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# Novel orally active morpholine *N*-arylsulfonamides γ-secretase inhibitors with low CYP 3A4 liability

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ABSTRACT

A new class of 2,6-disubstituted morpholine *N*-arylsulfonamide γ-secretase inhibitors was designed based on the introduction of a morpholine core in lieu or piperidine in our lead series. This resulted in compounds with improved CYP 3A4 profiles. Several analogs that were active at lowering Aβ levels in Tg CRND8 mice upon oral administration were identified.



Alzheimer's disease (AD), a neurodegenerative disease that affects mostly the elderly, remains at the top of the healthcare agenda due to its growing patient population, attached socioeconomic costs, and the limited therapeutic options that are currently available.[1] AD main histological features are the appearance of β-amyloid (Aβ) plaques and tau tangles in the brain of patients.[2] This has led many research groups to concentrate on the secretases[3] and tau kinases[4] believed to be responsible for their generation. Several drug candidates have recently entered in the clinic,[5] although many other areas remain under investigation in light of the complexity of the disease.[6] Among the secretases, γ-secretase remains an attractive target, due to the breadth of inhibitors and modulators,[7,8] that have been discovered so far and the fact that, despite γ-secretase involvement in other regulatory pathways such as Notch processing, early clinical results seem to indicate that a therapeutic window can be attained across several classes of compounds.[9]

Our previous investigations in the γ-secretase inhibitor field led us to report several series of piperidine sulfonamides γ-secretase inhibitors that were efficacious at lowering Aβ levels in Tg CRND8 mice.[7,10]

While the initial series typified by compound **1a** had potential drug–drug interaction due to high CYP 3A4 liability,[7] lowering the molecular weight and crowding the basic amine led to compounds (e.g., **2**) that showed better CYP profiles while retaining in vitro and in vivo Aβ40 lowering capability (Fig. 1).[10b]

Further analysis however indicated that compound **2** has a relatively short half-life in human hepatocytes due to metabolism occurring at the ethyl side chain and potentially at the piperidine core. Reengineering of the core especially at position 4 of the piperidine was therefore attempted and we pursued small molecule morpholine analogs (**I**) while others explored potential metabolites.[10c] One attractive feature of the morpholine core was its impact on lipophilicity that was shown to be a contributing factor to CYP 3A4 liability in our previous series.[10b]

We initially decided to introduce the morpholine core in the higher molecular weight aryl piperidine series that contained the worst CYP 3A4 offenders, to more clearly test the impact of such modification. For ease of synthesis, the *des*-cyclopropyl carbamate series was selected.[10a] Our initial synthetic scheme relied on the preparation of alcohol **4** from L-serine methyl ester, followed by condensation with a diazo ketone in the presence of indium(III) triflate.[11] Reduction followed by intramolecular Mitsunobu led to intermediate **6** that was converted to the carbamate used previously reported methods (Scheme 1).[10a] To our delight, the resulting compound **7** showed an improved CYP 3A4 profile and minimal

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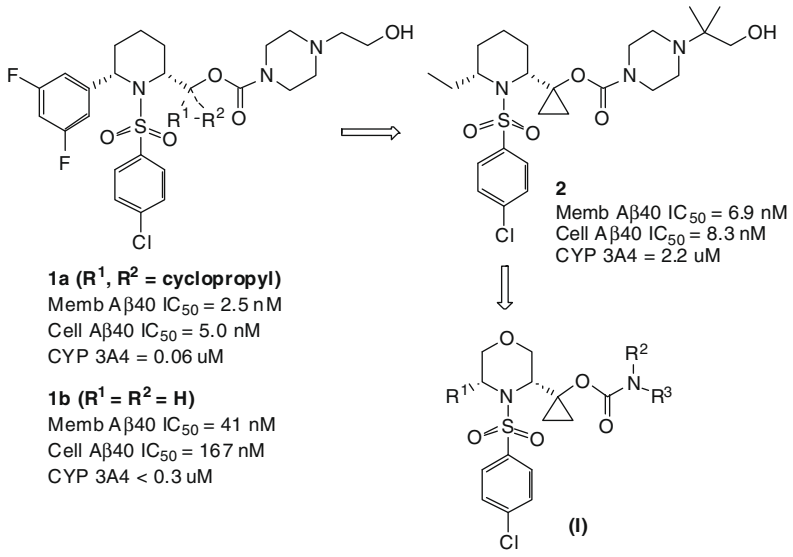
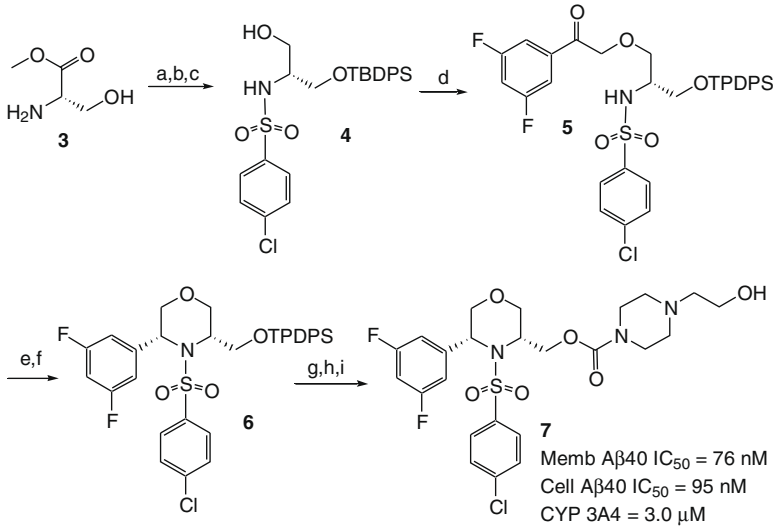



