Letters

Novel Orally Bioavailable γ-Secretase Inhibitors with Excellent in Vivo Activity

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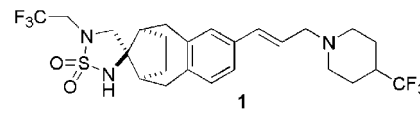
Abstract: The development of potent γ-secretase inhibitors having substituted heterocycles attached to a benzobicyclo[4.2.1]nonane core is described. This work led to the identification of [6*S*,9*R*,11*R*]-2′,3′,4′,5,5′,6,7,8,9,10-decahydro-2-(5-(4-fluorophenyl)-1-methylpyrazol-3-yl)-5′-(2,2,2-trifluoroethyl)spiro[6,9-methanobenzocyclooctene-11,3′-[1,2,5]thiadiazole] 1′,1′-dioxide (**16**), which has excellent in vitro potency (0.06 nM) and which reduced amyloid-β in APP-YAC mice with an ED_{50} of 1 mg/kg (po). **16** had a good pharmacokinetic profile in three preclinical species.

Alzheimer's disease (AD[a]) is a devastating neurodegenerative disorder and is the most common cause of senile dementia in the elderly. The disease is characterized by progressive cognitive and memory deterioration that leads to difficulty in carrying out everyday activities. As life expectancy in the developed world continues to increase, the number of cases of AD is predicted to rise significantly. Currently, the only treatments approved for AD are palliative, and chief among these are cholinesterase inhibitors.[1] A large research effort is focused on the discovery and development of therapies that could potentially halt, or even reverse, the progression of AD.

The main pathological features of AD are neurofibrillary tangles and neuritic plaques. The plaques are composed of 40- to 42-amino acid peptides known as amyloid-β (Aβ) which are generated from β-amyloid precursor protein (βAPP) by sequential cleavage by the enzymes β- and γ-secretase. It has been proposed that accumulation of Aβ is the trigger for progression of AD and therefore, inhibiting production of this protein or increasing its clearance could have therapeutic value.[2] This hypothesis has prompted the search for compounds that inhibit β-secretase[3] or γ-secretase.[4]

This communication describes a novel series of highly potent γ-secretase inhibitors that show excellent in vivo Aβ-lowering efficacy.

The development, in these laboratories, of a series of γ-secretase inhibitors having a bicyclo[4.2.1]nonane core has been described.[5,6] The most potent compound to be disclosed was the trifluoroethyl-substituted cyclic sulfamide **1** which inhibited γ-secretase in a SHSY5Y cell assay[7] with $IC_{50} = 0.24$ nM. In APP-YAC mice,[8] a transgenic line that overexpresses human βAPP, **1** had an ED_{50} of 17 mg/kg at 4 h for reduction of brain Aβ(40).



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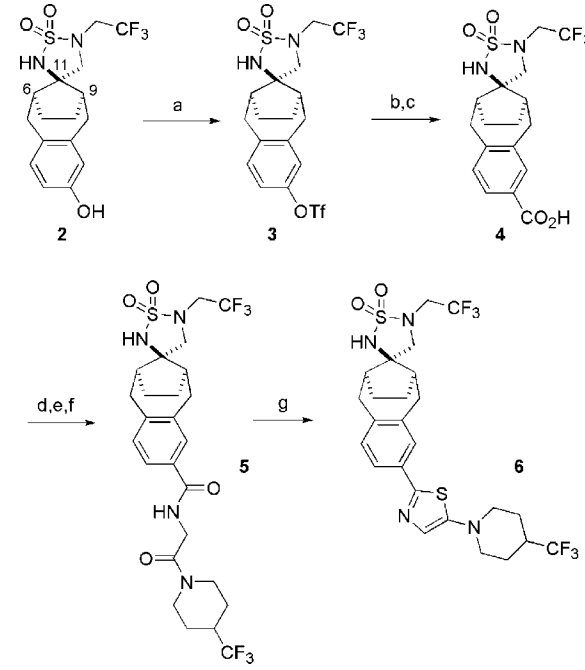
As part of a program to optimize the in vivo activity of **1**, compounds were targeted in which the allylic linker between the bicyclic core and the terminal cyclic amine was replaced by a heterocycle. In this communication, the synthesis and biological activity of γ-secretase inhibitors containing thiazole, triazole, imidazole and pyrazole linking groups will be described.

