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4-Substituted cyclohexyl sulfones as potent, orally active γ-secretase inhibitors

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Abstract—The protease γ-secretase plays a pivotal role in the synthesis of pathogenic amyloid-β in Alzheimer's disease. Here, we report a further extension to a series of cyclohexyl sulfone-based γ-secretase inhibitors which has allowed the preparation of highly potent compounds which also demonstrate robust Aβ(40) lowering in vivo (e.g., compound **32**, MED 1 mg/kg p.o. in APP-YAC mice).
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Alzheimer's disease (AD) is a devastating disorder of the aged population and with current palliative treatments being of modest efficacy, the need remains for a therapy able to alter the underlying pathophysiology of the disease. The predominantly 40–42 amino acid amyloid-β (Aβ) peptide is the major component of the extracellular proteinaceous plaques seen in Alzheimer's disease (Aβ) and much evidence suggests a pivotal role for Aβ in the disease process.[1] In particular, individuals possessing autosomal dominant mutations in the genes encoding for amyloid-β precursor protein (βAPP) or the membrane-bound protein homologs presenilin 1 and 2 have elevated Aβ levels and suffer from aggressive forms of early onset AD.[2,3] These observations have led to the hypothesis that Aβ, either in its soluble form or when aggregated into oligomers, fibrils, and subsequently plaques, is responsible for neuronal toxicity and cell death.[4]

Aβ is derived by processing of the 695–770 residue, type I transmembrane protein βAPP.[5] The major metabolic pathway of βAPP involves sequential cleavage by the proteases α-secretase and γ-secretase leading to non-amyloidogenic fragments. Alternative processing by stepwise cleavage mediated by β-secretase and γ-secretase leads to the production of Aβ and it is inhibitors of the latter enzyme that were targeted in the current work.[6,7]

γ-Secretase is a novel membrane-bound aspartyl protease complex formed from presenilin-1/2, nicastrin, Aph-1, and pen-2 whose catalytic center is unusual in likely being within transmembrane helices of the presenilin unit. As the role of γ-secretase has become better characterized, a variety of substrates[8] including the Notch receptor have been identified leading to the possibility of mechanism-based effects[9] or alternatively to additional therapeutic uses [10] for γ-secretase inhibitors.

Keywords: Alzheimer's disease; γ-Secretase; Protease inhibitor; Amyloid; In vivo.
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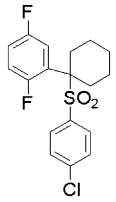

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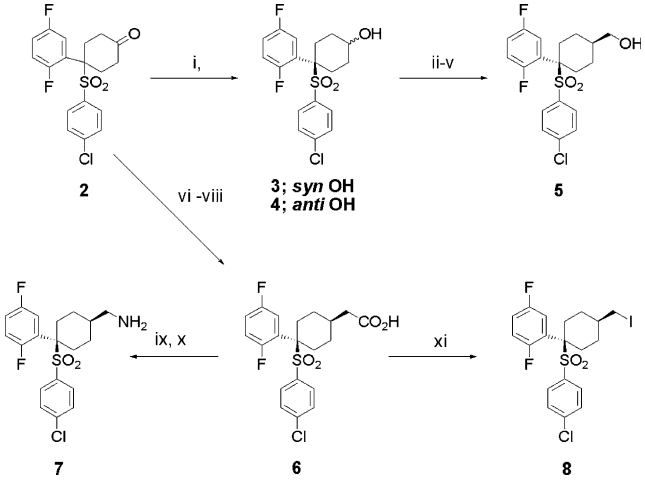
A whole-cell γ-secretase inhibition assay using SH-SY5Y neuroblastoma cells in which human γ-secretase catalyzes the breakdown of the overexpressed exogenous substrate A4CTF has been developed.[11] We have previously disclosed[12–15] a series of cyclohexyl sulfones which have demonstrated in vitro inhibition of Aβ(40) secretion in this assay. In this letter, we describe the further optimization of these leads resulting in the identification of highly potent analogs demonstrating in vivo reduction of brain Aβ(40) in a mouse model.