**Figure 1.** Compounds **1–2** and strategy for removing CYP 3A4 liability.



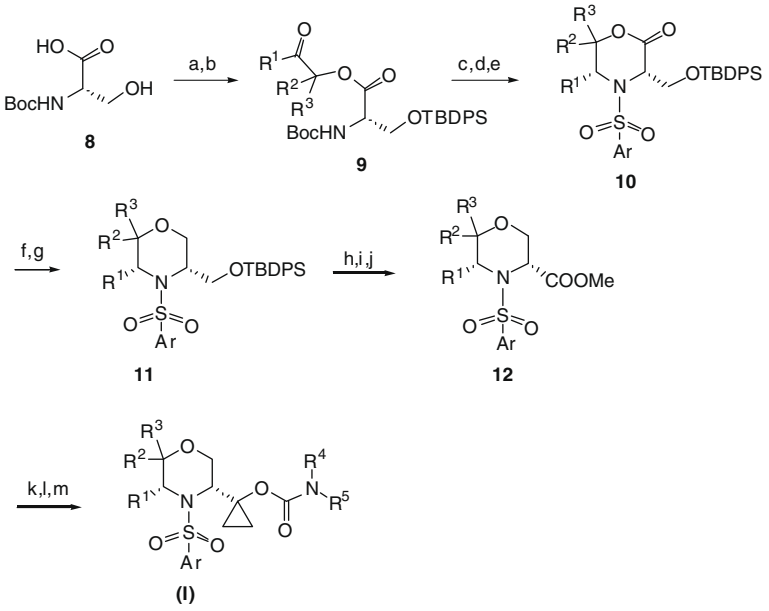

**Scheme 1.** Reagents: (a) 4-ClPhSO$_2$Cl, Et$_3$N; (b) TBDPSCl, imidazole; (c) LAH; (d) 2-diazo-1-(3,5-difluoro-phenyl)-ethanone, In(OTf)$_3$; (e) NaBH$_4$; (f) PPh$_3$, DEAD; (g) TBAF; (h) *p*-NO$_2$PhOC(O)Cl, pyridine; (i) 2-piperazin-1-yl-ethanol.

shift in cell versus membrane Aβ40 assay as compared to parent **1b**. Compound **7** also showed improved blood levels in rat following oral administration at 10 mg/kg ($AUC_{0-6h}$ = 4908 h ng/mL vs 826 h ng/mL for **1b**).

