Bioorganic & Medicinal Chemistry Letters 20 (2010) 2837–2842

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Piperazine sulfonamide BACE1 inhibitors: Design, synthesis, and in vivo characterization

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ARTICLE INFO

Article history:
Received 5 February 2010
Revised 8 March 2010
Accepted 9 March 2010
Available online 12 March 2010

Keywords:
Alzheimer's disease
β-Secretase
BACE
Aspartyl protease inhibitors
Peptidomimetics

ABSTRACT

With collaboration between chemistry, X-ray crystallography, and molecular modeling, we designed and synthesized a series of novel piperazine sulfonamide BACE1 inhibitors. Iterative exploration of the non-prime side and S2′ sub-pocket of the enzyme culminated in identification of an analog that potently lowers peripheral $A\beta_{40}$ in transgenic mice with a single subcutaneous dose.

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Alzheimer's disease (AD)[1] has become the sixth leading cause of death in the United States and is the most common form of dementia in the elderly. Despite significant research efforts in both industry and academia, there are currently no disease modifying therapies available to treat this illness. Significant evidence suggests that the pathology of AD is linked to generation of β-amyloid peptides ($A\beta_{40,42}$)[2] through proteolytic processing of amyloid precursor protein (APP), first by β-secretase (BACE1)[3] and subsequently by γ-secretase. BACE1 in particular offers an attractive drug target,[4] since BACE1 knockout mice are viable[5] and produce a significant reduction in disease pathology when crossed with transgenic AD mice.[6]

As part of our peptidomimetic BACE1 inhibitor efforts, we have previously reported on a series of novel piperazinone structures **1** (Fig. 1).[7] These cyclic amines achieved excellent BACE1 potency through a three point interaction in the active site: (1) requisite binding to the Asp32/Asp228 catalytic diad through the basic amine and hydroxyl group, (2) occupation of S2′ with substituents on the second ring nitrogen, and (3) hydrogen bonding to Thr72 NH of the flap from the ring carbonyl. X-ray crystal structures of the piperazinones bound to BACE1[8] suggested that a similar hydrogen bond to the flap could be achieved if the ring carbonyl were

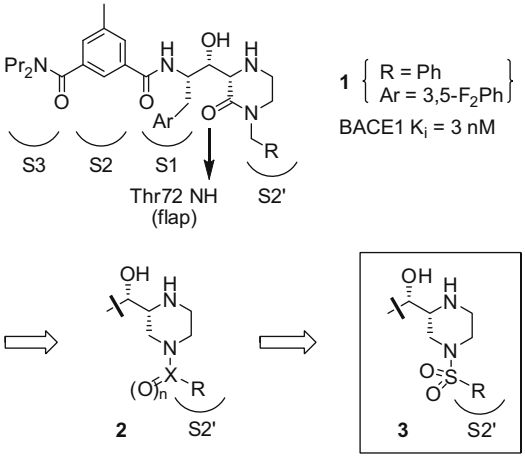

Figure 1. Initial design concepts of piperazine sulfonamide analogs **3** from earlier piperazinone series **1**.

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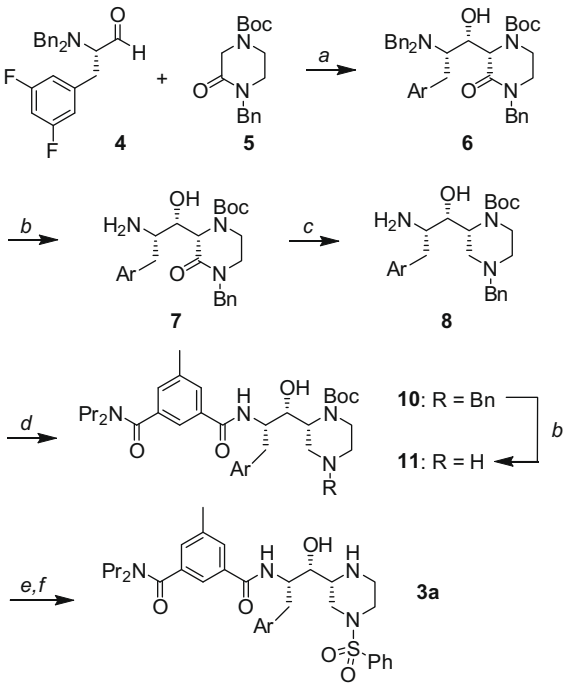
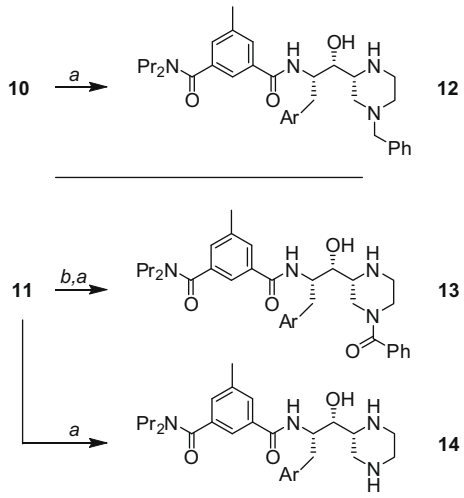
doi:10.1016/j.bmcl.2010.03.050