Scheme 1. Synthesis of Aminothiazole **6**[a]



[a] Reagents: (a) Tf_2O, pyridine, CH_2Cl_2, 0 °C to room temp; (b) CO, $Pd(OAc)_2$, dppp, Et_3N, MeOH, DMSO, 80 °C; (c) 4 M NaOH, THF, 60 °C; (d) $MeO_2CCH_2NH_2.HCl$, HBTU, $^iPr_2NEt$, MeCN, room temp to 40 °C; (e) 4 M NaOH, THF, 50 °C; (f) 4-trifluoromethylpiperidine, HBTU, $^iPr_2NEt$, MeCN, 40 °C; (g) Lawesson's reagent, toluene, reflux.

The synthesis of the 2,5-substituted thiazole **6** is outlined in Scheme 1. The previously described phenol **2**[6] was obtained in homochiral form by supercritical fluid chromatography of the benzyl ether derivative.[9] The phenol was converted to the triflate which was then carbonylated and hydrolyzed to give the

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[a] Abbreviations: AD, Alzheimer's disease; Aβ, amyloid-β; βAPP, β-amyloid precursor protein; APP-YAC, amyloid precursor protein−yeast artificial chromosome.

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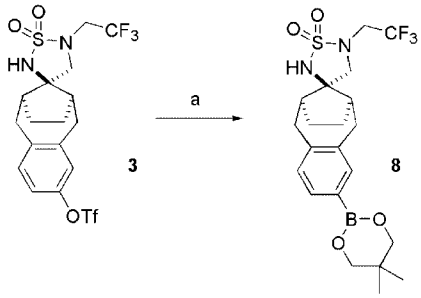
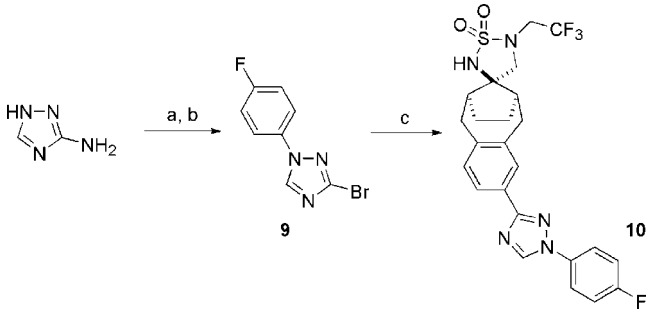
Scheme 2. Synthesis of Glycolatoboronate **8**[a]



[a] Reagents: (a) bis(neopentyl glycolato)diboron, [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with CH_2Cl_2, KOAc, 1,4-dioxane, DMSO, reflux, 88%.

Scheme 3. Synthesis of 1,2,4-Triazole **10**[a]



[a] Reagents: (a) $NaNO_2$, HBr, H_2O, 0 °C to room temp, 44%; (b) (4-F-Ph)$_3$Bi(OAc)$_2$, $Me_2NC(=NH)NMe_2$, $Cu(OAc)_2$, THF, 50 °C, air, 45%; (c) boronate **8**, $Pd(PPh_3)_4$, Cs_2CO_3, 1,2-dimethoxyethane (DME), H_2O, reflux, 73%.

carboxylic acid **4**. The absolute stereochemistry of the required enantiomer was determined by X-ray crystallographic analysis of the salt formed with (1*R*,2*S*)-ephedrine and is as shown in Scheme 1, namely (6*S*,9*R*,11*R*). Coupling of homochiral carboxylic acid with glycine methyl ester was followed by hydrolysis and a further amide coupling to give the acyclic precursor **5**; thiazole formation could then be achieved by heating with Lawesson's reagent.

