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(*S*)-*N*-(5-Chlorothiophene-2-sulfonyl)-β,β-diethylalaninol a Notch-1-sparing γ-secretase inhibitor

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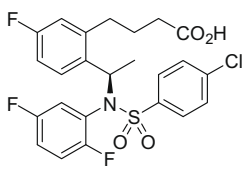
ABSTRACT

Accumulation of beta-amyloid (Aβ), produced by the proteolytic cleavage of amyloid precursor protein (APP) by β- and γ-secretase, is widely believed to be associated with Alzheimer's disease (AD). Research around the high-throughput screening hit (*S*)-4-chlorophenylsulfonyl isoleucinol led to the identification of the Notch-1-sparing (9.5-fold) γ-secretase inhibitor (*S*)-*N*-(5-chlorothiophene-2-sulfonyl)-β,β-diethylalaninol **7.b.2** ($A\beta_{40/42}$ EC_{50} = 28 nM), which is efficacious in reduction of Aβ production in vivo.

Amyloid β-peptide (β-amyloid, Aβ) plaques found in the brains of Alzheimer's disease (AD) patients are formed by aggregation of Aβ peptide, produced by sequential cleavage of amyloid precursor protein (APP), by two aspartic proteases. Initial cleavage by β-secretase (BACE-1) generates the membrane bound β-C-terminal fragment (β-CTF or C99). Subsequent cleavage within the transmembrane region by γ-secretase, a complex of proteins including, presenilin-1 (PS1), nicastrin, anterior parynx defective-1 (Aph-1), and presenilin enhancer-2 (Pen-2), results in formation of Aβ fragments, predominantly $A\beta_{40}$ and the more aggregatory $A\beta_{42}$.[1] Methods of lowering Aβ levels in brain are currently sought as novel disease modifying therapies for treatment of AD.[2] Despite the multitude of X-ray crystal co-structures determined for BACE-1 and its inhibitors, it has proven difficult to obtain potent inhibitors with suitable physical properties to allow brain penetration.[3] γ-Secretase on the other hand has proven more amenable to discovery of inhibitors and BMS-299897,[4,5] LY450139[6,7] and MK-0752[8] have entered clinical trials. However, γ-secretase inhibitors which also modulate the processing of other γ-secretase substrates, in particular Notch-1, have been shown to alter cell differentiation in tissues and cause goblet cell hyperplasia in intestinal epithelium.[9]



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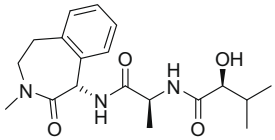
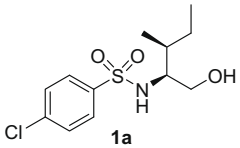
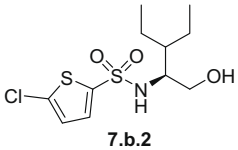


LY-450139



1a



7.b.2

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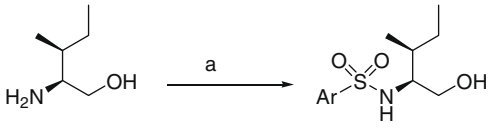
High-throughput screening of Wyeth and ArQule compound collections using a cell based $A\beta_{40/42}$ lowering assay, which uses a sandwich ELISA with 6E10 antibody for Aβ capture and $A\beta_{40}$ and $A\beta_{42}$ C-terminal specific antibodies for detection[10] led to the identification of (*S*)-4-chlorophenylsulfonyl isoleucinol **1a**.[11] Compound **1a** inhibits formation of both $A\beta_{40}$ and $A\beta_{42}$ almost equally (average $A\beta_{40/42}$ EC_{50} = 5.5 μM) while increasing β-CTF in a dose dependent manner without effecting APP, consistent with a γ-secretase inhibition mechanism.[12] Initial research of these leads by varying substitution on the benzene ring led to small increases in potency.[11] Methylation of the nitrogen (*NH* to *N-Me*) and the alcohol (*OH* to *OMe*), replacement of the sulfonamide with amide, urea and amine linkers all led to loss of activity, while conversion of the primary alcohol to secondary and tertiary alcohols also decreased activity.[11]

The focus of this manuscript is on the continued research on this γ-secretase inhibitor by varying the sulfonamide aryl group and the amino alcohol side chain. The initial array was prepared by reaction of sulfonyl chlorides with (*S*)-isoleucinol (Scheme 1). The 4-bromophenyl analog **1b** was about 2-fold more active, while the unsubstituted phenyl analog **1c** had equivalent potency (Table 1). While, the unsubstituted thiophen-2-yl analog **1d** had comparable activity to the phenyl analog **1c**, the 5-substituted thiophene-2-sulfonyl amides **1e**, **1f** and **1g** were 5- to 10-fold more potent than the corresponding 4-substituted phenyl analogs (**1a** and **1b**). Additional substitution on the thiophene ring in the 3- or 4-position as in **1h** and **1i** led to at least 6-fold loss in potency. The 5-chlorofuran-2-yl analog (**1j**) was considerably less active, as were other more polar aryl ring systems and aliphatic sulfonamides (data not shown).

