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Design and synthesis of tricyclic sulfones as γ-secretase inhibitors with greatly reduced Notch toxicity

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ABSTRACT

A novel series of tricyclic γ-secretase inhibitors was designed and synthesized via a conformational analysis of literature compounds. The preliminary results have shown that compounds in this new series have much improved in vitro potency and in vivo profiles. More importantly, they have greatly reduced Notch related toxicity that was associated with previous γ-secretase inhibitors.

Since the 40–42 amino acid amyloid peptides (Aβ) are critical factors in the onset and progression of Alzheimer's disease (AD),[1] reducing or eliminating the production of Aβ through inhibition of γ-secretase has been suggested to be a useful treatment for AD.[2] Previous sulfonamide γ-secretase inhibitors from our laboratories have persistent Notch related toxicity in addition to P-450 enzyme inhibition and liver toxicity liabilities although they have excellent in vitro potencies and in vivo efficacies.[3] To address this issue, we designed and synthesized a novel series of γ-secretase inhibitors via conformational analysis of an earlier sulfone lead. Compounds in this new series have comparable or better in vitro and in vivo profiles compared to the previous compounds. Additionally, they completely eliminated P-450 enzyme inhibition and liver toxicity problem of the sulfonamide series. More importantly, the compounds in this new series have greatly reduced Notch related toxicity.

Previously literature has disclosed an aryl sulfone series as potent γ-secretase inhibitor as exemplified by **1**.[4] A detailed conformational analysis of this compound led to the design of a novel bicyclic and tricyclic sulfone γ-secretase inhibitors as shown in Scheme 1.

The basic design was to form a covalent bond between two carbon atoms that are geometrically very close to each other. In order to quickly validate this new series with minimum synthetic effort, the original cyclohexane ring was broken open to form a bicyclic ring typified by **2**. After the bicyclic series was quickly synthesized and validated, the third ring was reintroduced to form tricyclic compound **3**.

The straight forward synthesis of key compounds in bicyclic series is shown in Scheme 2. Phenylsulfone **4** was synthesized in quantitative yield from 2,3,6-trifluorobenzyl bromide and was then alkylated with protected hydroxyalkyl bromide to provide **5**. The protecting group was removed and the product underwent SNAr reaction when treated with a base to provide bicyclic sulfone **6**. Alkylation at the 4-position of **6** with various alkyl halides gave compounds **7**. Alternatively, commercially available ketones **8** that have various substitutions on the A ring were treated with thiophenol and reducing reagent to generate sulfide which was then oxidized in situ to sulfones **9**. These compounds were similarly alkylated to give **10**. The same synthetic schemes could also be used to modify the A ring substitutions and B ring sizes.

In order to introduce substitutions at the 2- and 3-positions of the B ring, different synthetic routes were developed as shown in Scheme 3. Sulfone **4** was treated with *n*-butyl lithium, followed by an optionally substituted epoxide to form compound **11.** Compound **11** was converted to 2-substituted compound **12** and 2,4-disubstituted compound **13** using similar reaction sequences shown before. On the other hand, vinyl diester **14** was first synthesized by condensation of 2,3,6-trifluorobenzaldehyde with

