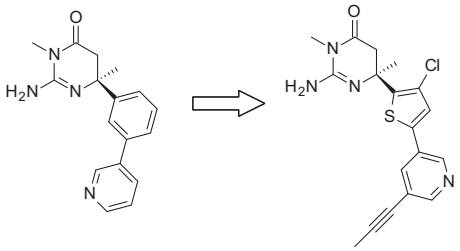
In an effort to boost the potency and access the P2′ pocket, Merck designed a series of conformationally constrained bicyclic-amino-dihydropyrimidinones.[50] Computationally aided design directed them to the series of pyrrolidine fused bicycles. The pyrrolidine nitrogen provided the optimal trajectory for the exploration of the P2′ pocket, which resulted in the identification of compounds such as **32**–**34** (Fig. 12).[50,51] By optimally substituting the pyrimidine and thus filling the P2′ pocket as in the case of **34**, Merck was able to remove the P3 substituent and maintain good potency.[51]



2, BACE1 K_i > 200 μM

12, BACE1 K_i = 3.7 μM

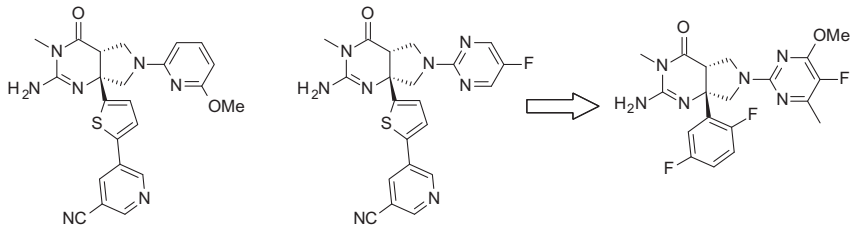
29, BACE1 K_i = 5.4 nM
Cell Aβ40 IC_{50} = 82 nM
clogP = 2.4
B/P ratio = 0.3
Rat $_{CSF\ A\beta40}$ = -54% @ 30 mg/kg po



30, BACE1 K_i = 670 nM
Cell Aβ40 IC_{50} = 460 nM

31, BACE1 K_i = 1.7 nM
Cell Aβ40 IC_{50} = 11 nM
F = 69%
Caco-2 = 153 nm.s^{-1}
PgP efflux = 2.4
B/P ratio = 3
Rat $_{cortex\ A\beta40}$ = -54% @ 10 mg/Kg po

Figure 11.

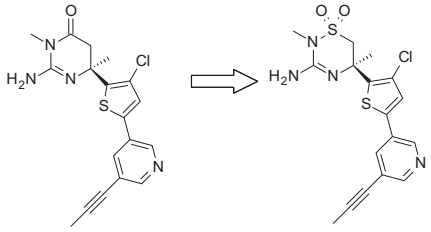


32, BACE1 K_i = 1 nM
Cell Aβ40 IC_{50} = 18 nM

33, BACE1 K_i = 4 nM
Cell Aβ40 IC_{50} = 16 nM F = 97%
Caco-2 = 151 nm.s^{-1}
PgP efflux = 1.9
B/P ratio = 0.1
Rat $_{CSF\ A\beta40}$ = -73% @ 30 mg/kg po
Rat $_{cortex\ A\beta40}$ = -42% @ 30 mg/kg po

34, BACE1 K_i = 8.5 nM
PgP efflux = 6.0

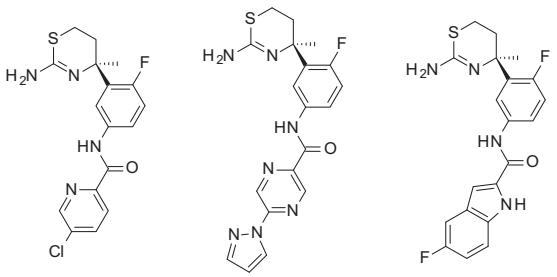
Figure 12.



35, BACE1 K_i = 1.7 nM
Pgp efflux = 2.4
Caco-2 = 153 nm.s^{-1}
Rat $_{cortex\ A\beta40}$ = -54% @ 10 mg/Kg po

36, BACE1 K_i = 2.4 nM
Pgp efflux = 1.6
Caco-2 = 278 nm.s^{-1}
Rat $_{cortex\ A\beta40}$ = -53% @ 10 mg/Kg po

Figure 13.