This result led us to refocus our efforts to the morpholine core. Due to a modest yield obtained in the conversion of **4** to **5**, an improved synthesis amenable to cyclopropyl carbamates was envisioned (Scheme 2). This methodology implied the condensation of a Boc-L-Serine carboxylate derivative with a α-bromo or α-hydroxy ketone to generate **9**.[12] Stereoselective intramolecular reductive amination then gave **10** that was opened and ring-closed to the morpholine core **11** under Mitsunobu conditions. Elaboration into ester **12** set the stage for the Kulinkovich reaction[10b,13] and derivatization into the final carbamates (**I**). This methodology allowed for the preparation of highly functionalized morpholine sulfonamides analogs. An alternative to the Kulinkovich strategy was also used involving reacting **12** with Tebbe's reagent followed by Simmons–Smith cyclopropanation and demethylation.[14]

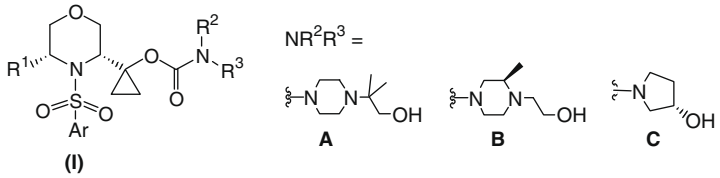
We first assessed high and low molecular weight morpholine analogs using standard carbamates identified in our earlier work.[10b] Compounds were evaluated for their in vitro γ-secretase inhibitory potency, CYP 3A4 liability and preliminary in vivo efficacy in our transgenic pre-plaque Tg CRND8 mice model of AD (Table 1). The standard 3,5-difluorophenyl was used at $R^1$ in the high molecular weight series while the cyclopropyl moiety was explored as a more metabolically stable but still potent alternative to the labile ethyl from compound **2** at $R^1$ for lower molecular weight analogs.

As shown in the table, both series showed comparable and modest CYP 3A4 liability. The 3,5-difluorophenyl series was slightly favored in terms of in vitro potency as compared to the cyclopropyl series, following a trend observed in the piperidine series. However, the cyclopropyl series seemed to have a slight advantage in vivo (compound **14** vs **13**), a feature attributed to the lower molecular weight. The later series was thus progressed further.



**Scheme 2.** Reagents: (a) TBDPSCl, imidazole; (b) $R^1C(O)C(R^2)(R^3)Br$, KOH; or $R^1C(O)C(R^2)(R^3)OH$, DCC; (c) TFA; (d) $NaBH(OAc)_3$; (e) 4-$ClPhSO_2Cl$, $Et_3N$; (f) $NaBH_4$; (g) $PPh_3$, DEAD; (h) TBAF; (i) $RuCl_3$, $NaIO_4$; (j) $SOCl_2$, MeOH; (k) EtMgBr, $Ti(OiPr)_4$; (l) *p*-$NO_2PhOC(O)Cl$, pyridine; (m) $NHR^4R^5$.

**Table 1**
Membrane γ-secretase inhibition, CYP 3A4 profile and preliminary in vivo profile for core morpholine analogs (**I**) (Ar = 4-ClPh)



| Compd | $NR^2R^3$ | $R^1$ | Memb Aβ40 $IC_{50}$[a] (nM) | CYP 3A4[b] (μM) | Tg CRND8 mice Aβ40 reduction[c] (%) |
|---|---|---|---|---|---|
| **13** | A | 3,5-$F_2$Ph | 1.4 | 4.1 | −68 |
| **14** | A | cPr | 9.5 | 2.5 | −78 |
| **15** | B | 3,5-$F_2$Ph | 5.5 | 3.8 | −87 |
| **16** | B | cPr | 37 | 4.1 | |
| **17** | C | 3,5-$F_2$Ph | 12 | 9.6 | +21 |
| **18** | C | cPr | 27 | >30 | |

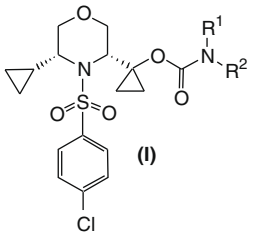
[a] Values are mean of two experiments.
[b] Values determined after 30 min pre-incubation with compound.
[c] Reduction in plasma Aβ40 at 3 h time point, following 30 mg/kg sub-cutaneous dosing.

A variety of cyclopropyl analogs were evaluated for their primary in vitro γ-secretase potency and CYP 3A4 liability, and rat pharmacokinetic (PK) data were obtained following 10 mg/kg oral administration when appropriate (Table 2). The best candidates were evaluated in the Tg CRND8 mice after acute oral administration. Following our exploration of CYP 3A4 mitigating approaches in the small molecule piperidine sulfonamides series,[10b] we directed our efforts towards candidates featuring hindered basic amines or non-basic amines at the right-hand side.

