Bioorganic & Medicinal Chemistry Letters 22 (2012) 240–244

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

# Discovery of pyrrolidine-based β-secretase inhibitors: Lead advancement through conformational design for maintenance of ligand binding efficiency

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ARTICLE INFO

*Article history:*
Received 9 October 2011
Revised 4 November 2011
Accepted 7 November 2011
Available online 12 November 2011

*Keywords:*
BACE-1
Inhibitor
Pyrrolidine
Ligand binding efficiency
Rational design
Conformational design

ABSTRACT

We have developed a novel series of pyrrolidine derived BACE-1 inhibitors. The potency of the weak initial lead structure was enhanced using library-based SAR methods. The series was then further advanced by rational design while maintaining a minimal ligand binding efficiency threshold. Ultimately, the co-crystal structure was obtained revealing that these inhibitors interacted with the enzyme in a unique fashion. In all, the potency of the series was enhanced by *4 orders of magnitude* from the HTS lead with concomitant increases in physical properties needed for series advancement. The progression of these developments in a systematic fashion is described.



Alzheimer's disease (AD) is rapidly becoming one of the largest unmet medical needs in the developed world. This issue is further exacerbated by the burgeoning population of the age group most at risk for the disease. More than 5 million Americans over the age of 65 are estimated to be affected, with costs for treatment to be upward of $20 trillion dollars over the next 40 years.[1] These facts coupled with the realization that there is currently no disease modifying treatments available serve as further impetus in the search for an effective therapeutic agent. As β-secretase (BACE-1) is responsible for the initial cleavage event in the catabolism of the amyloid precursor protein (APP) and the foundation of the amyloid cascade, it has gained a prominent role in the quest for a disease modifying treatment for AD.[2]

The first generation of BACE-1 inhibitors were mainly peptidic in character. The scissile amide bond was replaced with either a hydroxyethyl amine (HEA), hydroxyethylene (HE), or reduced amide transition state isostere. Inhibitors based on this design principle have historically displayed poor pharmacokinetic properties as well as P-glycoprotein (P-gp) efflux liabilities. In an effort to address these shortcomings, second generation BACE-1 inhibitors have centered on novel templates that rely on non-linear structures to fill the active site.[3] To identify novel non-peptidic scaffolds that have higher potential to achieve acceptable pharmacokinetic properties researchers have resorted to intensive screening of their internal compound collections while also leveraging fragment based screening and other novel methods for lead identification.

We have previously reported a novel series of 2-aminothiazole BACE-1 inhibitors that was identified using a high concentration high throughput screen (HTS) of the Merck sample collection.[4] In addition to this aforementioned described hit, 3,4-disubstituted pyrrolidine **1**, as a mixture of trans diastereomers, was also identified as a low potency inhibitor of BACE-1 (Fig. 1). While the intrinsic potency of **1** was very weak in the initial enzymatic assay ($IC_{50}$ = 240 μM) we viewed the unique pyrrolidine structural scaffold as having potential to achieve a final compound devoid of some of the physical property limitations present in other inhibitor series. The synthetic route also allowed for facile access to the pyrrolidine scaffold using a dipolar cycloaddition reaction of the appropriate *trans*-cinnamate ester with an azomethine ylide precursor.[5] Using the HTS hit **1** as a starting point we envisaged that the amide linkage would serve as an ideal starting point for rapid SAR development though library-based compound synthesis. However, the SAR

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doi:10.1016/j.bmcl.2011.11.024

