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Discovery of a novel [3.2.1] benzo fused bicyclic sulfonamide-pyrazoles as potent, selective and efficacious γ-secretase inhibitors

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ABSTRACT

Structure–activity relationship (SAR) of a novel, potent and metabolically stable series of benzo [3.2.1] bicyclic sulfonamide-pyrazoles as γ-secretase inhibitors are described. Compounds that are efficacious in reducing the cortical Aβx-40 levels in FVB mice via oral dose, as well as those with high selectivity over Notch, are highlighted.

Alzheimer's disease (AD) is the third most common cause of death, and the most common neurodegenerative disease, in the developed world.[1] The pathological hallmarks of AD are extracellular senile plaques, containing aggregated amyloid-β peptides (Aβ), and intracellular neurofibrillary tangles, containing hyper-phosphorylated tau. Aβ is a 36–43 aminoacid peptide generated by sequential cleavage of amyloid precursor protein (APP) by the proteases BACE and γ-secretase.[2,3] Inhibitors of these two proteases are being explored by several research teams, towards identifying potential treatments for AD.[4,5] γ-Secretase cleaves several non-APP substrates, including Notch, and inhibition of Notch cleavage leads to toxicity in peripheral organs.[6–8] Therefore, compounds selective for inhibition of APP cleavage over Notch cleavage are desirable. Herein we describe a new class of APP-selective γ-secretase inhibitors (GSIs), with improved selectivity for APP, high potency in vitro, suitable pharmacokinetics, and oral activity in rodents.

In several previous communications, we have reported very potent GSIs featuring sulfonamides and pyrazoles.[9,10] Two such GSIs, **1** and **2**, illustrated in Figure 1, are exceptionally selective for APP over Notch, measured using either biochemical[11] or cellular[12] assays.

Unfortunately, both **1** and **2** were metabolically unstable in microsomes and therefore not suitable for further development. Thus, we initiated an effort to identify related compounds with reduced oxidative metabolism. Studying the available SAR, we hypothesized the 3-fluorophenyl group in **1** and the phenyl group in **2** may contribute to the high APP selectivities of these two compounds. Based on this hypothesis, we imagined the benzo [3.2.1] bicycle **3,** illustrated in Figure 1, should maintain the high selectivity of **1** and **2**, but have greater oxidative metabolic stability due to hindrance of its saturated carbon atoms. We developed the synthetic approach illustrated in Scheme 1 to prepare a series of benzo [3.2.1] bicycles. The key intermediate **7** was formed in good yield using a hetero-Diels–Alder reaction. After pyrazole formation,

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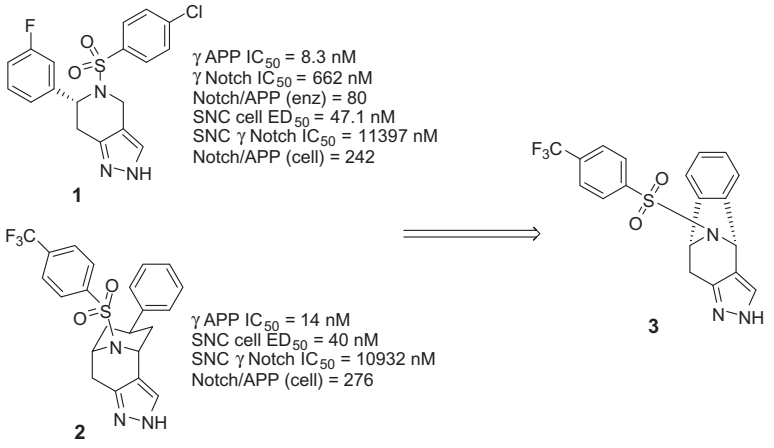
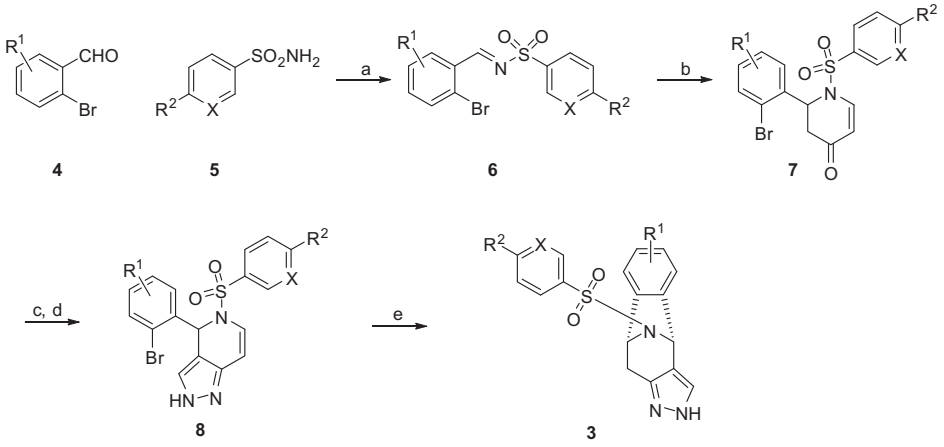
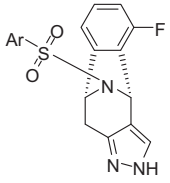