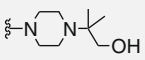
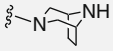
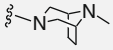
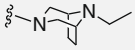
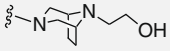
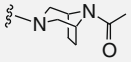
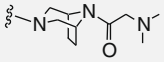
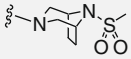
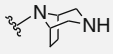
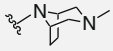
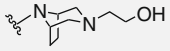
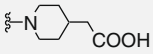
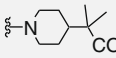
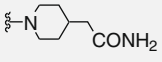
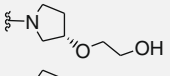
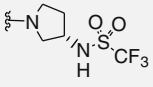
The best results were obtained using previously reported bridged piperazines.[10b] Compound **22** in particular showed good potency, excellent CYP3 A4 profile, good blood levels in rat and it greatly lowered plasma Aβ40 in CRND8 mice. Other basic amines variants in the series, for example, **19–21**, also featured excellent plasma Aβ40 efficacies and good to excellent rat PK levels albeit more modest CYP 3A4 profiles. Removing the basic amine led to reduction in either rat PK level (amides **23**, **24**) or potency (**25**). Moving the bridge away from the basic amine also resulted in loss of CYP 3A4 selectivity. As reported before, candidates devoid of basic amine functionality usually have much reduced rat PK levels and this was certainly observed with compound **32**. However, we were pleased to observe that acid variants **29 and 30** showed excellent CYP 3A4 profile and average to excellent rat AUCs. This was unfortunately accompanied by a drop in brain penetration and, as a consequence, the compounds were not progressed further in CNRD8 mice.

Armed with this data, we further explored modification at the morpholine core (Table 3). Introduction of an extra methyl resulted in improved potency for both *cis* and *trans* series but CYP 3A4 liability resurfaced, probably related to the increased lipophilic environment at the morpholine oxygen location. Rat blood levels also dropped substantially. In another attempt at trimming the scaffold at $R^1$ to lower CYP 3A4 liability, trimethyl morpholines analogs (e.g., **38**, **39**) were prepared but, while the *gem*-dimethyl substitution contributed in gaining potency, it was accompanied again by a drop in CYP 3A4 selectivity and/or rat blood levels.

**Table 2**
In vitro and AUC data in rat for series (**I**)

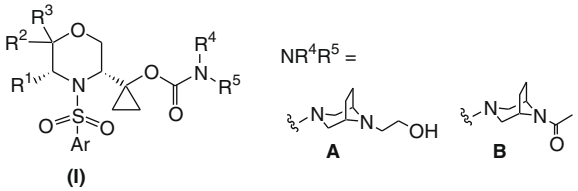


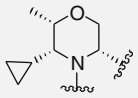
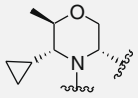
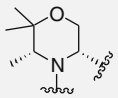

**(I)**

| Compd | $NR^1R^2$ | Memb Aβ40 $IC_{50}$[a] (nM) | Cell Aβ40 $IC_{50}$ (nM)[a] | CYP 3A4[b] (μM) | Rat $AUC_{0-6h}$[c] (h ng/mL) | Tg CRND8 mice reduction in plasma Aβ40 (30 mg/kg po, 3 h) |
|---|---|---|---|---|---|---|
| **14** |  | 9.5 | 9.0 | 2.5 | 446 | −78%[d] |
| **19** |  | 7.1 | 25 | 2.2 | 4365 | −90% |
| **20** |  | 4.6 | 20 | 3.2 | 939 | −88% |
| **21** |  | 10 | 25 | 3.4 | 1099 | |
| **22** |  | 7.9 | 15 | 20 | 1916 | −93% |
| **23** |  | 15 | 25 | >30 | 871 | |
| **24** |  | 8.9 | 31 | >30 | 567 | |
| **25** |  | 41 | | 16 | | |
| **26** |  | 17 | 72 | <0.3 | 4662 | |
| **27** |  | 2.4 | 17 | 0.5 | 2908 | |
| **28** |  | 15 | 27 | 1.0 | 1276 | |
| **29** |  | 24 | | >30 | 2463[e] | |
| **30** |  | 30 | | >30 | 703[e] | |
| **31** |  | 14 | 41 | 15 | 35 | |
| **32** |  | 20 | 31 | >30 | 11 | |
| **33** |  | 49 | | 11 | 870 | |

[a] Values are mean of two experiments.
[b] Values determined after 30 min pre-incubation with compound.
[c] Measured over 0–6 h after 10 mg/kg oral dosing in rat.
[d] Sub-cutaneous administration.
[e] Brain concentration <10 ng/mL at 6 h.

