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Piperazinyl pyrimidine derivatives as potent γ-secretase modulators

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ABSTRACT

The development of a novel series of piperazinyl pyrimidines as γ-secretase modulators for potential use in the treatment of Alzheimer's disease is disclosed herein. Optimization of a screening hit provided a series of potent γ-secretase modulators with >180-fold in vitro selectivity over inhibition of Notch cleavage.

Alzheimer's disease (AD) is a debilitating illness that represents a significant unmet medical need.[1] The number of people afflicted with the disease worldwide is expected to triple by the year 2050.[2] While there are symptomatic treatments available, there is currently no treatment that is considered disease-modifying.[3] The primary pathological event in sporadic and familial AD is the extracellular accumulation of amyloid-β (Aβ) peptides and formation of amyloid plaques.[4] One strategy for developing a disease-modifying AD therapy has been to reduce or eliminate the production of Aβ peptides through inhibition of γ-secretase, which generates Aβ peptides from the amyloid precursor protein (APP).

Several γ-secretase inhibitors are currently in clinical trials.[5] The major side effects observed with γ-secretase inhibitors are thought to be associated with blocking the processing of Notch, a transmembrane receptor which is also a substrate of γ-secretase.[6] To avoid Notch-related toxicity we pursued a strategy that focused on the discovery of a small molecule that modulates the processing of γ-secretase substrates, specifically targeting reductions in Aβ42. The pathogenic Aβ42 peptide fragment is thought to play a more significant role in AD pathology.[4] This approach was supported by recent reports that non-steroidal anti-inflammatory drugs reduce Aβ42 generation and spare Notch processing and Aβ40 production.[7,8]

We describe herein the synthesis and biological activity of a novel series of piperazinyl pyrimidine based γ-secretase modulators, which have been the subject of a recent patent application by our group.[9] As part of our screening campaign, we identified **1** which selectively inhibited the production of Aβ42 over Aβ40 (IC_{50} – Aβ42 = 1912 nM and IC_{50} – Aβ40 = 3736 nM; Figure 1).[10] Preliminary SAR studies led to the discovery of **2**, a more selective γ-secretase modulator (IC_{50} – Aβ42 = 169 nM and IC_{50} – Aβ40 = 2953 nM), which was identified as a lead candidate. Encouraged by the structural simplicity and degree of selectivity of this lead candidate, we initiated a lead optimization campaign.

The general synthetic approach employed to prepare analogs of **1** is outlined in Scheme 1. Regioselective nucleophilic addition of anilines and aminopyrazoles to 2,4-dichloropyrimidines **3** in the

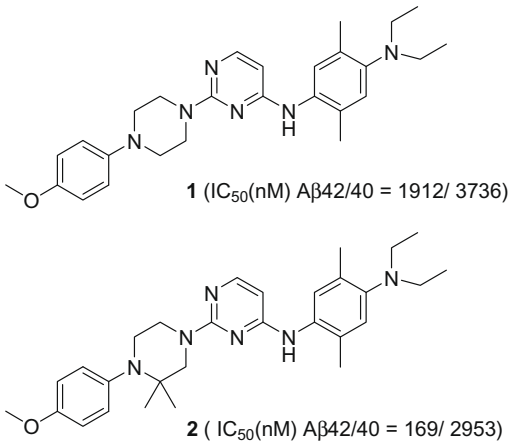

Figure 1. Novel class of piperazinyl pyrimidine γ-secretase modulators.

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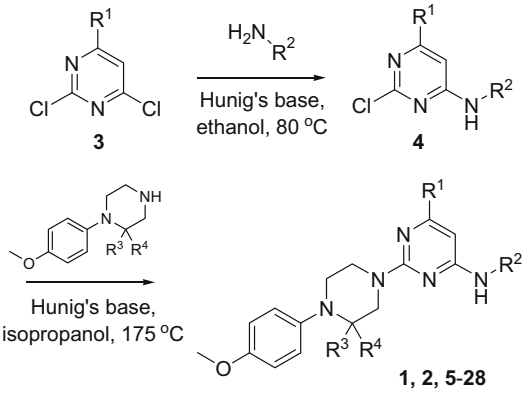


Scheme 1. Synthetic strategy for the preparation of 2,4-disubstituted and 2,4,6-trisubstituted pyrimidines.

presence of base afforded the corresponding 4-aminopyrimidines. A second addition of 4-methoxyphenyl piperazines to **4** using microwave irradiation gave the corresponding pyrimidines **1**, **2**, **5**–**28**.[11]

Initial SAR studies focused on improving potency, selectivity of Aβ42 against Aβ40, and the replacement of the electron rich anilines which are potential metabolic liabilities. SAR of the 4-methoxyphenyl piperazine of **1** showed this region to be very sensitive to modification. For example, attempts to migrate or replace the 4-methoxy group led to complete loss in potency. The piperazine ring proved to tolerate substitution, with minor changes often leading to very little change in potency; however, there was one important exception. Introduction of a *gem*-dimethyl group into the piperazine ring improved potency of Aβ42 inhibition but had very little effect on Aβ40 inhibition (Fig. 1, **1**→**2**). The conformation of the 4-methoxyphenyl piperazine of **1** and **2** sample different conformational spaces as shown in Figure 2.[12] This subtle difference in the conformational space, especially the orientation of the 4-methoxyphenyl group with respect to the aniline group, may contribute to the observed 17-fold Aβ42 selectivity of **2**. Given the improvement in selectivity, future SAR continued with **2** as the lead candidate.