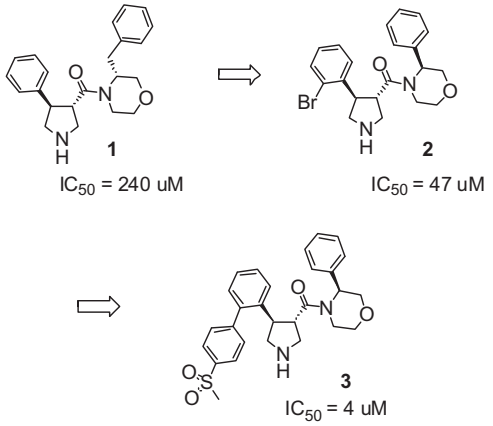



**Figure 1.** Elaboration of HTS hit.

proved very limited in this region and the 2-phenyl morpholine analog (**2**; $IC_{50}$ = 47 μM) was found to be the best fit during our screen. Concomitantly, we explored the effect of halogen substitution on the western phenyl ring which served two functions. First, bromine substitution would serve as a synthetic handle with which we hoped to further optimize the potency. Secondly, we hoped the electron density of a suitably positioned bromine atom would enable a better density fit should a low resolution co-crystal structure be obtained and would provide a vantage point to guide further analog design. With compound **2** in hand, we subsequently performed a Suzuki coupling library using the pendant aryl bromide as the functional handle as described above. Gratifyingly, compound **3** emerged from this screen with a 60-fold improvement in potency over the initial HTS lead **1**. Unfortunately, while improvements in intrinsic potency were realized the increased enzyme potency came at the expense of ligand binding efficiency. While HTS hit **1** possessed a LBE of 0.19, analog **3** exhibited a slightly lower LBE of 0.17. Since additional potency would need to be achieved before the series could be viewed as a serious lead class we viewed this drift in LBE as a move in the wrong direction and resolved to keep molecular weight low and focus our efforts with maximal ligand efficiency in mind going forward.

In the campaign advancing the series to biphenyl analog **3,** we continued to explore the eastern portion of the molecule through additional amide SAR. As noted above, the SAR was particularly restricted but we observed that in addition to the 2-substituted morpholine (**2**), the corresponding carbon analog, that is pyrrolidine **4**, possessed similar potency. Interestingly the 4-phenyl piperidine analog (**5**) also emerged from the scan of this region, albeit with an even more stringent SAR profile (Fig. 2). With these two analogs in hand we contemplated the effects of conjoining the two substitution patterns into a single molecular entity. We subsequently synthesized the hybrid *cis*-2,4-diphenyl analog **6** and were rewarded by a synergistic increase in potency to 600 nM, representing a 400-fold increase in potency from the starting HTS hit in addition to increasing the LBE to 0.25.

Cognizant of the lessons learned early in the series development, we did not want to pursue further potency enhancements at the expense of increasing molecular weight and additional biaryl bonds that could impart undesirable physical properties. As such, we approached future target design with a keen eye toward maintaining the ligand binding efficiency (LBE) represented by compound **6**. One way to effect increasing enzymatic potency is to reduce the entropy of binding thereby restricting the inhibitor into its bioactive conformation. Using this approach we were able to effect a 10-fold increase in binding potency from compound **7** to the 6-member spiro-fused analog **8** which resulted in an $IC_{50}$ = 4 μM whilst maintaining a favorable LBE of 0.26 (Fig. 3). Ultimately, by employing

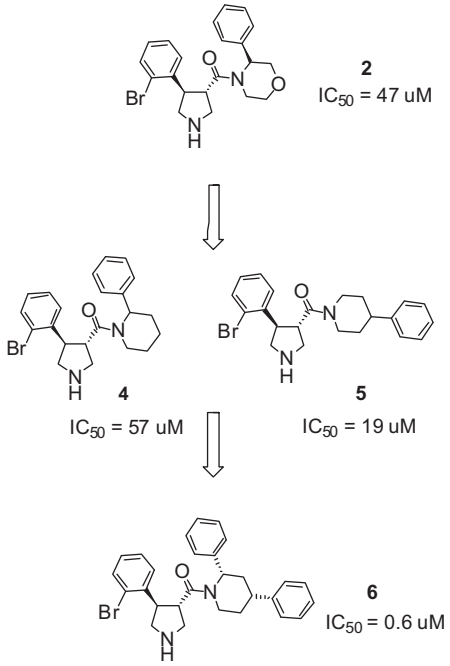



**Figure 2.** Synergistic binding potency via SAR combination.

the potency enhancing *cis*-2,4-bis substituted piperidinyl amide delineated in the non-fused system, an $IC_{50}$ of 0.15 μM was realized in compound **9** while achieving a further enhanced LBE of 0.28.