Figure 1. Sulfonamide-pyrazole GSIs with exceptional APP/Notch selectivity.



Scheme 1. Reagents and conditions: (a) $TiCl_4$, Et_3N, DCM, THF; (b) Danishefsky's diene [(*E*)-1-methoxy-3-trimethylsilyloxy-1,3-butadiene], PhMe; (c) Bredereck's reagent [*tert*-butoxy-bis(dimethylamino)-methane]; (d) hydrazine monohydrate, EtOH; (e) nBu_3SnH, AIBN, PhH.

Table 1
Sulfonamide substitution SAR



Compound	Ar	γ-APP IC_{50}[a] (nM)	γ-Notch IC_{50}[a] (nM)	Notch/APP ratio (enzy)	SNC cellular ED_{50}[a] (nM)	SNC cellular Notch IC_{50}[a] (nM)	Notch/APP ratio (cell)	Note
3a	4-CF_3Ph	0.4	36	90	2.2	250	114	Single ent.
3aa	4-CF_3Ph	674	30,305	45	2474	>40,000		Inactive ent. of **7a**
3b	2-CF_3-5-Pyridyle	0.4	18	45	1	146	146	Single ent.
3c	5-CF_3-Pyridyle	N/A	N/A		6.4	622	97	Single ent.
3d	5-Cl-Thiophenyl	N/A	N/A		4.7	497	106	Single ent.
3e	3,4-diFPh	N/A	N/A		2.5	467	187	Single ent.
3f	4-F	N/A	N/A		3.3	826	250	Single ent.
3g	2,4-diClPh	N/A	N/A		43.8	4101	94	Single ent.
3h	4-CF_2HOPh	N/A	N/A		33.1	3346	101	Single ent.
3i	4-CF_3OPh	N/A	N/A		7.1	971	137	Single ent.

[a] Values are means of three experiments.

reductive cyclization by a radical process formed the final products. The enantiomers of the final products were separated by chiral HPLC. In all cases, the separated enantiomers have very different bioactivities (see **3a** and **3aa** in Table 1), and the

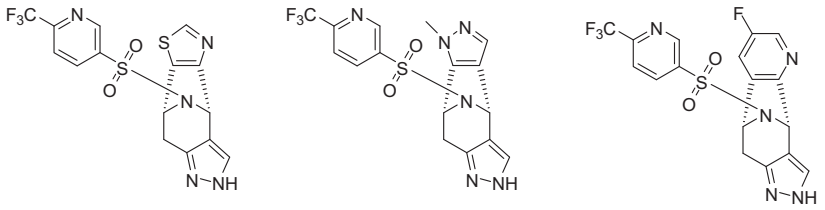


Figure 2. Heteroaromatic replacements for the benzo group.