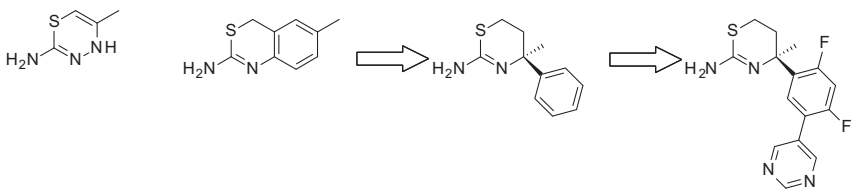
39, BACE IC_{50} = 18 nM **40**, BACE IC_{50} = 20 nM **41**, BACE IC_{50} = 20 nM

Figure 15.

The amino-dihydropyrimidinones were selected for their favorable pK_a range and their potential drugability. However, Merck also reported a modification of this series where the aminothiadiazine dioxide group was used as an isosteric replacement for the amino-dihydropyrimidinones. In this series, the sulfonamide was used to modulate the basicity of the amidine, while promoting good bioavailability and ligand affinity. Comparative data described show that the amino-thiadiazole oxides exhibit similar BACE1 affinity with a reduced Pgp efflux liability versus their amino-dihydropyrimidinone analogs (Fig. 13).[49,52]

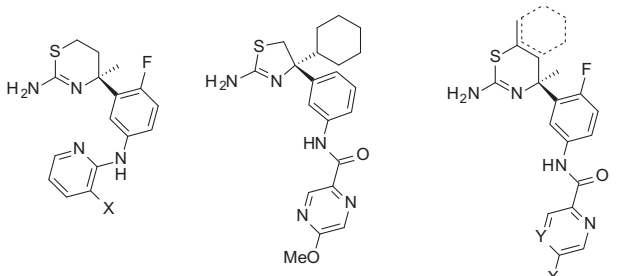
Eli Lilly identified isothiourea hits **3** and **4** in a fragment-based screening assay of approximately 8000 fragments (Fig. 14).[28] The original amino-benzothiazine **4** was a rigid bicyclic structure without a suitable vector into the P1/P3 region. Elimination of fused aromatic ring in **4** and subsequent substitution of newly formed sp^3 carbon resulted in the identification of amino-dihydrothiazine **37** with a 36-fold increase in potency. The phenyl ring of **37** occupies the P1 region and could be extended into the P3 pocket by substitution at the *meta*-position, resulting in sub-micro molar potency. Deactivation of the metabolically labile central aryl ring with fluorine atoms gave **38** (**LY-2811376**) (Fig. 14). This was the first BACE1 inhibitor of this amidine class to reach phase I clinical trials and showed prominent and long-lasting Aβ reductions in lumbar CSF after oral dosing of 30 and 90 mg in healthy volunteers.[28] Because of off-target retinal pathology, identified in long term preclinical toxicology studies, this compound was discontinued from further clinical evaluation.[28] Nevertheless, it showed the first evidence of a dose-dependent reduction of CSF APPβ in the human CNS by a BACE1 inhibitor and thus strengthened the theory of BACE1 being a promising target for treatment of AD.[30]

Already in 2007 Shionogi described BACE1 inhibitors with the same amino-dihydrothiazine warhead as used by Eli Lilly.[29] Interestingly Shionogi were the first to disclose the use of an amide linker between the two aryl rings as exemplified by **39** to **41** Figure 15. This linker resulted in a considerable boost in potency



3, BACE1 IC_{50} = 8200 μM **4**, BACE1 IC_{50} = 790 μM **37**, BACE1 IC_{50} = 22 μM **38**, BACE1 IC_{50} = 240 nM
Cell EC_{50} = 300 nM
LY-2811376

Figure 14.

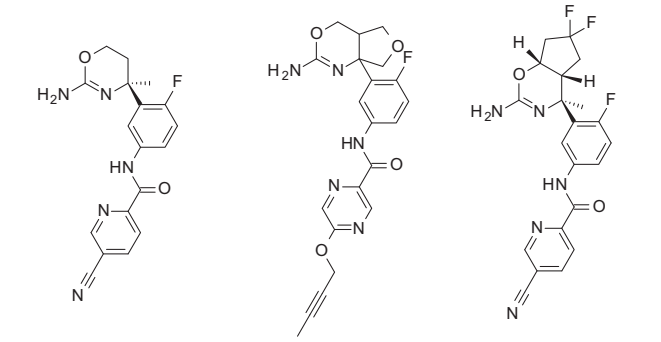


42, X = Cl
BACE1 IC_{50} = 83 nM
43, X = OMe
BACE1 IC_{50} = 52 nM

44, BACE1 IC_{50} = 514 nM

45

Figure 16.