More importantly, with compound **9** we were finally successful in obtaining a co-crystal in the BACE-1 active site.[6] Analysis of the crystallographic data revealed several interesting elements of the interaction between the inhibitor and the enzyme. First, while not surprising, it was apparent that the pyrrolidine nitrogen was positioned between the catalytic dyad interacting directly through a bidentate interaction with D32 and D228 (Fig. 4). This differs from previously published reduced amide isostere BACE-1 inhibitors,[7] in which the secondary amine hydrogen bonds only to the 'outside' oxygen (OD2) of D228.

The tetrahydronapthalene portion of the molecule was found to occupy the $S_1$ region of the enzyme, while the diphenyl piperidine resides in an area of the enzyme that is not accessed by the standard Schecter-Berger peptide subsites.[8] Here, the 4-phenyl group (Fig. 5) was neatly packed into a nascent binding pocket under the flap and adjacent to the $S_1$ pocket. This is created by an upward motion of the flap accompanied by an outward swing of the side-chain of Y71, which normally H-bonds to W76 in the flap-closed form of BACE. This readily explains the lack of substitution tolerated in this compacted region. Also apparent is that the 2-phenyl piperidine substituent is oriented toward the $S_2$' pocket, allowing

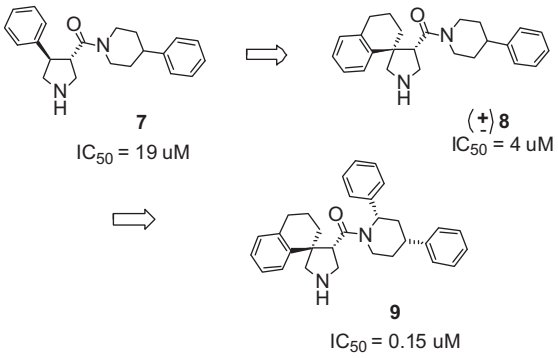



**Figure 3.** Spirocyclization leads to enhanced potency.

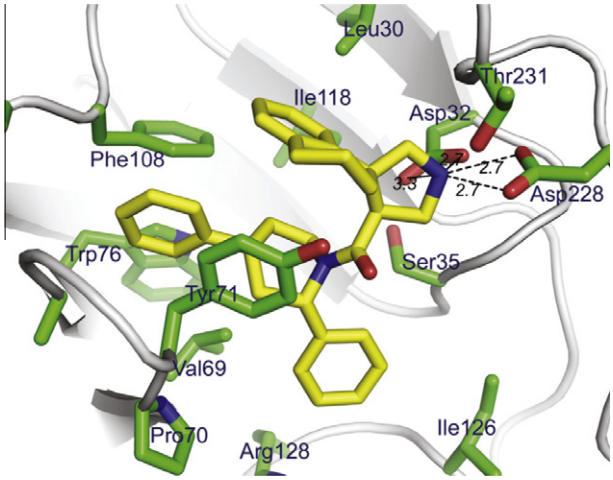



**Figure 4.** Interaction of pyrrolidine with catalytic aspartates.

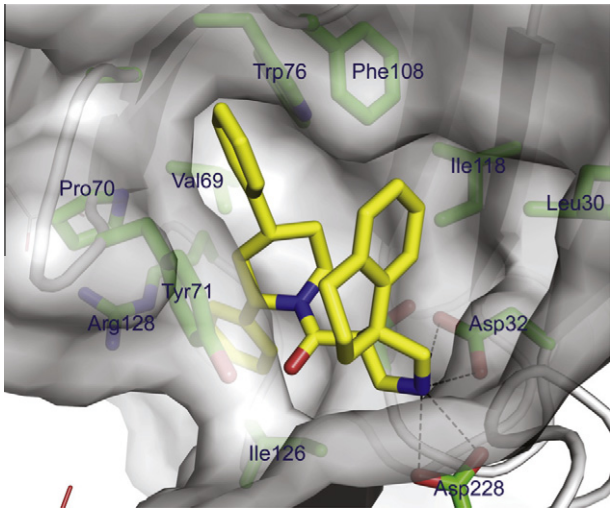



**Figure 5.** 4-Phenyl substituent occupies expanded $S_1$ pocket under the flap.

modest changes in structure, with some limitations due to the vector induced by the ring conformation imposed by the piperidine (vida infra).