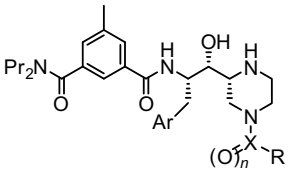


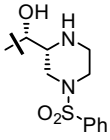
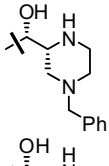
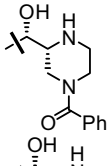
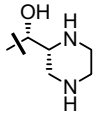
Scheme 1. Synthesis of BACE1 inhibitor **3a** as a representative example for generation of piperazine sulfonamide analogs **3** (Ar = 3,5-difluorophenyl). Reagents and conditions: (a) LDA + **5** then **4**, −78 °C, 41%; (b) H_2, $Pd(OH)_2$, HOAc, EtOH, ~90%; (c) $BH_3{\cdot}SMe_2$, THF, reflux, 78%; (d) **9**, EDCI, DIEA 81%; (e) RSO_2Cl, DIEA, 60–90%; (f) TFA, DCM, ~80%.



Scheme 2. Synthesis of reference BACE1 inhibitors **12**–**14** from intermediates **10** and **11** (Ar = 3,5-difluorophenyl). Reagents: (a) TFA, ~80%; (b) PhC(O)Cl, DIEA.

deleted and an oxo moiety introduced on the substituent at the 4-position of the ring (e.g., structure **2**, Fig. 1). From this initial concept, with extensive input from molecular modeling,[9] we designed piperazine sulfonamides **3**. The sulfonyl moiety in these analogs was intended to serve two functions. First, one of the sulfonyl oxygens would hydrogen bond with the Thr72 NH, and second, the sp^3 nature of the sulfonyl itself would provide the correct trajectory for the pendant aryl group to occupy the S2′ subsite.



Cmpd	Piperazine core	BACE1 IC_{50} (nM)
3a		5
12		120
13		1150
14		490

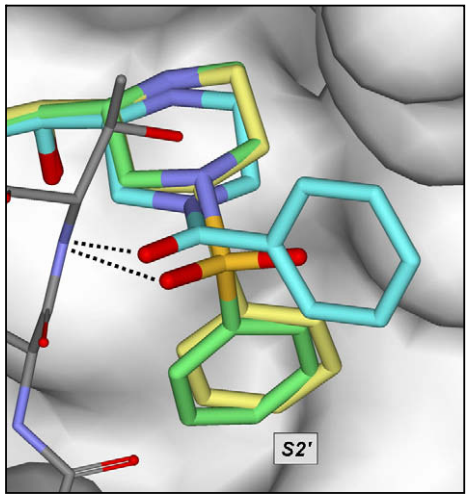


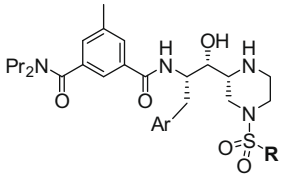
Figure 2. Initial SAR scan[14] of various piperazine core derivatives (Ar = 3,5-difluorophenyl), along with overlay of their X-ray crystal structures in BACE1. Only sulfonamide **3a** (green) simultaneously occupies S2′ and has a hydrogen bond to Thr72 NH of the flap (grey). *N*-Benzyl derivative **12** (yellow) only occupies S2′, and benzamide **13** (blue) only has the hydrogen bond to the flap.