46, BACE IC_{50} = 54nM **47**, BACE IC_{50} = 27nM **48**, BACE IC_{50} = 1.7nM

Figure 17.

when compared to the bis-aryl system. This boost is due to the formation of a favorable H-bond between the NH of the ligand and the carbonyl oxygen of back bone Gly[291].[28] Since the disclosure of this series, many R&D programs have incorporated these amide linkers into their series. Shionogi explored many amide-linked aromatic and alkylic systems, as exemplified by **40** and **41**, Figure 15.[29,53–55]

Shionogi also explored amine-linked bis-aryl-aminodihydrothiazine systems.[56] Contrary to the previous amide-linked systems, most P3 heteroaryl rings were substituted at the *ortho*-position (Fig. 16). In general, this modification appears a little less potent in vitro than the corresponding amide linked P1/P3 substituents.

While six-membered amino-dihydrothiazines were highly potent BACE1 inhibitors, five-membered analogues such as **44** demonstrated only moderate potency (Fig. 16).[57] Shionogi also explored modified versions of the cyclic isothiourea warhead with the introduction of *endo*- or *exo*-cyclic double bonds and additional ring fusions (Fig. 16).[57,58]

Replacement of sulfur with oxygen led to amino-dihydro-1,3-oxazines cores (**46**), a warhead which also appears in patent applications from multiple companies.[59] Eisai and Shionogi further refined both thiazine and oxazine series to explore bicyclic versions such as **47**.[60,61] Interestingly these are analogous to the bicyclic amino-dihydro-1,3-thiazines cores described by Eisai (Fig. 19, **53** and **54**) and Eli Lilly (Fig. 22 and **62**) vide infra. In addition to monocyclic amino-dihydro-1,3-oxazine warhead, Roche also described alternatively fused bicyclic derivatives, represented by **48**.[62,63] They further decorated the fused cyclo-pentane with a *gem*-difluoro functional group, supposedly to modulate the pK_a of the system.

Roche's investigation was initiated by the identification of a dihydrothiazine HTS hit (**7**, Fig. 3).[28] Modifications of the warhead allowed them to optimise physiochemical properties, such as pK_a, log D, solubility and permeability.[63] Their exploration showed that exceedingly basic compounds resulted in low activity in vivo, which was attributed to low exposure (Fig. 18).

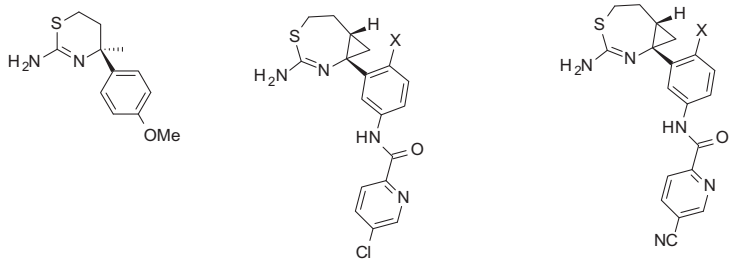
Roche also identified a series of seven-membered cyclopropyl-fused 1,3-thiazepines.[64] It is speculated that the fusion of the cyclo-propyl alters the bond angles, and thus has an effect of the orientation of groups in the P1 and P3 pockets. It also has an effect on the pK_a of the amino-thiazine functional group due to the electron-withdrawing properties of the cyclopropyl ring, resulting from its enhanced π-character (**49**, pK_a = 8.9). Interestingly, the introduction of a fluorine atom to the P1 phenyl results in a marked decrease in potency, which is perhaps due to steric clash between the cyclopropyl and the introduced fluorine. This series of compounds shows greater potency for BACE2 than for BACE1.[64]

Eisai described several series of bicyclic aminodihydrothiazines fused with unsaturated five- and six-membered rings, exhibiting high affinities for BACE1 (Fig. 19).[65–67]

Eisai recently published two process patent applications that highlighted warheads (**55**–**57**) and P3 substituents (**58**) of interest (Fig. 20).[68,69] It is possible that a combination of one of these warheads with **58** represents their clinical candidate, for example **59**.

Eisai further took advantage of the properties of the amino-dihydropyrimidinones integrating a ring fusion which incorporates a substituted tetrahydrofuran as in **60** (Fig. 21).[70] Merck applied the previously discussed replacement of the amino-dihydropyrimidinones with iminothiadiazine dioxides, to explore a series of analogous bicyclic derivatives exemplified by **61**.[71]

Similar to Eisai and Shionogi, Eli Lilly also explored a series of fused aminodihydrothiazine derivatives, which resulted in their

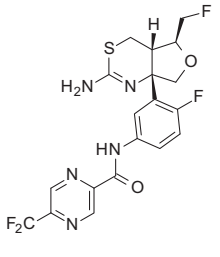


7, Cell IC_{50} = 41.2 μM

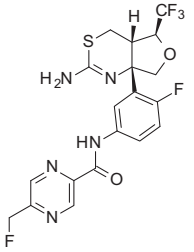
49, BACE1 X = H; Cell IC_{50} = 5 nM
BACE2 X = H; Cell IC_{50} = 2 nM
50, BACE1 X = F; Cell IC_{50} = 26 nM
BACE2 X = F; Cell IC_{50} = 2 nM

51, BACE1 X = H; Cell IC_{50} = 31 nM
BACE2 X = H; Cell IC_{50} = 1 nM
52, BACE1 X = F; Cell IC_{50} = 60 nM
BACE2 X = F; Cell IC_{50} = 5 nM

Figure 18.