Based upon the 1,1-disubstituted cyclohexane **1**, which has previously been shown to be a potent γ-secretase inhibitor (IC_{50} 3 nM),[12] we chose to probe the 4-position on the cyclohexane ring in order to improve both potency at γ-secretase and also ADME properties.

Investigation of substitution at the 4-position was facilitated by the ready availability, on scale, of the cyclohexanone derivative **2**.[12] This could be elaborated to provide a range of useful intermediates as shown in Scheme 1. Reduction of **2** gave predominantly the *anti*-alcohol **4** which underwent smooth cyanide displacement via a mesylate to give, after hydrolysis and reduction, *syn* hydroxymethyl **5**. Alternatively, Wadsworth–Horner–Emmons reaction followed by stereoselective reduction and hydrolysis gave the carboxylate **6**, which was elaborated to amine **7** via Curtius rearrangement or iodide **8** by Hunsdiecker-type radical iodination.[16]



Scheme 1. Reagents and conditions: (i) $NaBH_4$, EtOH, 10 °C (anti:syn 6:1); (ii) MsCl, Et_3N, DCM, −50 °C; (iii) Bu_4NCN, PhMe, 75 °C; (iv) AcOH/concd HCl, 110 °C; (v) $^{i}BuOCOCl$, Et_3N, THF then $NaBH_4$, H_2O; (vi) $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF; (vii) L-Selectride, THF, −40 °C; (viii) LiOH, aq MeOH; (ix) $(COCl)_2$, DMF, THF then NaN_3, Bu_4NBr, PhH then BnOH, reflux; (x) HBr/AcOH then NaOH; (xi) $PhI(OAc)_2$, I_2, PhH, reflux, hυ.

These intermediates could be further elaborated to a wide range of derivatives, a representative number of which are summarized in Table 1. Whilst the hydroxymethyl analog **5** showed activity similar to that of unsubstituted **1** only, formation of a carbamate (e.g., compound **9**) gave an increase in potency. Similarly, the poor activity of primary amine **7** could be improved markedly by acylation or sulfonylation with ureas, carbamates, and sulfamides all being well tolerated. Additionally, several heterocycles including succinimide provided compounds with good activity.

The iodide **8** also allowed ready access to either sulfonamides (via KNO_3/SO_2Cl_2 oxidation[17] of the intermediate thiol) or sulfones (via S_N2 displacement of the iodide and subsequent oxidation) as shown in Scheme 2. The in vitro potencies of a representative range of sulfonyl-containing analogs are summarized in Table 2.

The series of simple alkyl and aryl sulfones gave mostly modest inhibitory activity (data not shown) although cyclopropyl analog **16** was shown to be potent. However, the utilization of an aromatic substituent bearing a hydrogen bond acceptor at the *ortho* position proved to be advantageous; anisyl derivative **20**, in particular, showing high potency.

Sulfonamides were also well tolerated with azetidine derivative **24** being optimal in this series.

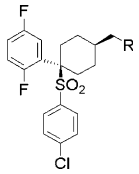
Whilst many of the 4-substituted cyclohexyl analogs prepared showed excellent in vitro potency, their in vivo efficacy profiles were often compromised by poor metabolic stability prompting us to investigate the cause of this issue. It was postulated that the activated methylene *exo* to the cyclohexyl core may be a site of metabolism and this was confirmed in the case of methyl analog **25** with the isolation of acid **26** as a major metabolite following in vitro incubation with rat liver microsomes (Fig. 1).

To address this potential route of metabolism, compounds lacking this labile methylene unit were next prepared. Initially, a wide range of functional groups were introduced directly linked to the cyclohexyl ring at the 4-position with the potency of a representative number summarized in Table 3. These compounds were available from the *syn* alcohol **3** (Scheme 1) or *syn* amine **27** (Scheme 3).