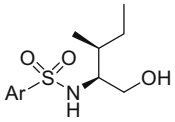
In order to vary the amino alcohol portion of the molecule 5-chlorothiophene-2-sulfonyl chloride was reacted with a set of amino alcohols, prepared by lithium aluminum hydride reduction of the corresponding amino acids (Scheme 2). Compared to the (L)-isoleucinol analog (**1f**; EC_{50} = 170 nM, Table 1) the (L)-leucinol amino alcohol (**2a**, Table 2) with a single substituent on the β-carbon of the amino alcohol had significantly lower activity, while the (L)-valinol analog **2b** with smaller methyl groups at the β-position was approximately 5-fold less active (EC_{50} = 820 nM) and the (L)-*tert*-butyl glycinol analog **2c** was 45-times less potent. The (L)-*allo*-isoleucinol analog **2d** with opposite (*R*)-stereochemistry at the β-center had similar activity to **1f**. Taken together these results suggest a requirement for two alkyl substitutents at the amino alcohol β-center, with chirality of this center having little effect. In contrast, the (D)-isoleucinol analog **2e** with (*R*)-stereochemistry at the α-center is much less active (EC_{50} > 7.6 μM) indicating the strict requirement for the correct (*S*)-stereochemistry at the α-center. Additional substitution at the α-center led to a slight increase in potency, compare **2b** and **2f**.

In order to gain a more thorough understanding of the requirement for two substituent at the β-center while maintaining the α-center in the *S*-configuration, we used the asymmetric amino acid synthesis protocol developed by Hruby to prepare an array of β-branched amino alcohols (Scheme 3).[13] Cinnamic acids were coupled with (*R*)-4-benzyl-2-oxazolidinone to give the alkenoyloxazolidin-2-one products **3**. Cuprate reagents prepared in situ by addition of Grignard reagents to copper bromide dimethylsulfide under carefully controlled temperature conditions, underwent Mi-



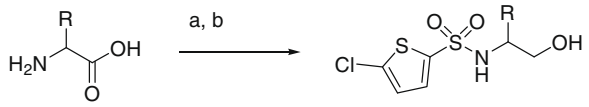
Scheme 1. Reagents: (a) $ArSO_2Cl$, Et_3N, THF.

Table 1
Mean $A\beta_{40/42}$ EC_{50} values for arylsulfonyl (*S*)-isoleucinol derivatives



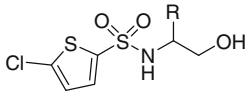
Compound	Ar	EC_{50}[a] (nM)
1a	4-Chlorophenyl-	5500
1b	4-Bromophenyl-	2200
1c	Phenyl-	4700
1d	Thiophen-2-yl-	7000
1e	5-Fluorothiophen-2-yl-	460
1f	5-Chlorothiophen-2-yl-	170
1g	5-Bromothiophen-2-yl-	160
1h	4,5-Dichlorothiophen-2-yl-	1050
1i	3-Bromo-5-chlorothiophen-2-yl-	>30,000
1j	5-Chlorofuran-2-yl-	>30,000

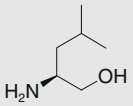
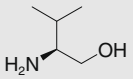
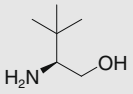
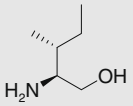
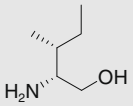
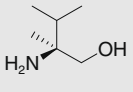
[a] Values are the average of $A\beta_{40}$ and $A\beta_{42}$ inhibition (typically with <30% difference).



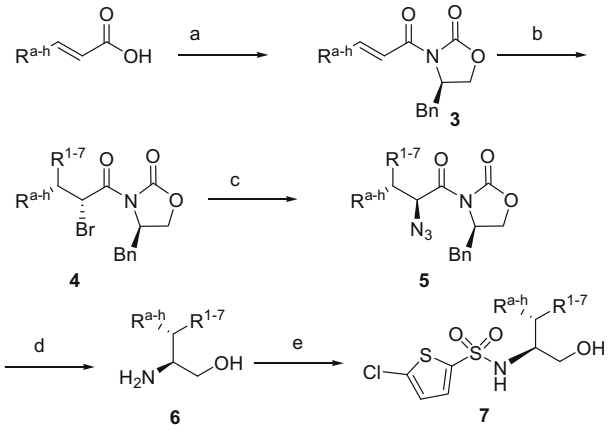
Scheme 2. Reagents and conditions: (a) $LiAlH_4$, THF, 60 °C; (b) 5-chlorothiophene-2-sulfonyl chloride, Et_3N, THF.