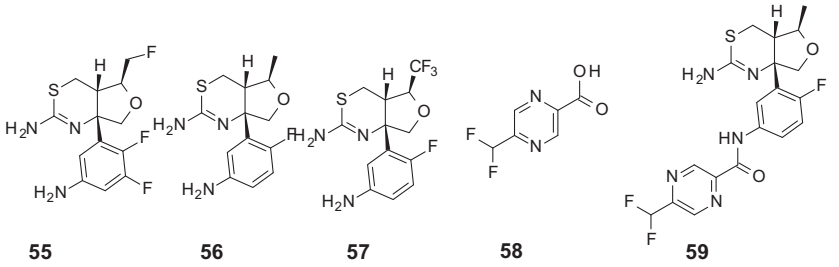


53, BACE1 IC_{50} = 7 nM
$Rat_{brain\ A\beta40}$ = -64% @ 10 mg/Kg po, 6h
$Rat_{CSF\ A\beta40}$ = -70% @ 10 mg/Kg po, 6h
[Plasma] = 570 nM, 6h
[Brain] = 570 nM, 6h
B/P = 0.7
Pgp efflux = 20.7



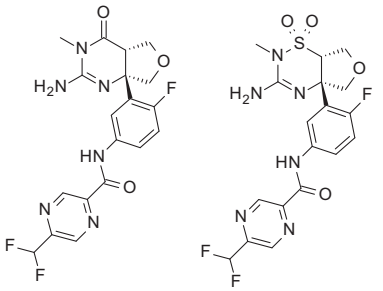
54, BACE1 IC_{50} = 6 nM
$Rat_{brain\ A\beta40}$ = -72% @ 10 mg/Kg po, 6h
$Rat_{CSF\ A\beta40}$ = -74% @ 10 mg/Kg po, 6h
[Plasma] = 229 nM, 6h
[Brain] = 845 nM, 6h
B/P ratio = 3.7
Pgp efflux = 1.7

Figure 19.



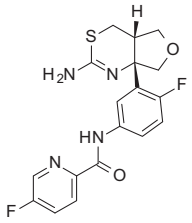
55 **56** **57** **58** **59**

Figure 20.



60

61, BACE1 Ki = 27.2 nM
BACE2 Ki = 17.4 nM



62, BACE1 IC_{50} = 20.3 nM
LY-2886721

Figure 22.

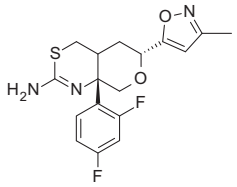
Figure 21.

second BACE1 inhibitor tested in the clinic, **62** (**LY-2886721**).[72] In June 2013 the clinical investigation of this compound was stopped due to abnormal liver function; the company's press release suggests that this is not target mediated so interest in the target remains.[73]

Pfizer also described some bicyclic aminodihydrothiazine derivatives, however in their examples the pyran ring was substituted with a heteroaromatic, such as isoxazole in **63** (Fig. 23).[74] This additional substitution apparently boosted the potency sufficiently to allow truncation of the molecule and removal of the P3 substituent. This had a favorable effect on the TPSA of the system and as a consequence may enhance permeability.

Further to the 1,3-oxazine warhead (Fig. 17), the 1,4-oxazine warhead has been independently exemplified in various patent applications and publications by Janssen,[75] Novartis,[76] Roche[77] and AstraZeneca.[25] Based on the filing dates of the various applications, the investigations were ongoing simultaneously. The P1/P3 investigations scan all chemical space and examples of amide (**64**),[77] amino linked (**65**)[78] and directly linked (**66**)[79] P1/P3 systems are described in the various patents (Fig. 24). Roche published a broad exploration of 1,4-oxazines investigating both the six- and seven-membered systems. They seem to concentrate their efforts on seven-membered systems, in which they explore many substitution patterns on the warhead.[80]

Analogously to their work on the seven-membered cyclopropyl-fused-1,3-thiazepine Roche also explored cyclopropyl-fused-1,4-homo-oxazepine (Fig. 25).[64,81] However, the seven-membered cyclopropyl-fused-1,3-thiazepine are more potent against BACE1 than the oxygen analogs in cell based assays (**50**, IC_{50} = 26 nM, **67**, IC_{50} = 73 nM). Interestingly the oxygen analogs showed an inversed selectivity over BACE2 compared to the amino-thiazine analog **50**. Other substitution patterns have been explored as