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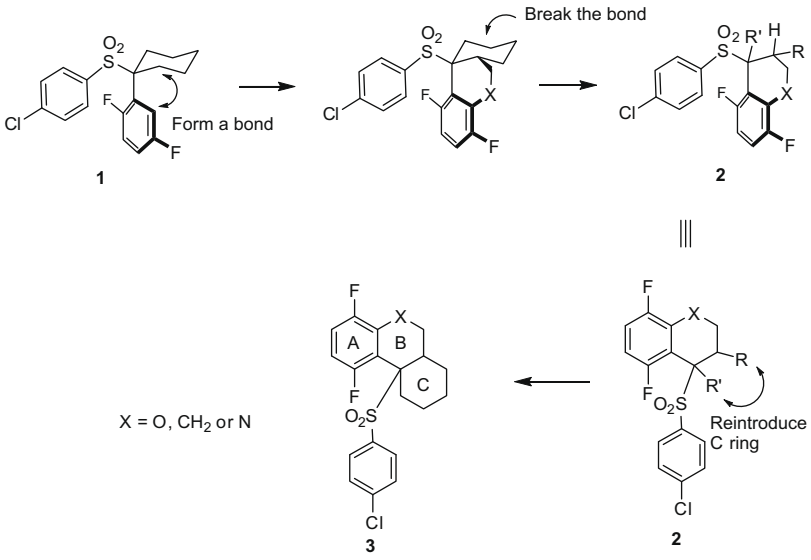
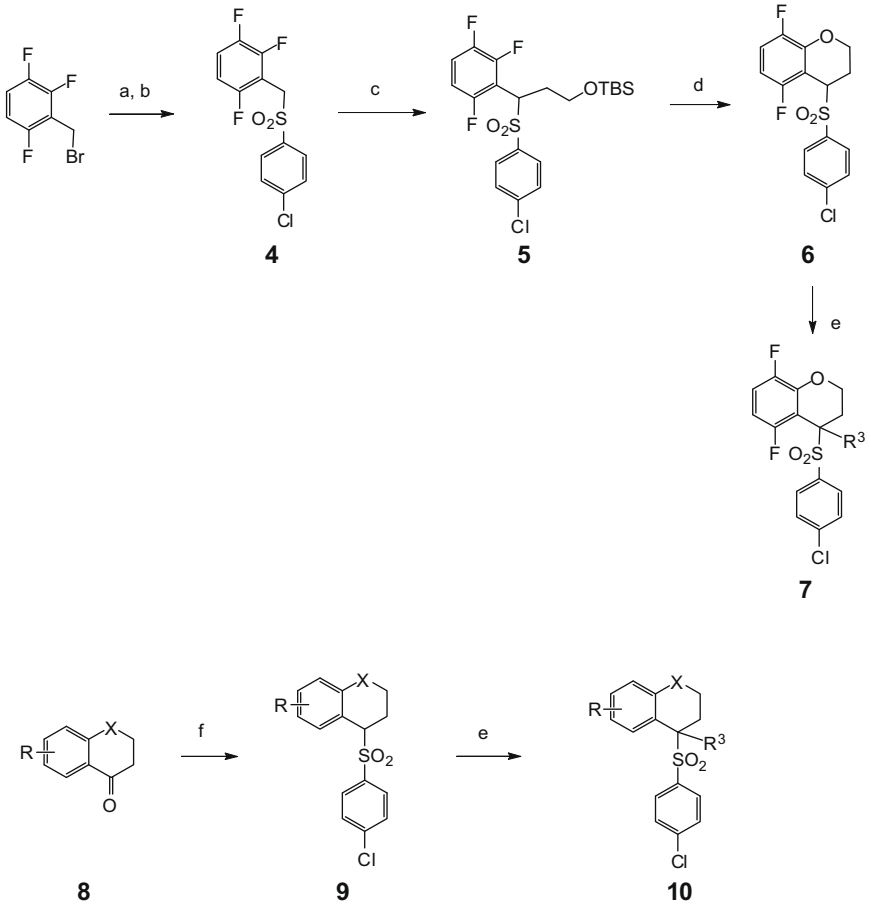