Having obtained the co-crystal structure, we subsequently 'walked' a bromide around the constrained system to confirm whether the binding vectors remained similar to the non-constrained system. Through this exercise we were able to effect a further 2-fold enhancement of activity to produce compound **10** with an $IC_{50}$ = 0.07 μM by the addition of a 4-bromo substituent (Fig. 6). Surprisingly, the optimal position for bromine placement in the spiro system was the 4-position in contrast to that of the non-fused congener that preferred the 2-position. Upon analysis of the co-crystal structure of **10**, the additional potency was easily rationalized as it fit into a small hydrophobic pocket packing neatly against the enzyme.

It was also readily apparent from the crystal structure that the disubstituted piperidine ring occupied a twist-boat conformation. We had assumed this to be the case prior to obtaining the co-crystal structure based on the prevailing literature on 2-substituted piperidyl amides.[9] In these type of systems containing a 2-subtituted *N*-acyl piperidine, the prevailing $A_{1,3}$-strain interaction between the acyl group and the 2-substituent forces the adjacent 2-substituent into an axial orientation. With the additional 4-substituent aligned *cis* this would result in an unfavorable di-axial arrangement.

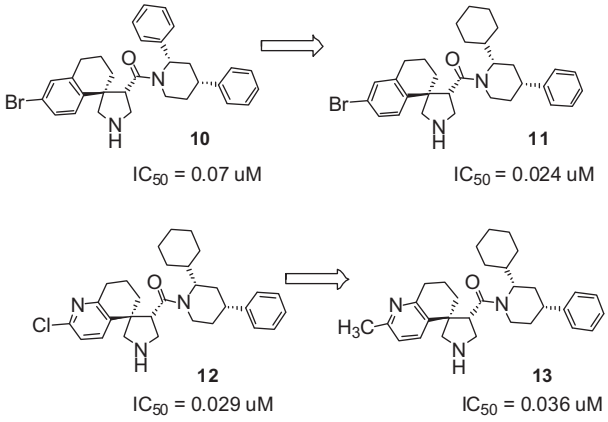
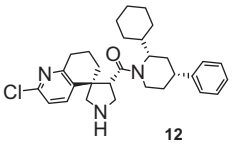



**Figure 6.** Optimization of spirocyclic ring system.

To relieve the strain from this type of configuration the ring must adopt a twist-boat conformation as its low energy state. As such, we conducted an investigation of various 2-piperidine substituents to exploit the effects of $A_{1-3}$-strain on the enforcement of the twist boat conformation in the non-spiro system. We found the 2-cyclohexyl substituent to be the optimal group as far as enforcement of the twist boat conformation in addition to more efficiently filling the hydrophobic pocket created in the region.[10] We incorporated the newly optimized 2-cyclohexyl substituent into the spiro-system and synthesized compound **11** which showed an $IC_{50}$ of 0.024 μM.