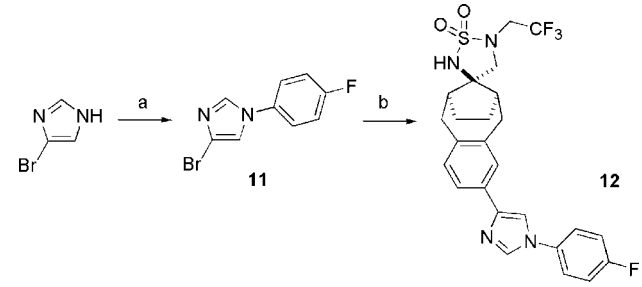
Thiazole-containing compounds were synthesized having a cyclic amine or a 4-fluorophenyl ring as the terminal substituent. The 4-fluorophenyl thiazole (**7**) was synthesized in a manner similar to that outlined in Scheme 1, with the acyclic precursor being the product of coupling between homochiral acid **4** and 2-amino-4′-fluoroacetophenone hydrochloride.

The application of cross-coupling strategies proved vital for rapid exploration of heterocycle structure−activity relationships, and a key synthetic intermediate was the glycolatoboronate **8** derived from triflate **3** as shown in Scheme 2.

The synthesis of the 1,3-diaryl 1,2,4-triazole **10** via a Suzuki coupling is shown in Scheme 3. The preparation of the bromide coupling partner utilizes methodology developed by Finet et al.[10] for the N-phenylation of azoles by triphenylbismuth derivatives in the presence of catalytic copper diacetate. 3-Bromo-1,2,4-triazole was synthesized by diazotization and subsequent bromination of 3-amino-1,2,4-triazole. Reaction with bis(acetato)tris(4-fluorophenyl)bismuth[11] under the published conditions gave bromotriazole **9** which was coupled with the homochiral boronate **8** to give the diaryltriazole **10**.

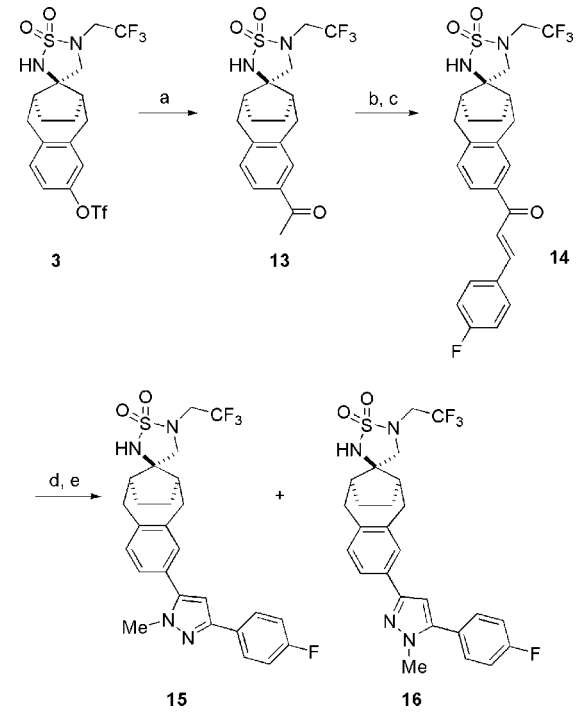
The final step in the synthesis of imidazole **12** (Scheme 4) was again a Suzuki coupling, and, in this case, the required aryl heterocycle was prepared using the copper-catalyzed N-arylation protocol developed by Collman.[12]

Scheme 4. Synthesis of Imidazole **12**[a]



[a] Reagents: (a) $[Cu(OH)\cdot TMEDA]_2Cl_2$, 4-fluorophenylboronic acid, CH_2Cl_2, O_2, 46%; (b) boronate **8**, $Pd(PPh_3)_4$, Cs_2CO_3, DME, H_2O, reflux, 88%.