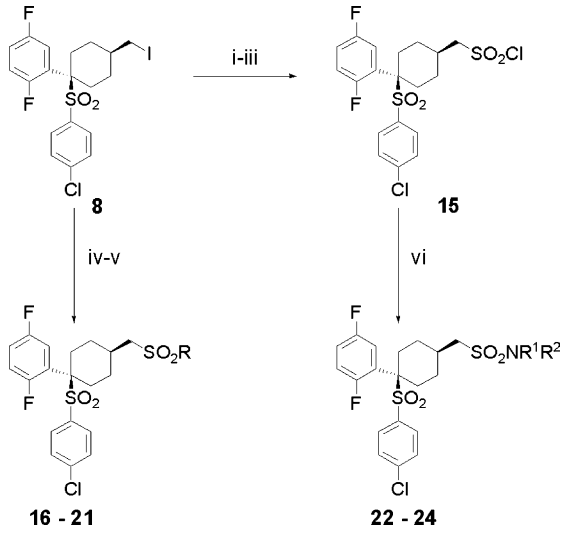
In most cases, the presence of a polar group directly attached to the cyclohexyl core was attended with inferior in vitro potency (note, in particular, the parent alcohol **3** and amine **27**). Acylation of **3** or **27** was also not productive, except in the case of carbamate **28** where potency was at best improved only to the level of unsubstituted **1**.

In marked contrast to acylated amino analogs, extending this work to a series of sulfonamides and sulfamates gave compounds with increased potency.

Table 1. In vitro γ-secretase inhibition of compounds **5**–**14**



Compound	Preparation	R	IC_{50} (nM)
5	Scheme 1	OH	4.7 ± 1.7
6	Scheme 1	CO_2H	21.2 ± 1.5
7	Scheme 1	NH_2	148 ± 28
8	Scheme 1	I	14.3 ± 7.2
9	**5**, 4-Nitrophenyl chloroformate, pyr, THF then $MeNH_2$/EtOH	OCONHMe	0.51 ± 0.15
10	**7**, Ac_2O, DMAP, Et_3N, DCM	NHAc	1.3 ± 0.2
11	**6**, DPPA, Et_3N, PhMe, 110 °C then NH_3/dioxan, rt	$NHCONH_2$	1.0 ± 0.1
12	**7**, MeO_2CCl, Et_3N, DCM, 0 °C	$NHCO_2Me$	2.2 ± 0.1
13	**7**, catechol sulfate, THF then ethylamine/dioxan	$NHSO_2NHEt$	1.7 ± 0.3
14	**5**, MsCl, Et_3N, DCM then succinimide, NaH, DMF, 80 °C		1.0 ± 0.4

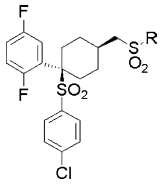


Scheme 2. Reagents and conditions: (i) KSAc, DMF; (ii) NaOH, aq MeOH; (iii) KNO_3, SO_2Cl_2, MeCN; (iv) RSH, KOH, EtOH, reflux; (v) cat. RuO_2, $NaIO_4$, EtOAc/H_2O; (vi) R_1R_2NH, THF.

In the series of sulfonamides, alkyl substituents were well tolerated with *n*-propyl (**31**) and trifluoromethyl (**32**) substituents preferred. A series of aryl sulfonamides was also prepared and whilst the observation noted earlier of the favorable effect of introduction of *ortho*, electronegative substituents appeared not to translate to this series, several potent heterocyclic analogs (e.g., **33**) could be prepared. With some parallel to the earlier series of sulfamides (Table 2), this functional group was further utilized to give potent compounds with the azetidine derivative **35** giving extremely high levels of activity.

On consideration of in vitro potency and ADME profiles of the potent inhibitors, the trifluoromethane sulfonamide **32** was selected for further work. Compound **32** inhibited the generation of Aβ(40) and Aβ(42) with very similar IC_{50}s[18] and inhibited the cleavage of Notch receptor[19] at concentrations similar to those required for inhibition of APP processing,[20] consistent with the model that γ-secretase is required for both of these processes.

Table 2. In vitro γ-secretase inhibition of compounds **16**–**24**



Compound	R	IC_{50} (nM)
16	^cPr	1.4 ± 0.3
17	CF_3	21.5 ± 5
18	Ph	8.8 ± 1.7
19		0.65 ± 0.1
20		0.51 ± 0.02
21		2.1 ± 0.2
22	NH_2	4.2 ± 1.4
23	NMe_2	1.1 ± 0.3
24		0.36 ± 0.02

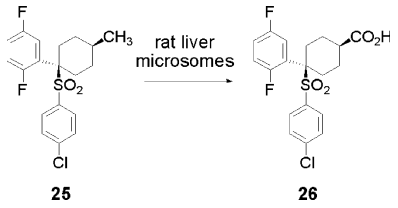


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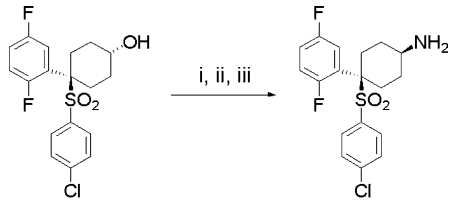
Figure 1. In vitro metabolism of methyl analog **25**.