63, BACE1 IC_{50} = 59 nM
BACE2 IC_{50} = 4.55μM

Figure 23.

exemplified by **68**–**70**. Efforts seem to concentrate around smaller electron-withdrawing substituents, which is presumably to control the pK_a of the amidine, which for **69** has been measured at a pK_a >10.[63]

Novartis and Janssen concentrated their research efforts on six-membered 1,4-oxazine derivatives. Interestingly, they both focus on electron withdrawing substituents which may have a positive effect over the pK_a of the amidine (Fig. 26).[82]

Novartis explored the addition of placing fluorine atoms on the quaternary methyl, presumably in an attempt to modulate the pK_a of the system. Interestingly the mono-fluoro-methyl (**72**) and the difluoromethyl (**73**) are more potent that the corresponding methyl analog (**71**), while the trifluoromethyl derivative (**74**) is less potent.

Novartis explored other ways to control the pK_a of the amidine, one other example is the introduction of a CF_3 alpha to the amidine as in **75**–**77**.[82,83] Compound **75** is also the subject of a recent process patent for the isolation of a crystalline derivative, which suggests a high level of interest in this particular molecule.[84]

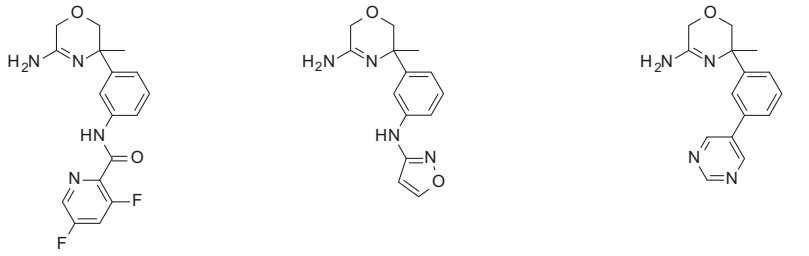
Janssen's patent applications concentrate on the introduction of electron withdrawing groups on the morpholino 6-position to modulate the pK_a of the system. Their modifications include the addition of mono-fluorine **78**, difluoromethyl **79**, trifluoromethyl **80** and also trifluoromethyls and fluorine combined as in **81**.[85,86]

Novartis disclosed a potent series of BACE1 inhibitors based on a tetrahydropyrazine warhead (**82**, Fig. 27).[87] The addition of a carbonyl to this system was investigated by Janssen and Novartis,[87–89] with representative examples **84**–**85** from Janssen in Figure 27.[90] In an attempt to improve the properties of their initial series of 2-amino-3,4-dihydroquinazolines **83** which exhibited high lipophilicity and basicity, they adopted an in silico virtual screen to triage de novo ideas. This led to the identification of the two regio-isomeric forms of the piperazinone warhead **84** and **85**. Predicted and measured pK_a values were found to be consistent with the required protonation at the site of action.

Janssen continued their interest in piperazine warheads and published several patent applications around piperazine derivatives fused to five-membered electron deficient heteroaromatics **86**–**87** (Fig. 28).[91,92] Shionogi and Schering also explored the introduction of an electron deficient heteroaromatic ring, however their respective series fuse the ring to an amino-dihydropyrimidine warhead, with representative structure **88** shown below.[93,94]

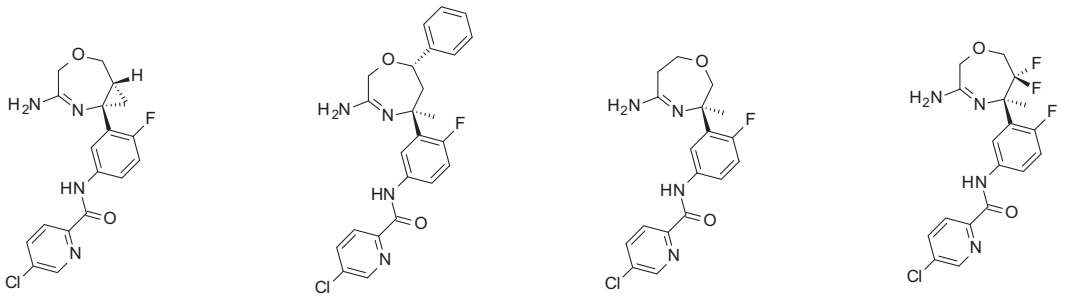
As yet another morpholino alternative, Merck reported a series of oxidized thio-1,4-oxazine cores **89**–**90** where the electron withdrawing sulfone group will result in a reduction in pK_a of the amidine.[95,96] It appears that to optimize for potency, the substitutes on the C-6 position should be limited to smaller groups. In essence, this can also be considered a modification of the previously described iminothiadiazine dioxides warhead, exemplified by **91**. This compound is specifically claimed in WO-2011/044181 and may well represent or resemble the structure of MK-8931 (Fig. 29).[49]