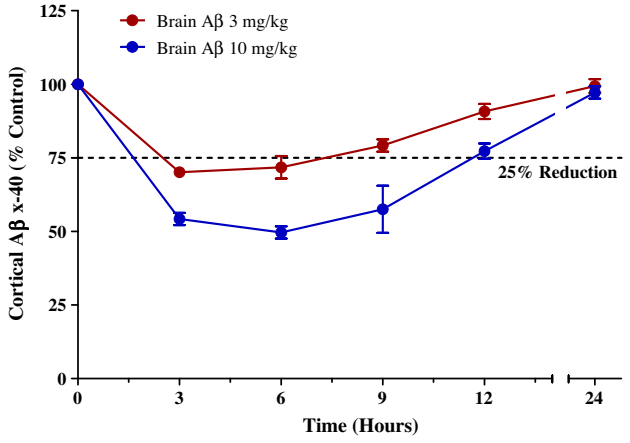


Figure 3. Reduction of cortical Aβx-40 in FVB mice following single oral doses of **3a**.

configurations reported here are assigned by referring to SAR among earlier sulfonamide-pyrazole GSIs, for which small molecule X-ray structures are available.[9,13]

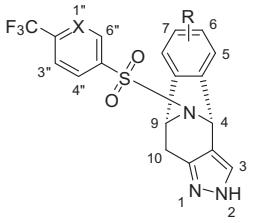
Substituents on the sulfonamide aryl ring were studied first, and the results are listed in Table 1. As observed with earlier sulfonamide-pyrazole GSIs, 4-substituted benzene-sulfonamides are generally preferred. 5-Cl-thiophene-sulfonamides have potency comparable to 4-substituted benzene-sulfonamides, but the former are less stable to oxidative metabolism. Overall, 4-trifluoromethyl-benzenesulfonamides and 2-trifluoromethyl-5-pyridine-sulfonamides exhibited the best in vitro potencies.

Substituents on the benzo group were explored next. Compounds with chlorine substitution on C-5 (**3k**) or C-6 (**3l**) were more potent than the analog with hydrogen atoms at both of these positions (**3j**). Chlorine on C-5 affords slightly higher potency than chlorine on C-6. Chlorine substitution on C-7 (**3m**) has little effect on potency, but chlorine substitution on C-8 (**3n**) leads to a more than 60-fold decrease in potency.

Fluorine substitution on C-5 (**3a**) maintained the potency of chlorine substitution on C-5 (**3k**). Surprisingly, fluorine substitution on C-8 (**3o**) did not reduce potency as did chlorine substitution on C-8 (**3n**). Two di-fluoro compounds (**3p**, **3q**) were prepared, and both displayed excellent potencies. Compounds substituted with cyclopropyl (**3r**), trifluoromethyl (**3t**, **3u**) and 4-fluorophenyl (**3s**) were significantly less potent than the chlorine and fluorine substituted analogs.

Using a synthetic route similar to that illustrated in Scheme 1, several compounds with heteroaromatic rings in place of the benzo

Table 2
Benzo substitution SAR



Compound	X	R	γ-APP IC_{50}[a] (nM)	γ-Notch IC_{50}[a] (nM)	Notch/APP ratio (enzy)	SNC cellular ED_{50}[a] (nM)	SNC cellular Notch IC_{50}[a] (nM)	Notch/APP ratio (cell)	Note
3j	CH	H	1.9	117	62	4.2	595	142	Single ent.
3k	CH	5-Cl	0.22	14	64	0.93	113.33	122	Single ent.
3l	CH	6-Cl	0.57	45	79	2.1	268	128	Single ent.
3m	CH	7-Cl	1.86	165	89	14.2	1831	129	Single ent.
3n	CH	8-CL	122	6998	57	844	>40,000		Single ent.
3a	CH	5-F	0.4	36	90	2.2	250	114	Single ent.
3b	N	5-F	0.4	18	45	1	146	146	Single ent.
3o	N	8-F	1	49	49	4.2	522	124	Single ent.
3p	N	5,8-diF	0.3	12	40	1.6	158	99	Single ent.
3q	CH	6,7-diF	1.1	127	115	8	1093	137	Single ent.
3r	N	5-cPr	4.6	190	41	63.5	5621	89	Single ent.
3s	N	5-(4′-FPh)	60.9	2827	46	364	>40,000		Single ent.
3t	CH	6-CF_3	N/A	N/A		1236	>40,000		Single ent.
3u	CH	7-CF_3	N/A	N/A		58	8043	139	Single ent.