4 27

Scheme 3. Preparation of amine **27**. Reagents and conditions: (i) MsCl, Et_3N, DCM, −50 °C; (ii) NaN_3, DMF, 90 °C; (iii) PPh_3, aq THF.

This compound was profiled in vivo in the APP-YAC mouse model[21] and demonstrated a robust decrease of DEA-extractable brain Aβ(40) levels[22,23] 4 h after oral dosing with a minimum effective dose of 1 mg/kg (Fig. 2). A time-course study at 10 mg/kg showed excellent duration of action with a significant lowering of brain Aβ(40) levels throughout a 24 h period.

In summary, we have developed a series of 4-substituted cyclohexyl sulfones which inhibit γ-secretase in vitro in the low to sub-nanomolar range. This high level of in vitro potency could be coupled with good pharmacokinetics to produce potent, orally active γ-secretase inhibitors, such as **32**, which was shown to have excellent efficacy in reducing central Aβ(40) levels in APP-YAC mice with a minimum effective dose of 1 mg/kg p.o.

Acknowledgments

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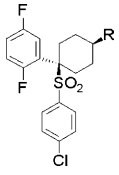
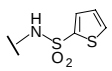
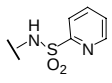
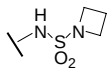
Table 3. In vitro γ-secretase inhibition of compounds **27**–**35**



Compound	Preparation	R	IC_{50} (nM)
3	Scheme 1	OH	10.7 ± 1.7
28	**3**, $ClSO_2NCO$, THF, 0 °C	$OCONH_2$	1.9 ± 0.5
27	Scheme 3	NH_2	510 ± 10
29	**27**, Ac_2O, Et_3N, DCM	NHAc	35 ± 3
30	**27**, MsCl, Et_3N, DCM	$NHSO_2Me$	4.2 ± 1.2
31	**27**, $PrSO_2$ Cl, Et_3N, DCM	$NHSO_2Pr$	0.82 ± 0.22
32	**27**, Tf_2O, Et_3N, DCM	$NHSO_2CF_3$	0.65 ± 0.13
33	**27**, $ArSO_2Cl$, Et_3N, DCM		0.25 ± 0.07
34	**27**, $ArSO_2Cl$, Et_3N, DCM		0.66 ± 0.01
35	**27**, [reagent from azetidine/SO_2Cl_2], Hunig's base, MeCN		0.15 ± 0.07

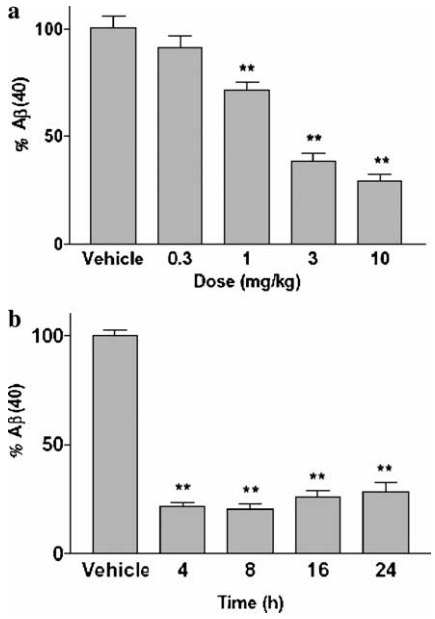


Figure 2. Effects of **32** on DEA-soluble brain Aβ(40) in APP-YAC mice. (a) Dose–response following p.o. dosing of **32** with analysis 4 h post-dose. (b) Time-course following 10 mg/kg p.o. dose. $n = 5$ per group. Compound was dosed as a suspension in 0.5% methocel solution.

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