Chronologically, the warhead motif has increased in complexity, looking for ever more inventive ways to combine the properties required for high BACE1 affinity, while not infringing on the intellectual space of another. This has been made necessary by the limited chemical space available for potent BACE1 inhibitors



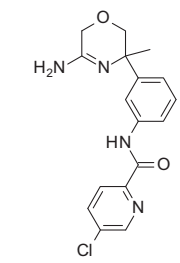
64, BACE1 IC_{50} = 120 nM **65**, BACE1 IC_{50} = 580 nM **66**, BACE1 IC_{50} = 710 nM

Figure 24.

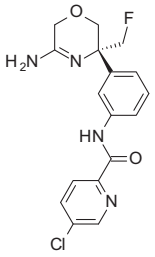


67, BACE1 Cell IC_{50} = 0.073 μM **68**, BACE1 Cell IC_{50} = 142.0 μM **69**, BACE1 Cell IC_{50} = 0.011 μM **70**, BACE1 Cell IC_{50} = 0.001 μM
BACE2 Cell IC_{50} = 11.06 μM BACE2 Cell IC_{50} = 5.02 μM BACE2 Cell IC_{50} = 0.011 μM BACE2 Cell IC_{50} = 0.005 μM

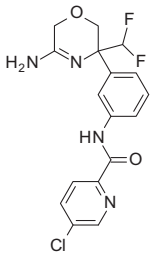
Figure 25.



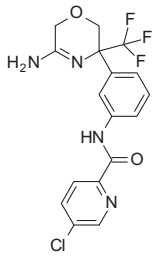
71, BACE1
IC_{50} = 240 nM



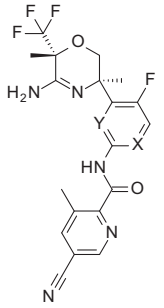
72, BACE1
IC_{50} = 26 nM



73, BACE1
IC_{50} = 30 nM



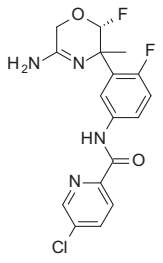
74, BACE1
IC_{50} = 420 nM



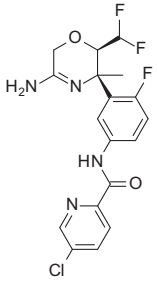
75, X = CH, Y = CH
BACE1 IC_{50} = 10 nM

76, X = N, Y = CH
BACE1 IC_{50} = 2 nM

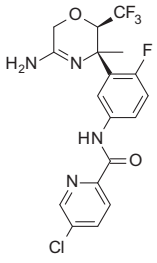
77, X = CH, Y = N
BACE1 IC_{50} = 77 nM



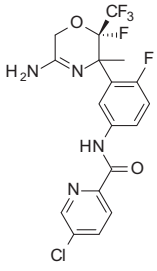
78, BACE1
IC_{50} = 20 nM



79, BACE1
IC_{50} = 40 nM

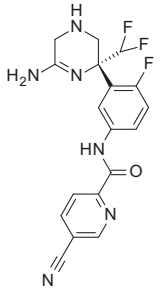


80, BACE1
IC_{50} = 34 nM

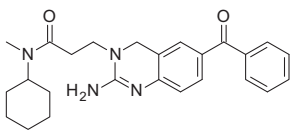


81, BACE1
IC_{50} = 25 nM

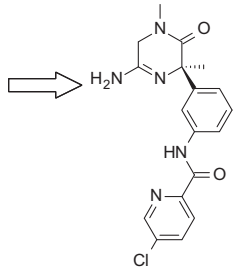
Figure 26.



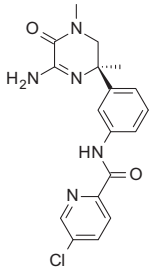
82, BACE1
IC_{50} = 28 nM



83

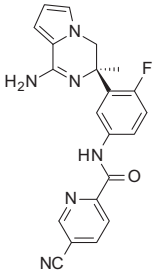


84, BACE1
IC_{50} = 0.324 μM
pKa = 7.8

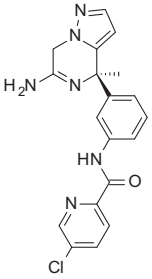


85, BACE1
IC_{50} = 0.295 μM
pKa = 7.6

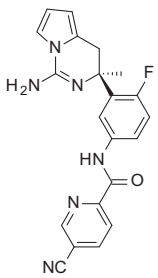
Figure 27.