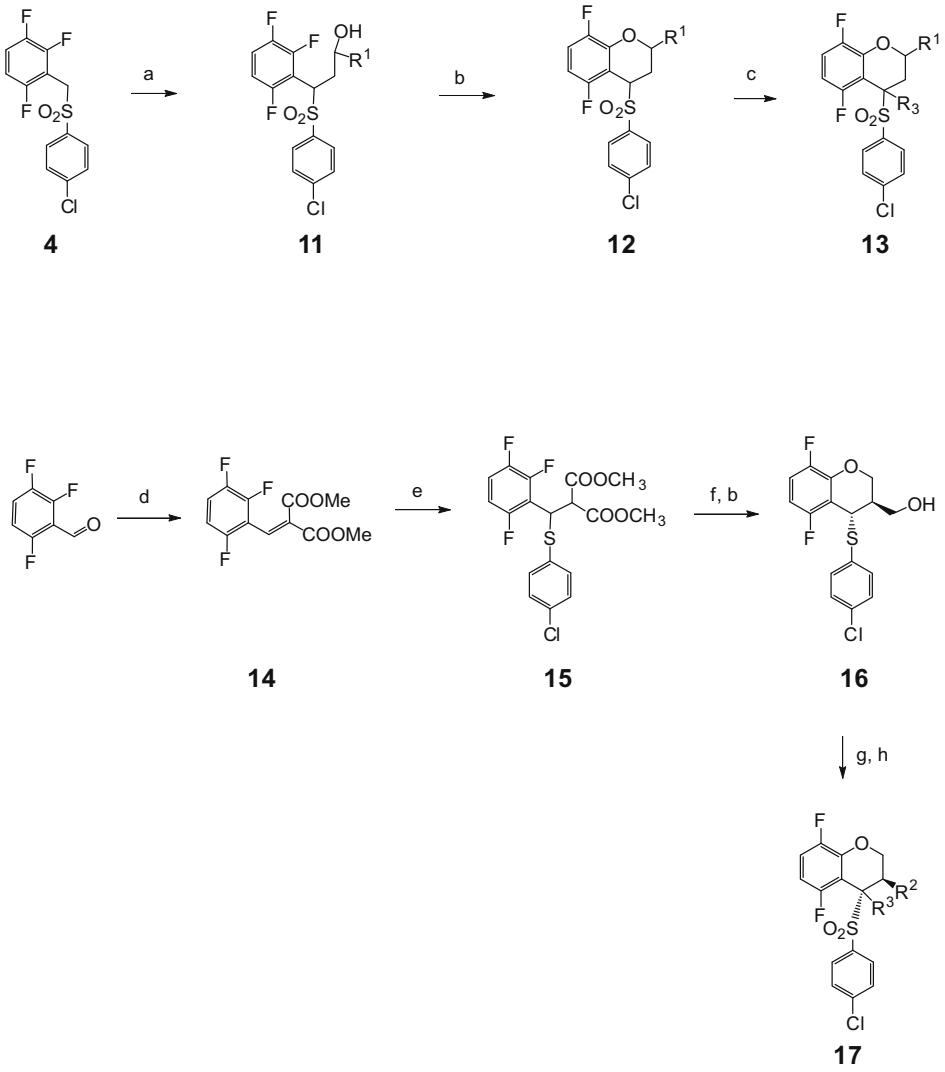
Scheme 1.



Scheme 2. Reagents and conditions: (a) 4-chlorobenzenethiol, NEt_3; (b) MCPBA 98% for two steps; (c) $BrCH_2(CH_2)_nOTBS$, THF, KO*t*-Bu, 28%; (d) TBAF; then NaH, 67%; (e) alkyl bromide, KO*t*-Bu, THF; (f) 4-chlorothiophenol, BH_3, then MCPBA.

dimethylmalonate and then reacted with 4-chlorothiophenol in the presence of potassium carbonate to give **15**. Reduction of the diester **15** with DIBALH, followed by the SNAr reaction gave compound **16**. During the B ring closure reaction, only the desired trans configuration between the thioether and hydroxyl methyl groups was formed. The thioether was oxidized to the sulfone, and standard functional group manipulation generated compound **17** with various substitutions on the 3- and 4-positions of B ring.

Considerable effort went into the development of a synthetic route to synthesize the rather complex tricyclic compounds. The most practical synthesis from this effort is shown in Scheme 4. Chromene **22** was synthesized according to a patent procedure[5]