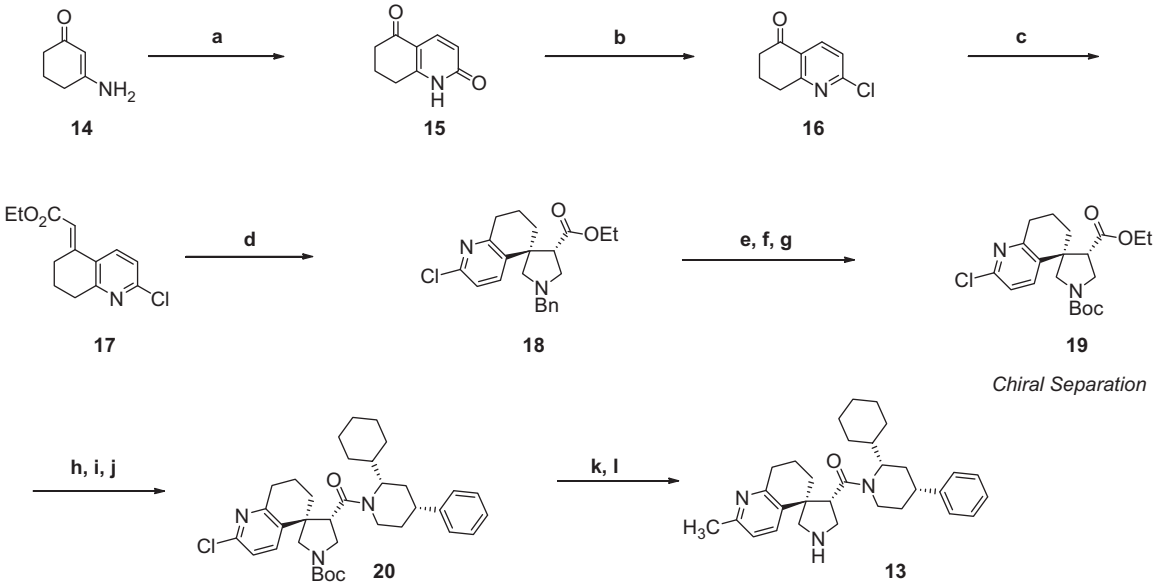
Unfortunately, while substantial progress was realized with respect to intrinsic potency toward the enzyme, there was a pronounced potency shift when the compounds were assessed in our cellular assay. As a matter of reference, while compound **11** exhibited an intrinsic potency of 24 nM, its functional activity in a cell-based assay was shifted more than 71-fold to 1700 nM. Since these compounds are very hydrophobic this was not particularly surprising since initial efforts were grounded in optimization of the binding which is often most readily accomplished through hydrophobic interactions.

Having secured a competent and enzymatically potent lead series we next sought to introduce a measure of 'subtle polarity' in order to decrease the effective shift between the enzymatic and cellular assay. To accomplish this we introduced a nitrogen atom into the fused aromatic tetrahydronapthalene ring of **11**. Analysis of the co-crystal structure revealed no significant negative interaction perceived by the introduction of the pyridyl nitrogen. We also replaced the 2-bromo substituent, with a chlorine atom, as an optimized binding element that would have reduced potential for untoward electrophilic reactivity. These modifications resulted in chloropyridine **12** in which the enzymatic potency remained relatively constant at 29 nM, however the corresponding cellular $IC_{50}$




| *Assay* | *Value* |
|---|---|
| BACE-1 $IC_{50}$ | 29 nM |
| sAPPβ_NF $IC_{50}$[a] | 570 nM |
| BACE-2 $IC_{50}$ | 29 nM |
| Renin $IC_{50}$ | 106000 nM |
| Cat D $IC_{50}$ | 6212 nM |
| Clearance (rat)[b] | 15 mL/min/kg |
| %F (rat)[c] | 44% |
| LBE | 0.3 |