[a] Values are means of three experiments.

Table 3
In vitro DMPK properties of selected compounds

Compound	γ-APP IC_{50}[a] (nM)	Glucuron. microsom. % remain[b] (h, d, r, m)	Oxidative metabol % remain[c] (h, d, r, m)	Papp (nm/s)	Pgp efflux	Sol (μM)
3a	0.4	100, 79, 45, 53	77, 78, 33, 53	158	0.8	65
3b	0.4	100, 88, 71, 100	65, 66, 37, 46	172	1.5	100
3j	1.9	75, 85, 77, 87	47, 57, 27, 53	193	1.1	15

[a] Values are means of three experiments.
[b] Percentage of compound (2 μM) remaining after 30 min incubation in liver microsomes (0.5 mg protein) supplemented with 1 mM UDPGA, 100 mM $MgCl_2$, and 25 mg/mL alamethacin at 37 °C in phosphate buffer.
[c] Percentage of compound (1 μM) remaining after 30 min incubation in liver microsomes (0.5 mg protein) supplemented with 1 mM NADPH at 37 °C in phosphate buffer.

Table 4
Summary of cross species in vivo PK parameters of **3a**

Parameter	SD rat	Beagle dog	Cynomolgus monkey
CL (L/h/kg)	2.9	0.8	0.7
V_{ss} (L/kg)	3.5	1.8	2.5
$AUC_{0-inf.}$ (ng h/mL)	346	1324	1430
%F	17[a]	26[a]	16[b]

[a] *%F* values based on a 2 mg/kg oral administration.
[b] *%F* value based on a 1 mg/kg oral administration.

group in **3** were prepared (Fig. 2). All of these compounds are potent GSIs, but the five-membered heteroaromatics are less potent than the six-membered heteroaromatics.

Several examples of the new GSIs described in this Letter have fair in vitro DMPK properties as illustrated in Table 3.

Encouraged by **3a**'s favorable in vitro properties, we studied the pharmacokinetics in multiple species. Consistent with the in vitro properties, **3a** exhibited moderate to high clearance in rat and moderate clearance in dog and monkey. The bioavailability ranged between 16% and 25% across species (Table 4).

Compound **3a** was tested for bioactivity in FVB mice. As illustrated in Figure 3, 3 h after a single 3 mg/kg dose of **3a**, the cortical Aβx-40 level was reduced by 30%, and the Aβ level returned to baseline within 24 h. Six hours after a single 10 mg/kg dose of **3a**, the cortical Abx-40 level was reduced by 60%, and the Aβ level returned to baseline within 24 h. The brain to plasma exposure ratios of this compound ranged from 0.9 to 1.1.

It is interesting to compare the biochemical and cellular APP/Notch selectivities of **3a** (Table 2) with those of three previously reported sulfonamide GSIs, **BMS-708163**,[14,15] **GSI-953**,[16] and **ELND006**[17] (Fig. 4). Tested side by side, **3a** is 6- to 14-fold more selective than any of these compounds in our biochemical assay, and 1.5- to 2.5-fold more selective than any of these compounds in our cellular assay. We do not have a hypothesis for non-correlation of biochemical and cellular selectivities, but this has been a reproducible finding in our hands.