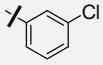
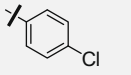
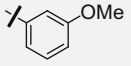
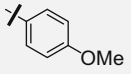
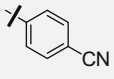
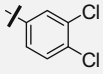
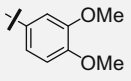
Synthesis of these novel inhibitors began with a stereoselective Aldol addition of the conjugate base of piperazinone **5** to (*S*)-α-amino aldehyde **4**[10] (Scheme 1). Adduct **6** was the major product of this reaction. The desired absolute and relative stereochemistry of the newly formed hydroxyl group was set under non-chelation control enforced by the *N*,*N*-dibenzyl groups on the amino aldehyde.[11,12] Debenzylation of adduct **6** via palladium-mediated hydrogenation proceeded smoothly to give primary amine **7** that was then subjected to borane reduction to give piperazine **8**. The primary amine of intermediate **8** was coupled to 5-methyl-*N*,*N*-dipropylisophthalic acid (**9**)[13] in the presence of EDCI and HOBt to give amide **10**. The piperazine *N*-benzyl group was removed with a second hydrogenation to give core **11**. Sulfonylation of this key intermediate followed by removal of the Boc group gave BACE1 inhibitors **3**, with phenylsulfonamide **3a** shown as a representative example.

In addition to sulfonamides, we also made analogous benzyl, benzamide, and N-unsubstituted analogs **12**–**14** (Scheme 2) for baseline comparison to sulfonamide **3a**. The BACE1 potencies[14] of these four compounds, in conjunction with their X-ray crystal structures (Fig. 2),[15] clearly show the synergy of simultaneous contact with the S2′ pocket and the flap. Sulfonamide **3a** is the only analog that can achieve both of these interactions and is by far the most active compound. It is 24-fold more potent than benzyl analog **12** that only occupies S2′, albeit with excellent overlap. In addition, sulfonamide **3a** is 230-fold more potent than benzamide **13** that has a hydrogen bond to the flap but, due to the sp^2 nature of the amide linker, cannot occupy S2′.

With the piperazine sulfonamide core validated, we turned our attention to optimization of the prime and non-prime side substitution patterns of these inhibitors. On the prime side, there was a relatively steep SAR around substitution on or replacement of the S2′ phenyl group, reflecting the relatively defined and rigid nature of that sub-site. In fact, most substitutions of the phenyl group caused a loss in BACE1 activity (Table 1) relative to unsubstituted phenyl **3a**. The exceptions to this general trend, with BACE1 K_i values <10 nM, were *ortho*-tolyl, *meta*-tolyl, and *meta*-chlorophenyl inhibitors, **3b**, **3c** and **3f**. The *meta*-tolyl compound **3c** in particular gave the best overall K_i and cell potency of these analogs.

Table 1
SAR of the piperazine sulfonamide S2′ substituents with the *N*,*N*-dipropyl isophthalamide non-prime side binding motif (Ar = 3,5-difluorophenyl)[14]



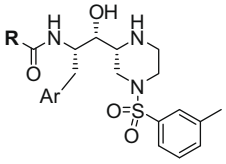
Compd	R	BACE1 K_i (nM)	Cell IC_{50} (nM)	Compd	R	BACE1 K_i (nM)	Cell IC_{50} (nM)
3a		3	120	**3m**		3	57
3b		5	64	**3n**		7	100
3c		1	48	**3o**		8	83
3d		60	1300	**3p**		10	400
3e		400		**3q**		53	2900
3f		7	240	**3r**		240	
3g		42	1700	**3s**		970	
3h		20	440	**3t**		1200	
3i		120		**3u**		5800	
3j		310		**3v**	Me	73	2300
3k		73	1500	**3w**	Pr	95	1800
3l		45	1300				

All *para* substitution (examples **3d**, **3g**, **3i**, **3j**) produced a drop in potency of at least 10-fold, and addition of a *meta* substituent to these analogs to give 3,4-disubstitution (compounds **3k**, **3l**) did not significantly rescue the lost activity.

Further expanding the scope of S2′ SAR, several heterocycles (Table 1, **3m**–**3p**) gave BACE1 K_i values ⩽10 nM, though did not offer improvements in cell potency over *meta*-tolyl analog **3c** despite their increased polarity. A variety of other heterocycles were less well tolerated (**3q**–**3u**, BACE1 K_i >50 nM). Finally, small alkyl sulfonamides **3v** and **3w** that did not display significant lipophilicity to S2′ showed only modest potency improvements (~5-fold) over unsubstituted piperazine **14**.