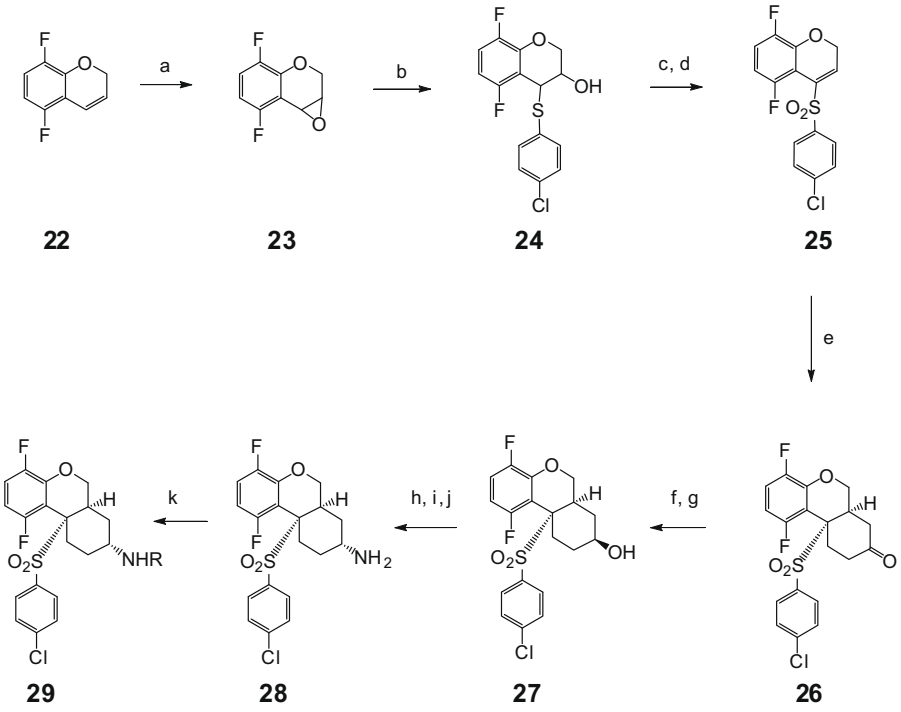
Scheme 3. Reagents and conditions: (a) *n*-BuLi, substituted epoxide, 52% when R^1 = Me; (b) NaH; (c) alkyl bromide NaH, THF; (d) dimethylmalonate, NEt_3; MsCl; (e) 4-chlorothiophenol, K_2CO_3, 72% for two steps; (g) MCPBA, 80%; (h) functional group manipulation and alkylation.

and was then oxidized with MCPBA gave the epoxide **23**. 4-Chlorothiophenol was then added to **23** in a *regio* selective fashion in the presence of indium chloride,[6] resulted in the desired *regio* isomer **24** as the sole product. Oxidation of the thioether **24** to the sulfone, followed by dehydration gave the vinyl sulfone **25**. The key step in this synthesis was the Diels–Alder reaction of compound **25** with 2-trimethylsilyloxy 1,3-butadiene.[7] After an acidic work up of this reaction, a single *regio* isomer ketone **26** was obtained. The ketone was reduced to the hydroxyl group with sodium borohydride at low temperature to yield only the desired *trans* isomer **27**. It was determined early on that the biological activities of these compounds resided mainly in one of the enantiomers, so pure enantiomers were needed to fully evaluate these compounds. In the absence of a viable asymmetric synthesis, chiral separation was used to obtain pure enantiomers. After evaluating all intermediates leading to the final product for feasibility of chiral separation, it was found that the hydroxyl compound **27** gave the best separation with all existing chiral columns. The two enantiomers were separated at this stage using chiral HPLC, and the hydroxyl group of the desired enantiomer was converted to amino group in three steps to yield amine **28**. It should be noted that the C-8 chiral center was completely inverted at the step when the mesyl group was replaced by the azide group. As a result, only the compound with the desired cis configuration between amino and sulfone groups was obtained. Further derivatives of amine **29** were synthesized as usual.

The absolute configuration of the active enantiomer was determined by X-ray crystallography of triflic amide (**29c**, R = $CpSO_2$) and the result is shown in Figure 1.[8] Compounds with this absolute stereo configuration were more active in our biological assays. Thus, the detailed in vivo studies were all performed using only pure enantiomers with this absolute configuration.

The bicyclic compounds were synthesized to validate this new series and to optimize the type and size of newly formed B ring. Since A ring substitutions were well defined by literature precedents and our in-house data,[3,4] they were kept constant at this time and B ring substitutions were varied. The $A\beta_{40}$ IC_{50}s of selected compounds in this series are listed in Table 1.