**Figure 7.** Detailed profile of BACE-1 inhibitor **12**. [a]cell-based assay.[11] [b]2 mpk iv dose. [c]10 mpk po dose.




**Scheme 1.** Reagents and conditions: (a) methyl propiolate, 170 °C, neat, 33%; (b) $POCl_3$, 62%; (c) triethylphosphonoacetate, NaH, THF, 23% *E*; (d) *N*-(methoxymethyl)-*N*-(trimethylsilylmethyl)benzylamine, cat. TFA, $CH_2Cl_2$, 100% ; (e) 1-chloroethyl chloroformate, $CH_2Cl_2$; (f) MeOH; (g) $BOC_2O$, THF, 87% over 3 steps; (h) 2 N NaOH, THF/MeOH, 50 °C; (i) oxalyl chloride, DMF, $CH_2Cl_2$; (j) 2-cyclohexyl-4-phenyl-piperidine, triethylamine, $CH_2Cl_2$, 70% over 2 steps; (k) $NiCl_2$(dppp), MeMgBr, THF, 43%; (l) TFA, $CH_2Cl_2$, 73%.

was improved to 570 nM representing a reduced shift between the two assays of 19-fold (compared to a 71-fold shift for **11**). Compound **12** also had the additional attributes of selectivity toward other related aspartyl proteases, modest in vivo clearance in rats (15 mL/min/kg) and a respectable oral bioavailability of 44% (Fig. 7). Finally, replacement of the chloride with a 2-methyl substituent provided the more basic pyridyl derivative **13**, which again retained a favorable enzymatic potency with an $IC_{50}$ = 36 nM, but now the corresponding cellular potency was even further improved to 100 nM—a nominal 3-fold shift in potency between the enzymatic and cellular assays.

The synthesis of compound **13** is depicted in Scheme 1. 3-Aminocyclohex-2-en-1-ol (**14**) was condensed under thermal conditions with methyl propiolate to provide pyridinone **15**.[12] Transformation of **15** to chloropyridine **16** was readily accomplished through the action of phosphorus oxychloride. Horner-Emmons olefination of ketone **16** produced the unsaturated ester **17** as a 3:1 mixture of *E* to *Z* isomers that were readily separable by flash chromatography. Formation of the tetrahydronapthalene was then be realized via a 1,3-dipolar cycloaddition employing the azomethine ylide generated from *N*-(methoxymethyl)-*N*-(trimethylsilylmethyl)benzylamine with TFA as the catalyzing agent. Typically, cycloadditions of tri-substituted olefins are very difficult without at least two electron withdrawing groups. We were pleased to find that compound **17** underwent facile cycloaddition to give pyrrolidine quinoline **18**. In contrast, cycloadditions of earlier congeners were much more sluggish and excess equivalents of the azomethine ylide were necessary to effect full conversion. 1-Chloroethyl chloroformate mediated *N*-debenzylation of **18** and conversion to the corresponding *N*-Boc derivative resulted in compound **19**. This transformation resolved any issues with late-stage hydrogenative removal of the *N*-benzyl group in the presence of an aryl chloride, such as in compound **12**. Hydrolysis of the ester, activation of the carboxyl as the acid chloride, and amide formation produced the corresponding amide **20**. Finally, nickel mediated methylation followed by Boc deprotection produced the desired compound **13**.

In summary, we have developed a novel series of pyrrolidine derived BACE-1 inhibitors. The potency of the weak initial lead structure was dramatically enhanced using library-based SAR methods. The series was further advanced by maintaining a minimal ligand binding efficiency threshold in compound design. Ultimately, the co-crystal structure was obtained and the co-crystal structure revealed that these inhibitors interacted with the enzyme in a unique fashion and allowed for potent binding in a non-traditional fashion. To address pharmacokinetic concerns, a weakly basic nitrogen was introduced resulting in a potent, cell active inhibitor with respectable clearance and bioavailability. In all, the potency of the series was enhanced by *4 orders of magnitude* from the HTS lead with concomitant increases in physical properties needed for series advancement. Further optimization and in vivo activity will be the focus of a future disclosure.

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