Using the optimized *meta*-tolyl sulfonamide piperazine core, we next examined a number of isophthalamide variations that have found wide use in the literature as non-prime side binding motifs (Table 2). In particular, methoxymethyl pyrrolidine amide **3x**[16] and sultam **3z**[17] were both very potent against BACE1 (K_i ~1 nM) with exceptional cellular activity (IC_{50} <10 nM). While some truncation of these moieties was tolerated in vitro (e.g., analogs **3y**, **3aa**,[18] **3bb**,[19] BACE K_i ⩽ 3 nM), these modifications all resulted in at least fourfold loss of cellular potency (IC_{50} ⩾ 38 nM). Further reduction in the size of the non-prime side group, as exemplified by lactam **3cc** and simple acetamide **3dd**, resulted in larger losses of both in vitro and cellular potency.

Table 2
SAR of non-prime side isophthalamide variations with the *meta*-tolyl sulfonamide core (Ar = 3,5-difluorophenyl)[14]



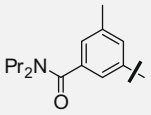
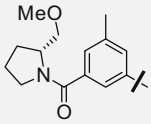
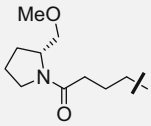
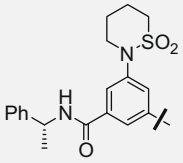
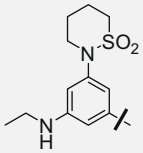
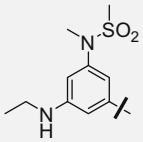
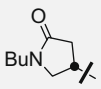
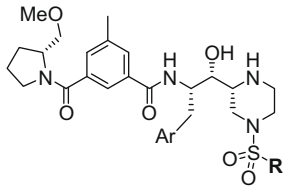
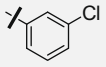
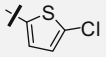
Compd	R	BACE1 K_i (nM)	Cell IC_{50} (nM)
3c		1	48
3x		1	7
3y		3	270
3z		1	8
3aa		1	62
3bb		1	38
3cc		14	1350
3dd	Me	365	4350

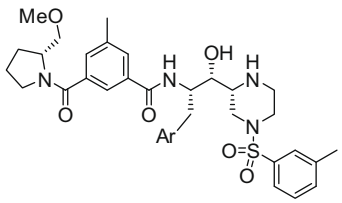
Table 3
SAR of the sulfonamide substituents occupying the S2′ subsite with the methoxymethyl pyrrolidine non-prime side binding motif (Ar = 3,5-difluorophenyl)[14]



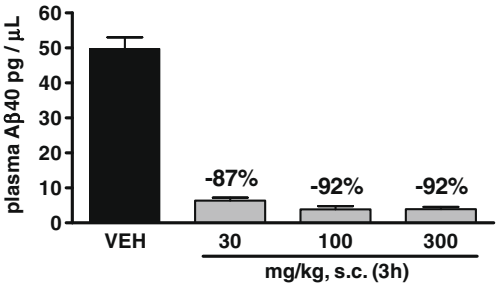
Compd	R	BACE1 K_i (nM)	Cell IC_{50} (nM)
3ee		1	13
3ff		1	22
3gg		2	70
3hh		1	68
3ii		2	53
3jj		1	14
3kk	c-Pr	7	100
3ll	Me	13	220

Of the two potent lead sulfonamides **3x** and **3z** identified above, the former was chosen for additional SAR development based on its superior physico-chemical properties, specifically its lower molecular weight and fewer hydrogen bond donors and acceptors.[20] With this optimal methoxymethyl pyrrolidine isophthalamide group on the non-prime side, re-examination of the S2′ pocket showed that a wide variety of aryl sulfonamide substituents **3ee**–**3jj** (Table 3) gave excellent BACE1 activity (K_i ⩽ 3 nM) and good cellular potencies (IC_{50} <70 nM). Truncated alkysulfonamides **3kk** and **3ll** were also now fairly well tolerated, as opposed to the earlier S2′ SAR (c.f. analog **3ll** in Table 3 vs analog **3v** in Table 1).