The data for compounds **13a**–**13c** show that substitution at the 2-position of B-ring is not tolerated. Even the small alkyl or hydroxymethyl groups dramatically reduced the potency of resulting compounds. On the other hand, small alkyl groups on the 3- and 4-positions improved the potency dramatically. A simple addition of an ethyl group at the 4-position improved the membrane $A\beta_{40}$ IC_{50} from 1600 nM (**6**) to 72 nM (**7b**). A *trans* methyl group at 3-position improved the membrane $A\beta_{40}$ IC_{50} from 1600 nM



Scheme 4. Reagents and conditions: (a) MCPBA 60%; (b) *p*-chlorothiophenol, $InCl_3$, 57%; (c) MCPBA; (d) mesyl chloride, NEt_3, 64% for two steps; (e) 2-trimethylsilyoxy 1,3-butadiene, trifluorotoluene, 160 °C, 48%; (f) $NaBH_4$, −78 °C to rt, 67%; (g) Chirl OD Column; (h) mesyl chloride, NEt_3; (i) NaN_3; (j) PPh_3, H_2O, 44%for three steps; (k) various conditions.

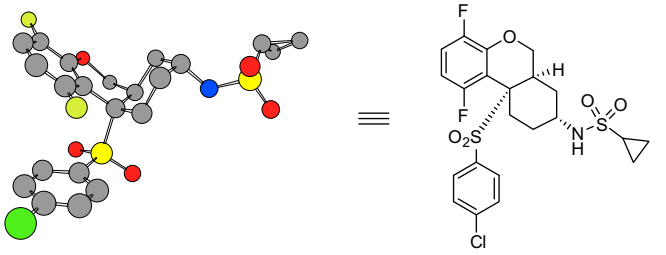


Figure 1. The crystal structure of **29c**.

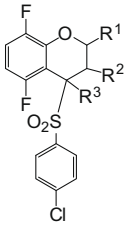
(**6**) to 64 nM (**17b**). Unfortunately, all attempts to further improve the potency of the bicyclic compound was not successful. Introducing other substitutions on other positions of A and B ring also resulted in less active compounds.

Compounds such as **7b** and **17b** were potent enough to warrant further studies, especially when they were resolved to the corresponding pure enantiomers. Unfortunately, further studies revealed that all these compounds have poor pharmacokinetic profiles most likely due to their high Log *P* values. Attempts to reduce their Log *P* by introducing polar groups at 3- and 4-positions yielded only less active compounds. Since both literature and our in-house work had shown that the *p*-chlorophenyl sulfone is one of the best substitutions at bottom portion of the molecule, we kept this part of the molecule constant for the scope of this study. Additionally, a pyran ring proved to be optimal in a limited exploration of various B rings. When the oxygen atom of the B ring was replaced by carbon, nitrogen and sulfur, the resulting compounds were much less active in general.[9] This reason, coupled with the fact that pyran B ring was much easier to access synthetically, prompted us to pursue only the pyran as B ring in all future studies.

Once the design of the new series was validated in the bicyclic series, we turned our attention to tricyclic compounds. The synthetic effort of this complex ring system paid off in the end since the resulting compounds were among the best we have obtained in this series. A large library of compounds with substitutions at 8-position, including amides, sulfonamides, urea, carbonamides, sulfones and heterocycles was synthesized and evaluated. The biological data for selected compounds are listed in Table 2.

As the data in Table 2 shows, many different substitutions at the 8-positon of the C ring were tolerated, resulting in compounds with good to excellent in vitro activities. This provided a very good

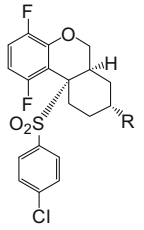
Table 1
Membrane $A\beta_{40}$ IC_{50} data for selected bicyclic compounds

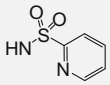


Compd[a]	R^1	R^2	R^3	Mem. IC_{50}[b] (nM)
6	H	H	H	1600
7a	H	H	CH_3	808
7b	H	H	CH_2CH_3	72
13a	*trans* CH_3	H	CH_3	1200
13b	*trans* CH_2CH_3	H	CH_2CH_3	830
13c[c]	CH_2OH	H	CH_2CH_3	620
17a	H	*trans* CH_2OH	H	510
17b	H	*trans* CH3	H	64
17c	H	*trans* CH_2OCH_3	CH_3	270
17d	H	*trans* CH_3	CH_3	110
17e	H	*trans* CH_3	CH_2CH_3	97

[a] All compounds are racemic unless otherwise indicated.
[b] Values are means of two experiments.
[c] Mixture of diastereomers.