Scheme 5. Synthesis of Pyrazole Regioisomers **15** and **16**[a]



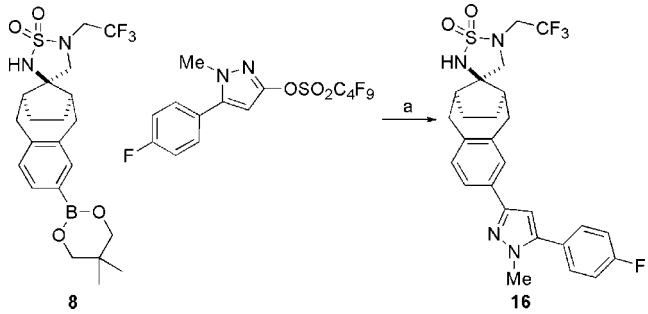
[a] Reagents: (a) (i) $Bu_3SnC(OEt)=CH_2$, $Pd(OAc)_2$, LiCl, PPh_3, DMF, 100 °C, (ii) 1 M $HCl_{(aq.)}$, 84%; (b) LHMDS, 4-fluorobenzaldehyde, THF, −78 °C, 86%; (c) $CeCl_3\cdot 7H_2O$, NaI, MeCN, reflux, 69%; (d) $MeNHNH_2$, EtOH; (e) DDQ, THF, 86% over two steps.

The syntheses outlined in Schemes 3 and 4 were used to generate a number of analogues and were found to be amenable to scale-up.

The fourth heterocycle to be highlighted is the methyl-3,5-diarylpyrazole. This was initially synthesized as a 1:1 mixture of regioisomers by condensation of methylhydrazine with the appropriate racemic 1,3-diketone precursor (not shown). The ratio of isomers could be improved to 5:1 in favor of regioisomer **16** by condensation of methylhydrazine with enone **14**, available in homochiral form, followed by oxidation of the crude product mixture (Scheme 5).

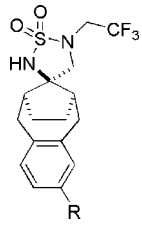
Methylpyrazole regioisomer **16** could be synthesized selectively (Scheme 6) via a Suzuki coupling of boronate **8**. In this case the required coupling partner was 1-methyl-3(5)-(4-fluorophenyl)pyrazole nonaflate which was synthesized regioselectively using methodology described in a publication from these laboratories.[13] Suzuki couplings were utilized to give multigram quantities of a number of halogenated phenylpyrazoles.

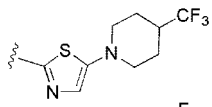
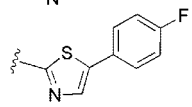
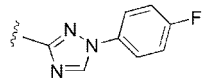
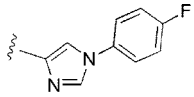
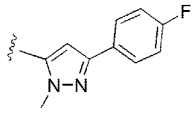
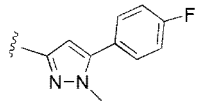
Scheme 6. Optimized Synthesis of Methylpyrazole **16**[a]



[a] Reagents: (a) $Pd(PPh_3)_4$, Na_2CO_3, DMF, 100 °C, 100%.

Table 1. Inhibitory Activities of Heterocycles



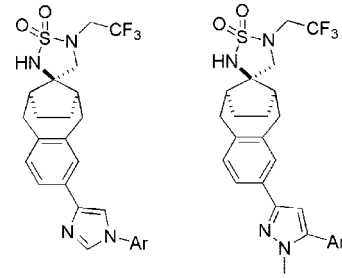
compound[a]	R	IC_{50}, nM (n)
6		0.34 (3)
7		0.42 (4)
10		0.15 (2)
12		0.03 (2)
(±)-15		1.65 (2)
16		0.06 (7)

[a] All compounds are homochiral unless otherwise noted.

All of the heterocycle-linked compounds described above were found to be potent inhibitors of γ-secretase in vitro (Table 1) with subnanomolar IC_{50} values.