Table 4
In vitro, counterscreening, and permeability profile of piperazine sulfonamide **3x** (Ar = 3,5-difluorophenyl)



Assay	
BACE1 K_i (nM)	0.8
BACE2 K_i (nM)	5.4
Cell IC_{50} (nM)	7.0
Cathepsin D K_i (nM)	200
Cathepsin E K_i (nM)	86
Pepsin K_i (nM)	73
Caco-2 AP-BL (efflux ratio)	1 (177)



Dose (mg/kg)	Brain (ng/g)	Plasma (ng/mL)	B/P
30	133	615	0.2
100	248	1506	0.2
300	657	1432	0.5

Figure 3. Acute dose–response of piperazinone sulfonamide **3x** in 6-week-old pre-plaque CRND8 mice along with average compound levels measured in brain and plasma for each dose (eight animals per dose, administered subcutaneously, $A\beta_{40}$ and compound levels measured at 3 h post-dose. Changes in plasma $A\beta_{40}$ are expressed as mean ± standard error as a percent of vehicle. For additional general experimental details, see Ref. 21).

Even with this subsequent exploration of the S2′ subsite, however, sulfonamide **3x** (in vitro profile in Table 4) still represented the best lead in this series and was evaluated in vivo in 6-week-old, pre-plaque CRND8 mice.[21] This potent compound reduced plasma $A\beta_{40}$ levels to background with single subcutaneous[22] doses at or above 30 mg/kg (Fig. 3). Interestingly, these single doses produced an effect that was not only robust at the 3 h time point, but also persistent. The effect lasted 6 h for the 30 mg/kg dose, at least 24 h for the 100 mg/kg dose, and the 300 mg/kg dose still showed more than 90% reduction of plasma $A\beta_{40}$ at 12 h (Fig. 4). Unfortunately, there was no effect on $A\beta_{40}$ levels in the cortex of the mice at any of these doses or time points. Like many hydroxyethylamine-based peptidomimetics,[16b,23,24] sulfonamide **3x** is a substrate for P-gp (Caco-2 efflux ratio >100). Despite this high ratio, subcutaneous dosing generated modest levels of compound in the brain (Fig. 3). With mouse plasma protein binding of approximately 95%, however, the fraction of compound unbound in the brain is only at or slightly above the cellular IC_{50}, likely accounting for lack of efficacy in that compartment.

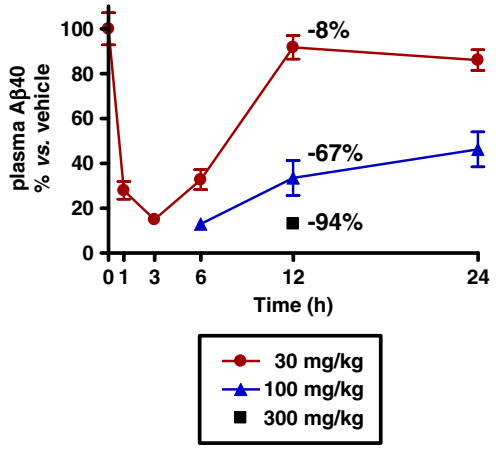


Figure 4. Time course of changes in plasma $A\beta_{40}$ following a single dose of piperazinone sulfonamide **3x** in 6-week-old pre-plaque CRND8 mice (three to eight animals per dose, compound administered subcutaneously, $A\beta_{40}$ levels for each dose were measured at time points shown. Changes in $A\beta_{40}$ levels are normalized relative to vehicle (100%) and are expressed as mean ± standard error. For additional general experimental details, see Ref. 21).

In conclusion, using an iterative structure-based approach, we have designed a series of novel peptidomimetic piperazine sulfonamide BACE1 inhibitors. The sulfonamide portion of these structures achieves both occupancy of S2′ and capture of a key hydrogen bond to the flap of the active site. These interactions, in conjunction with optimal non-prime side binding motifs, gave compounds with excellent BACE1 potency, both in vitro and in cells. In addition, analog **3x** produced a robust and persistent lowering of peripheral Aβ in a transgenic mouse model following a single subcutaneous dose. However, these compounds, as with many peptidomimetics, are substrates for P-pg, and this liability limits their oral bioavailability, brain penetration, and ultimately their central efficacy. Based on these observations, we have directed significant effort toward identification of non-peptidic BACE1 inhibitors, and results will be reported in due course.[25]

Acknowledgments

Use of the Advanced Photon Source at Argonne National Laboratory was supported by the US Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. DE-AC02-06CH11357. Use of the IMCA-CAT beamline 17-ID at the Advanced Photon Source was supported by the companies of the Industrial Macromolecular Crystallography Association.

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