Table 2
Biological data for tricyclic compounds



Compd[a]	R	IC_{50}[b] (nM) Mem.	IC_{50} [b] (nM) Cell $A\beta_{40}$, $A\beta_{42}$	CYP 3A4[c] (μM)	RR AUC (h ng/ml)	CRND8 $A\beta_{40}$ at 3 h[d] Inh. Plasma, Cortex
26	=O	13	17, 12	1.2	60	19% (Plasma)
27	OH	28	25, 18	9.1	164	44%, 55%
28	NH2	1700	—	—	—	—
29a[e]	NHTf	27.1	108, 46	>30	5052	90%, 42%
29b	NHAc	107	145, 107	—	1725	—
29c[e]		8.4	9.3, 4.3	>30	1909	80%, 58% at 6 h
29d[e]		7.6	68, 26	—	—	19% (Plasma)

[a] All compounds are racemic unless otherwise indicated.
[b] Values are means of two experiments.
[c] Values determined after 30 min pre-incubation with compound.
[d] Dosed orally at 30 mg/kg.
[e] Pure enantiomer, absolute structure is indicated in Figure 1.

handle to fine tune the overall profile of the final compounds. Further testing has shown that these compounds had no CYP or liver toxicity issues as seen in the earlier series. Selected compounds were also tested in an acute efficacy assay in our CRND8 mouse model.[10] Overall, compound **29a** had the best efficacy in this assay, and it inhibited plasma $A\beta_{40}$ by 90% and cortex $A\beta_{40}$ by 42% three hours after a single oral dose of 30 mg/kg. This compound also significantly lowered plasma and cortical $A\beta_{40}$ after chronic dosing in the CRND8 mouse and did not produce Notch related GI toxicity under conditions where our earlier sulfonamide series produced significant GI toxicity.[11] The full results of this study will be published in due course.

In summary, a novel series of tricyclic sulfone γ-secretase inhibitors was designed and synthesized. Compounds in this new series have comparable or better in vitro activities and in vivo efficacies compared to our previously reported compounds. Additionally, they have completely eliminated P-450 enzyme inhibition and liver toxicity problem that was seen in our previous sulfonamide series. More importantly, further studies revealed that they have greatly reduced mechanism based Notch side effects compared to other γ-secretase inhibitors.

Acknowledgements

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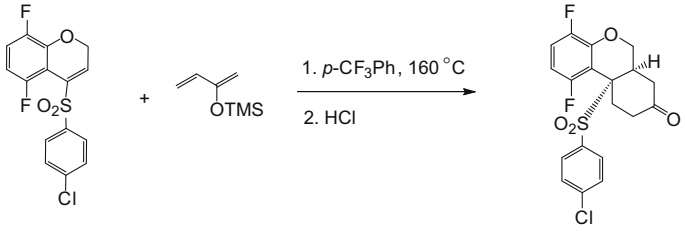

25 **26**

Compound **25** (22 g, 64 mmol) was dissolved in 300 ml trifluorotoluene and 2-trimethylsilyloxy-1,3-butadiene (36.5 g, 260 mmol) was added. The reaction was heated to 160 °C in a sealed tube for 7 h. The reaction was cooled to room temperature and solvent was removed. The residue was dissolved in 200 ml THF and 5 ml 1 N HCl solution was added. The reaction was stirred for 0.5 h at room temperature. EtOAc (200 ml) was added and the organic layer was washed with water (200 ml), brine (200 ml), dried over Na_2SO_4 and concentrated. The residue was purified by column (EtOAc/hexane from 0/100 to 50/50 in 45 min). Yield: 12.6 g, 48%. ^1H NMR ($CDCl_3$ 400 MHz) δ 7.63 (d, J = 8.1 Hz, 2H), 7.52 (d, J = 8.8 Hz, 2H), 7.14 (m, 1H), 6.64 (m, 1H), 5.27 (dd,

J = 11.7 and 2.9 Hz, 1H), 4.13 (d, J = 11.7 Hz, 1H), 3.18 (d, J = 12.4 Hz, 1H), 2.79 (dt, J = 12.4 and 3.7 Hz, 1H), 2.4–2.57 (m, 4H), 2.04–2.17 (m, 1H).
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