86, BACE1
pIC_{50} = 7.71

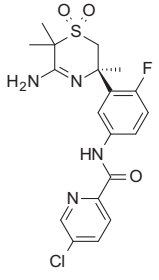


87, BACE1
pIC_{50} = 7.31

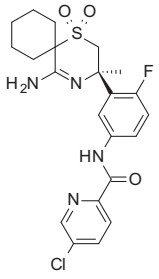


88, BACE1
IC_{50} = 36 nM

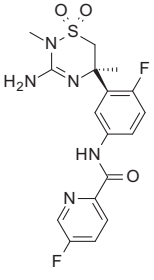
Figure 28.



89, BACE1
IC_{50} = 1.0 nM



90, BACE1
IC_{50} = 8.4 nM



91

Figure 29.

and the high level of activity within the field. Figure 30 gives an overview of the chemo-types explored and the companies involved in their exploration. While not exhaustive, it gives an idea of the competitiveness of the field and the overlap between various R&D investigations.

The need for an amidine-based functional group in this class of BACE1 inhibitors has created its own unique challengers for development, including Pgp and hERG. The basic center is required for the affinity to the catalytic dyad and as such needs to be protonated at the site of action. Chronologically, through the various R&D investigations we have seen the modulation of this basic center in ever more inventive ways, while maintaining the possibility to protonate as required.

All non-peptidomimetic small molecule BACE1 inhibitors have had to deal with the very large active site. The class of inhibitors described in this review has delivered the optimal vectors, aided by structure based design. This led to the discovery of the quaternary center and the possibility to fill/explore the P1/P3 and the P2′ pockets optimally. The identification of the H-bond with Gly[291], created by the amide linker between the P1 and P3 substituents led to a boost in potency. The need to combine all these components has led to compounds with TPSA's close to and above 100 Å.[2] Nevertheless, many of them have demonstrated good permeability and brain penetration. This may be explained by the masking of the amide polar surface area by an intra-molecular hydrogen bond with the nitrogen of the heterocycle P3 targeting substituent.[97]

In general, the selectivity of this amidine based class of BACE1 inhibitors over similar proteases such as cathepsin D and E, renin, and pepsin has been reported to be excellent. On the other hand, selectivity over the more homologous BACE2 enzyme appears to be more difficult to achieve, and many are reported to have the same level of potency for BACE1 and BACE2. BACE2 has recently been identified as being involved in the regulation of pancreatic β cell mass and function and BACE2 inhibition has been postulated as a mechanism to treat Type II diabetes.[55] BACE2 has also been recognized as an efficient Aβ protease.[98] BACE2 has further been identified to play a role in the processing of pigment cell-specific Melanocyte Protein (PMEL), revealing the importance of BACE2 in pigmentation.[99] These roles for BACE2 may hint to possible toxic consequences of chronic, non-selective BACE1 inhibition.

While BACE1 inhibition clearly results in reduced formation of Aβ, investigations are also ongoing to understand the possible toxic consequences of BACE1 inhibition itself. Initial reports suggested the existence of a subtle phenotype for the BACE1 KO animals, which reinforced the attraction of BACE1 inhibition as a therapy for AD.[100–104] However, the identification of more than 60 BACE1 substrates including neuregulin, cast doubt on these preliminary observations.[105,106] Neuregulin-1 or at least the Ig-containing β1 NRG1 (IgNrgβ1) isoform, has been identified as a substrate for BACE1. It has been found to regulate the muscle spindle physiology and maintain motor coordination.[106] It is thus possible that extended inhibition of BACE1 may result in disrupted muscle spindle functions and may lead to impaired movement.[106] Cai et al.