Table 2
Mean $A\beta_{40/42}$ EC_{50} values for 5-chlorothiophene-2-sulfonyl amino alcohol derivatives



Compound	Amino alcohol	Amino acid	EC_{50} (nM)[a]
2a		L-Leucine	>30,000
2b		L-Valine	820
2c		L-*tert*-Leucine	7500
2d		L-*allo*-Isoleucine	140
2e		D-Isoleucine	7600
2f		L-α-Methyl-valine	490

[a] Values are the average of $A\beta_{40}$ and $A\beta_{42}$ inhibition (typically with <30% difference).



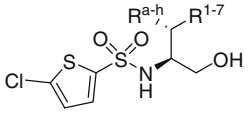
Scheme 3. Reagents and conditions: (a) i—(*R*)-4-benzyl-2-oxazolidinone, $(CH_3)_3CCOCl$, Et_3N, THF, (ii) *n*-BuLi, THF; (b) i—$R^{1\text{-}7}MgBr$, CuBr.DMS, THF −40 to −15 °C; ii—NBS, −78 °C; (c) *N,N,N′,N′*-tetramethylguanidinium azide, ACN, rt; (d) $LiAlH_4$, THF, 60 °C; (e) 5-chlorothiophene-2-sulfonyl chloride, Et_3N, THF.

chael addition and the anion was trapped with *N*-bromosuccinimide to give the α-bromo derivatives **4**.[14] Displacement of the bromide with *N,N,N′,N′*-tetramethylguanidinium azide yielded the corresponding azides **5**.[13] Simultaneous reduction of amide and azide moieties by treatment with lithium aluminum hydride yielded the corresponding amino alcohols **6**, which were reacted with 5-chlorothiophene-2-sulfonyl chloride to give the desired 5-chlorothiophene-2-sulfonyl amino alcohols **7**.

The β,β-diethyl analog **7.b.2** was the most active (EC_{50} = 28 nM, Table 3). While one larger alkyl group was tolerated especially in the pro-S side chain, for example, compare **7.b.6** versus **7.g.2**. The presence of a phenyl (see **7.c.7** or **7.h.1**) or two larger alkyl substituents (see **7.e.4**) was detrimental to activity.

Compound **7.b.2** was further profiled for selectivity in a Notch-1 cleavage assay[12] and found to have an EC_{50} = 266 nM or 9.5-fold selectivity ratio between β-CTF and Notch-1 cleavage. Compound **7.b.2** had good solubility (37 ng/mL at pH 7.4) permeability (13×10^{-6} m/s at pH 7.4) and did not inhibit the cytochrome P_{450} enzymes (2C9, 3A4, 2D6) at 50 μM. Unfortunately, **7.b.2** was rapidly metabolized in rat, mouse and human liver microsomes with half-life of 1, 2 and 8 min, respectively, due to rapid hydroxylation of the methyl groups.

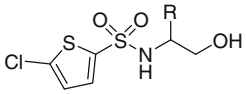
Table 3
Mean $A\beta_{40/42}$ EC_{50} values for 5-chlorothiophene-2-sulfonyl β,β-disubstituted amino alcohol derivatives

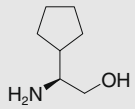
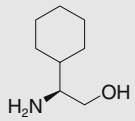
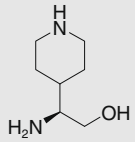


EC_{50} (nM)[a]	R^1 = Me	R^2 = Et	R^3 = *n*-Pr	R^4 = *n*-Bu	R^5 = *i*-Bu	R^6 = *n*-Hexyl	R^7 = Ph
R^a = Me					115	212	
R^b = Et		28			28	43	
R^c = *n*-Pr	103	29	111		253	826	4055
R^d = *i*-Pr						71	
R^e = *n*-Bu				4180			
R^f = *n*-Pentyl	264	72			20,708	>30,000	>30,000
R^g = *n*-Hexyl		429					
R^h = Ph	3906						> 30,000

[a] Values are the average of $A\beta_{40}$ and $A\beta_{42}$ inhibition (typically with <30% difference) and mean of three experiments for each protein with a standard deviation of <20%.