**Table 3**
Impact of morpholine core modification




| Compd | Core | $NR^4R^5$ | Memb Aβ40 $IC_{50}$[a] (nM) | CYP 3A4[b] (μM) | Rat $AUC_{0-6h}$[c] (h ng/mL) |
|---|---|---|---|---|---|
| **34** |  | A | 1.5 | <0.3 | |
| **35** | | B | 5.4 | 4.8 | 178 |
| **36** |  | A | 0.9 | 0.5 | |
| **37** | | B | 4.0 | 24 | 23 |
| **38** |  | A | 1.1 | <0.3 | 1372 |
| **39** | | B | 4.6 | 6.7 | 6 |

[a] Values are mean of two experiments.
[b] Values determined after 30 min pre-incubation with compound.
[c] Measured over 0–6 h after 10 mg/kg oral dosing in rat.

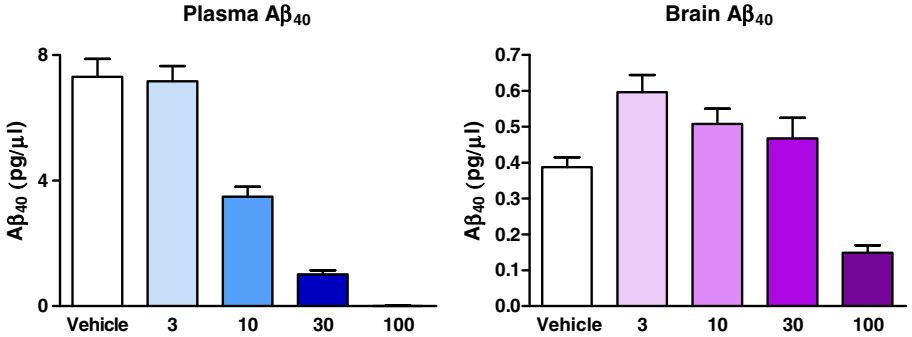



**Figure 2.** Reduction in Aβ40 production in CRND8 mice following 5 days oral administration with compound **22**.

Our best compound **22** was dosed orally once a day in Tg CRND8 mice at various doses ranging from 3 to 100 mg/kg and over a period of 5 days. Plasma and brain Aβ40 levels were collected at the end of the experiment (Fig. 2). This compound again showed a robust reduction in plasma Aβ40 with an $ED_{50}$ = 10 mg/kg. Reduction in brain A40 required higher dose however, with an $ED_{50}$ estimated at 100 mg/kg. The compound had an $IC_{50}$ = 194 nM for Notch processing in whole cells, indicative of a ~13-fold selectivity for inhibition of Aβ40 formation versus this process.

In summary, replacement of the core piperidine into morpholine in our lead series resulted in compounds with improved CYP 3A4 profile. Further modification of the core to optimize brain penetration and potency will be reported in due course.

## Acknowledgments

The authors wish to thanks Drs. A. Buevich, T.-M. Chan, and A. Evans for their help in confirming many structures by NMR as well as for many helpful discussions.

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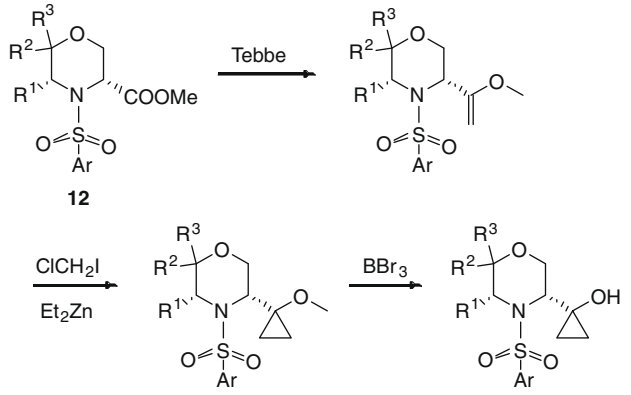
14. The following route was used prior to steps l-m of Scheme 2:




This route used processes derived from: Pine, S. H. *Org. React.* **1993**, *43*, 1; Denmark, S. E.; Beutner, G. In *Cycloaddition Reactions in Organic Synthesis*; Kobayashi, S., Jorgensen, K. A., Eds; Wiley-VCH Verlag GmbH & Co KGaA: Weinheim, Germany, 2002; p 85.