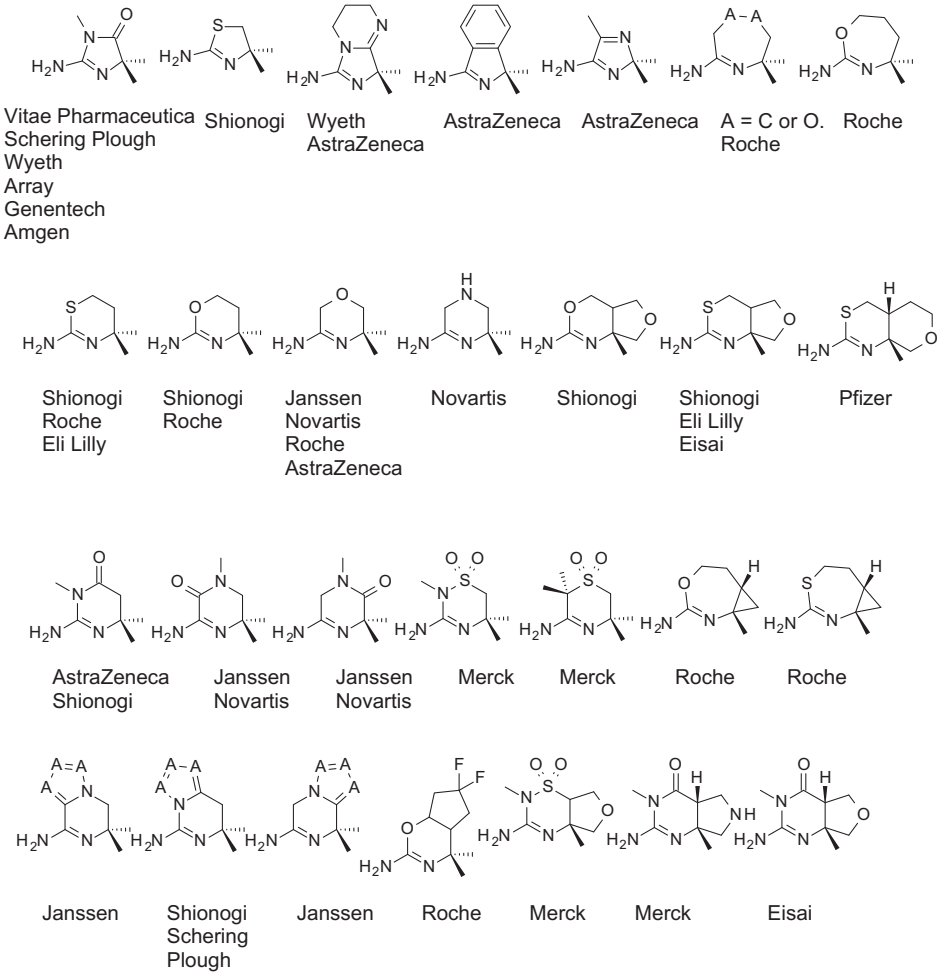


Figure 30.

described retinal pathology resulting from BACE1 inhibition.[107] The retinal toxicity observed in BACE1 KO mice includes retinal thinning, vascular abnormalities and an increase in age pigment. These critical findings and the fact that BACE1 inhibitors will need to be chronically dosed, highlight the need to monitor for adverse toxicity during the development of BACE1 inhibitors and the determination of a therapeutic window.

While it may be possible to design an orthosteric BACE1 inhibitor with selectivity over BACE2, substrate, that is APP, selectivity may not be possible with an orthosteric inhibitor, especially one whose binding mode is associated to the catalytic dyad. Novel modes of modulation of BACE1 activity may be required to selectively target APP processing.

This latest generation of BACE1 inhibitors has clearly been more successful and achieved more advanced milestones, than the earlier incarnations. Of this generation of BACE1 inhibitors seven have successfully reached clinical analysis. There have been some failures as with the two compounds from Eli Lilly (**LY-2811376** & **LY-2886721**), **AZD-3839** from AstraZeneca vide supra, and **RG-7129** from Roche which was recently terminated in phase I. Many other companies still have BACE1 inhibitors undergoing clinical analysis. The most advanced of these is **MK-8931** from Merck, which has started recruiting for the phase III trial in prodromal and mild to moderate AD in patients from 55 to 85 years old.[108] Should the readout of **MK-8931** be successful, Merck will likely have the first oral disease modifying therapeutic for mild to moderate Alzheimer's disease. Merck is not alone in the clinic and is followed by compounds from Eisai (**E2609**) and AstraZeneca (**AZD-3293**). Presumably, after the successful data generated during the various phase I clinical trials, Eisai will imminently start their phase II analysis of **E2609**. Eisai is currently analyzing the metabolism and elimination in an open label single dose of [14C]-**E2609**, in healthy volunteers.[109] AstraZeneca is progressing **AZD-3293** through several phase I trials, of which the first has been completed. They are currently recruiting for two phase I clinical trials to assess the effect of cytochrome 3A4 inhibitors on the pharmacokinetics of **AZD-3293** in healthy volunteers, and to assess the safety and effect of **AZD-3293** in healthy elderly and AD patients.[110,111]

The results of these clinical trials may validate not only the amyloid hypothesis but also whether extended inhibition of BACE1 is therapeutically viable as a treatment of AD. Questions to be answered by these trials will be the required level of BACE1 inhibition, and the optimal moment of intervention in the disease progression. Future BACE1 inhibitors are likely to require a better profile, being more selective for not only BACE1 but also APP.

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