Table 4
Mean $A\beta_{40/42}$ EC_{50} values for cyclic aminoalcohol replacements



Compound	Amino alcohol	Amino acid	EC_{50} (nM)[a]
8a		L-Cyclopentylglycine	580
8b		L-Cyclohexylglycine	820
8c		(*S*)-2-Amino-2-(piperidin-4-yl)acetic acid	>30,000

[a] Values are the average of $A\beta_{40}$ and $A\beta_{42}$ inhibition (typically with <30% difference) and mean of three experiments for each protein with a standard deviation of <20%.

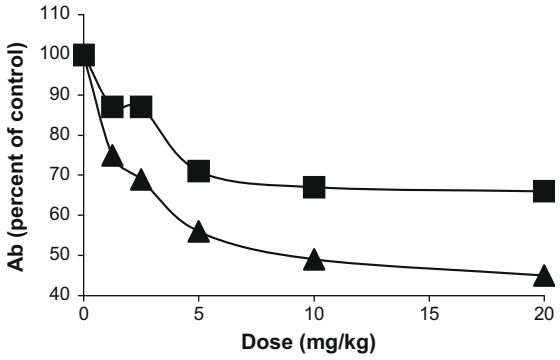


Figure 1. Reduction of $A\beta_{40}$ (▲) and $A\beta_{42}$ (■) levels in Tg2576 mouse by **7.b.2** dosed sub-cutaneously.

In an attempt to prevent rapid metabolism of the alkyl chains, a set of 5-chlorothiophene-2-sulfonyl amino alcohols with cyclic side chains were prepared using the procedure outlined in Scheme 2. However, all these amino alcohol derivatives had significantly weaker activity relative to the β,β-diethyl analog **7.b.2**. The cyclopentyl and cyclohexylglycinol analogs **8a** and **8b** had EC_{50} = 580 and 820 nM, respectively (Table 4), while the 4-piperidine glycinol analog **8c** was greater than 30 μM.

Poor oral bioavailability of **7.b.2** precluded oral testing, however when dosed 20 mg/kg sub-cutaneously in Tg2576 mouse **7.b.2** led to a 55% reduction in brain $A\beta_{40}$ levels (Fig. 1). Sub-chronic dosing of 1 mg/kg sub-cutaneously for 5 days resulted in 25 and 15% reduction in brain $A\beta_{40}$ and $A\beta_{42}$.[12]

In summary, a series of 5-chlorothiophene-2-sulfonylamido alcohols were synthesized based on the HTS hit **1a**. Research varying the aryl and aminoalcohol moieties of the series using iterative parallel synthesis lead to **7.b.2**, with 28 nM cellular potency in the γ-secretase inhibition assay. We have demonstrated the requirements for (*S*)-configuration at the α-center and bis-substitution with small alkyl groups at the β-center. Despite its poor oral PK, **7.b.2** was efficacious in Aβ lowering experiments in vivo. Future publications will focus on more detailed in vivo characterization of **7.b.2** and on improving the microsomal stability and oral PK of this very attractive series of γ-secretase inhibitors.

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14. Representative procedure for synthesis of (*R*)-4-benzyl-3-(2-bromo-3-ethyl-pentanoyl)-oxazolidin-2-one. To CuBr.DMS complex (34.5 g, 168 mmol, 1.2 equiv) in THF/DMS (2:1, 2 L), cooled to −40 °C was added EtMgBr (112 mL 3 M solution in THF, 336 mmol, 2.4 equiv). The solution was allowed to warm to −15 °C, stirred for 15 min, then re-cooled to −40 °C. (Note: it is important that the temperature does not go above −10 °C). A solution of (*R*)-4-benzyl-3-pent-2-enoyl-oxazolidin-2-one (36.27 g, 140 mmol) in THF (280 mL) was added. The solution was allowed to warm to room temperature and stirred for 8–16 h. The solution was re-cooled to −78 °C and a slurry of *N*-bromosuccinimide (31 g, 168 mmol, 1.2 equiv) in THF (280 mL) was added. The solution was allowed to warm to 0 °C and stirred at 0 °C for 3 h, then quenched with a 1:1 solution of saturated ammonium carbonate and 0.5 N potassium bisulfate (1 L). The organic phase was decanted off, dried and concentrated. The product was purified by flash chromatography on silica gel with 20% ethyl acetate in hexanes to give (*2R,4R*)-4-benzyl-3-(2-bromo-3-ethyl-pentanoyl)-oxazolidin-2-one (29.5 g) as slightly green oil almost exclusively as a single isomer by NMR.