The thiazole analogue **6** of the allylic amine **1** retained in vitro potency (IC_{50} = 0.34 nM), and further changing the structure to replace the terminal piperidine by a substituted phenyl moiety (e.g. 4-fluorophenyl, **7**) was also tolerated. It was observed that, in general, the aryl-substituted thiazoles were less active than the aminothiazoles in ion channel counterscreens and therefore, in subsequent investigation of heterocyclic linkers, compounds having a terminal aromatic ring were targeted. For ease of comparison, only the 4-fluorophenyl-substituted heterocycles are shown in Table 1; this substitution pattern was generally well tolerated in vitro. The 1,3-disubstituted 1,2,4-triazole **10** was found to be a potent γ-secretase inhibitor (IC_{50} = 0.15 nM) while the N-substituted imidazole **12** was found to be more potent still (IC_{50} = 0.03 nM). The *N*-methylpyrazole regioisomers **15** and **16** inhibited the γ-secretase enzyme to different extents with the more potent isomer **16** (IC_{50} = 0.06 nM) having in vitro activity comparable to that of the imidazole. On the basis of these results, aryl-substituted imidazoles and pyrazoles were synthesized and representative examples are shown in Table 2. It can be seen that 4-substitution is generally preferred and that several disubstitution patterns are also well tolerated. Further in vitro experiments showed that pyrazole **16**, a representative example of this class of compounds, inhibited production of Aβ(40) and Aβ(42) with similar IC_{50} values and also showed no selectivity over Notch cleavage.[14]

Table 2. Inhibitory Activities of Various Aryl-Substituted Imidazoles and Pyrazoles[a]



Ar	IC_{50}, nM (*n*) imidazoles	pyrazoles
2-fluorophenyl	0.17 (2)	0.21[b] (2)
3-fluorophenyl	0.82 (2)	0.46[b] (2)
4-fluorophenyl	0.03 (2)	0.06 (7)
2,4-difluorophenyl	0.06 (2)	0.06 (2)
3,4-difluorophenyl	0.12 (3)	0.06 (3)
4-chlorophenyl	0.08 (4)	0.07 (2)
3,4-dichlorophenyl	0.42 (4)	0.41 (2)

[a] All compounds are homochiral unless otherwise noted. [b] Given IC_{50} values are for racemic compounds.

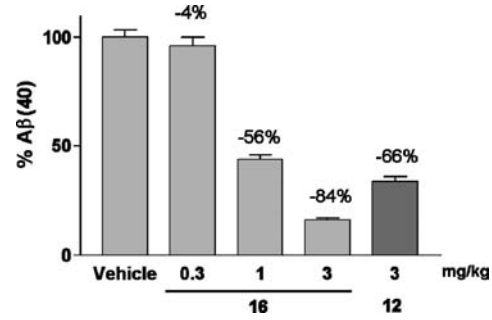


Figure 1. Reduction of brain Aβ(40) 4 h after oral dosing of imidazole **12** and pyrazole **16** to APP-YAC mice.

Two of the most potent compounds in vitro, the 4-fluorophenyl-substituted imidazole **12** and pyrazole **16**, were evaluated in vivo. The compounds were dosed orally, as suspensions in 0.5% methocel, at 3 mg/kg to APP-YAC mice[8] and, 4 h after dosing, the level of soluble Aβ(40) in the forebrain was measured. As shown in Figure 1, the imidazole **12** caused a 66% reduction and the pyrazole **16** an 84% reduction in brain Aβ(40) relative to vehicle-treated animals.

Compound **16** was also dosed orally at 0.3 and 1 mg/kg to APP-YAC mice, and a dose-dependent reduction in Aβ(40) was observed (Figure 1) with an ED_{50} of approximately 1 mg/kg.

Pharmacokinetic evaluation of **16** in rat ($F = 35\%$, $t_{1/2} = 2$ h), dog ($F = 42\%$, $t_{1/2} > 10$ h), and rhesus ($F = 45\%$, $t_{1/2} = 16$ h) demonstrated that the compound had a good pharmacokinetic profile across the range of preclinical species. Further in vivo studies will be reported elsewhere.

In conclusion, a number of heterocycle-containing compounds have been shown to be potent inhibitors of γ-secretase in vitro. Furthermore, the imidazole **12** and pyrazole **16** were highly efficacious in APP-YAC mice. The pyrazole **16**, with an ED_{50} of approximately 1 mg/kg for reduction of Aβ(40) in this model, is one of the most potent γ-secretase inhibitors reported to date.

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Supporting Information Available: Experimental details and spectral data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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