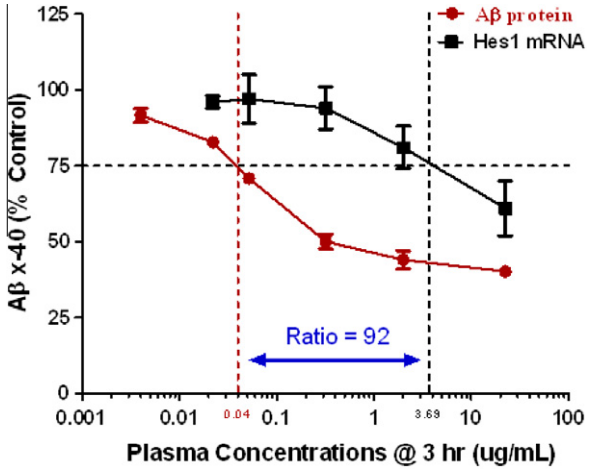
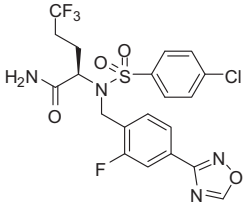


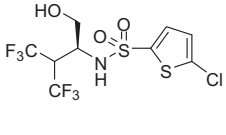
Figure 5. PK-PD in FVB mice: effects of **3a** on Aβ protein in cortex and Hes1 mRNA in thymus 3 h post dose.

Earlier, we reported for **ELND006** a Hes1 mRNA biomarker can be used to determine an acute in vivo APP/Notch selectivity.[17a] In that study, the plasma concentration of **ELND006** required for a 25% reduction of cortical Aβ was 57-fold lower than that required for a 25% reduction of Hes1 mRNA in thymus. We have tested **3a** in a similar PK-PD experiment, in which FVB mice were treated with 0.3, 1, 3, 10, 100 or 300 mg/kg **3a**, followed by quantifying **3a** in plasma, Aβ in cortex, and Hes1 mRNA in thymus. As illustrated in Figure 5, **3a** requires a 92-fold lower plasma concentration for a 25% reduction of Aβ than for a 25% reduction of Hes1 mRNA. The slightly higher in vivo selectivity of **3a**, compared to **ELND006**, correlates more closely to the slightly higher cellular selectivity of **3a**, than to the significantly higher enzyme selectivity of **3a**.

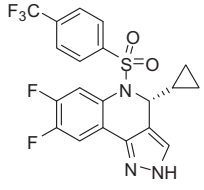
In summary, we designed and prepared a novel series of potent and APP/Notch selective sulfonamide-pyrazole GSIs, featuring a



BMS-708163
γ APP IC_{50} = 0.13 nM
γ Notch IC_{50} = 1.5 nM
Notch/APP (enz) = 12
SNC cell ED_{50} = 1.2 nM
SNC γ Notch IC_{50} = 71 nM
Notch/APP (cell) = 59



GSI-953
γ APP IC_{50} = 1.6 nM
γ Notch IC_{50} = 10.5 nM
Notch/APP (enz) = 6.6
SNC cell ED_{50} = 7.8 nM
SNC γ Notch IC_{50} = 353 nM
Notch/APP (cell) = 45



ELND006
γ APP IC_{50} = 0.34 nM
γ Notch IC_{50} = 5.3 nM
Notch/APP (enz) = 16
SNC cell ED_{50} = 1.1 nM
SNC γ Notch IC_{50} = 82 nM
Notch/APP (cell) = 74

Figure 4. In vitro potencies and selectivities of **BMS-708163**, **GSI-953**, and **ELND006**.

benzo [3.2.1] bicycle framework. The DMPK properties of current scaffold were improved compared to the earlier series (exemplified by compounds **1** and **2**). The in vitro properties (i.e., suggestive to good CNS penetration and moderate to good in vivo CL) were in fact corroborated by in vivo cross species PK behaviors. The presence of a benzo group on the 3.2.1 bicycle framework may contribute to the enhanced APP/Notch selectivity, measured in vitro and in vivo. Efforts to identify analogs with further improved stability to oxidative metabolism will be reported in a subsequent Letter.

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