We then turned our attention toward exploring the SAR profile of the 1,4-benzenediamine moiety (Table 1). Removal of the 4-diethylamino group led to a loss in potency that could be partially recovered via introduction of a 4-ethoxy group (**5**, **6**). Interestingly, removal of the 5-methyl group led to a 10-fold loss in potency, whereas introduction of an isopropyl group in the same position resulted in a 10-fold improvement in potency (**7**, **8**). Using this observation to our advantage, we were able to remove the 4-ethoxy group while maintaining potency by introduction of a *tert*-butyl group at the 5-position (**9**). The introduction of a phenyl

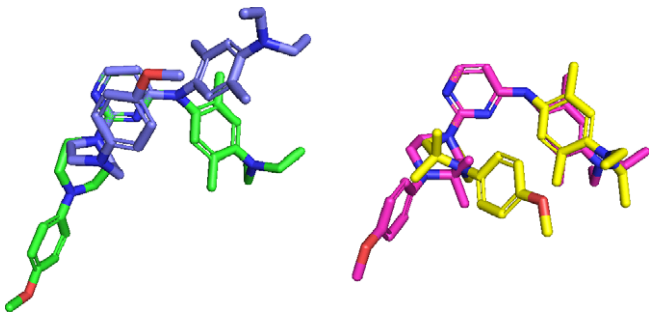
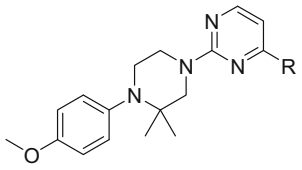
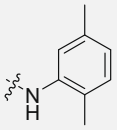
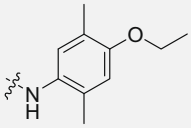
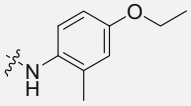
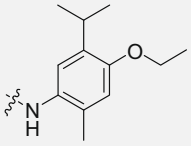
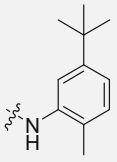
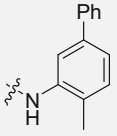
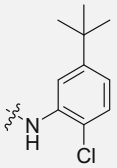
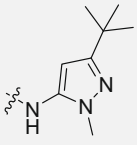
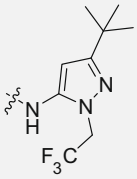


Figure 2. Two most diverse representative conformers of **1** (left) and **2** (right) are shown above.[12]

Table 1
In vitro activity of 2,4-disubstituted pyrimidines against Aβ42 and Aβ40



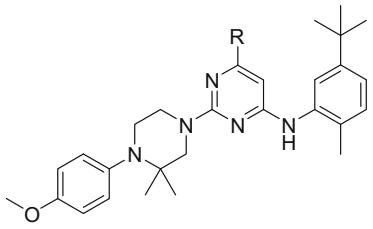
Compound	R^1	Aβ42 IC_{50}[10] (nM)	Aβ40 IC_{50}[10] (nM)
5		1004	4579
6		534	6303
7		3444	10,000
8		35.6	737
9		255	5565
10		410	2282
11		695	1000
12		150	1938
13		272	5087

ring at the 5-position led to a loss of potency (**10**). The methyl group in the 2-position proved to be important for both potency and selectivity (**11**). The importance of the 5-*tert*-butyl and 2-methyl groups for potency and selectivity is further supported by the fact that the 3-*tert*-butyl-1-methyl-1*H*-pyrazol-5-amine is a good isostere for the 5-*tert*-butyl-2-methylaniline (**9** vs **12**). We

continued our SAR studies with **9** since 5-*tert*-butyl-2-methylaniline was potentially less prone to metabolism than 4-ethoxyaniline or 1,4-benzenediamine moieties.

Preliminary SAR studies showed that the 6-position of the pyrimidine ring of **9** was a permissive region for polar groups. With this in mind, we focused our SAR efforts in this area with the goal of concurrently improving potency and the physical parameters of **9** (Table 2). In our survey we found that polar groups were well tolerated in this region with potency retained and physical parameters improved. Of all the polar substitutions examined, hydroxymethyl **19** was the most potent which may be due to the gain of a hydrogen bond interaction.

Table 2
In vitro activity of 2,4,6-trisubstituted pyrimidines against Aβ42 and Aβ40



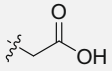
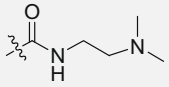
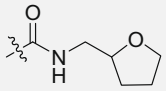
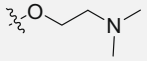
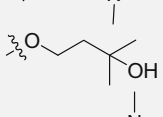
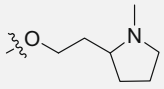
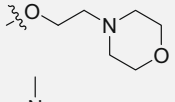
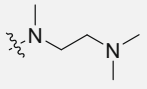
Compound	R^1	Aβ42 IC_{50}[10] (nM)	Aβ40 IC_{50}[10] (nM)
14		348	6461
15		400	5780
16		234	1927
17		572	6408
18		85	2289
19		34	823
20		307	2985
21		112	1712
22		194	8120
23		262	5682
24		108	1586
25		216	5452
26		235	5598
27		263	6151
28		213	1970
29		88	830

Table 3
In vitro activity of 2,4,6-trisubstituted pyrimidines against Notch cleavage

Compound	Notch IC_{50}[13] (nM)	Notch IC_{50}/Aβ42 IC_{50}
19	6140	180
22	>50,000	>250
23	>50,000	>190
24	28,840	270
25	>50,000	>230
26	>50,000	>210
27	>50,000	>190
29	34,430	390

Several lead compounds were profiled in a Notch cleavage assay[13] and found to have at least a 180-fold selectivity ratio between inhibition of Aβ42 and Notch cleavage (Table 3).

In summary, a novel class of piperazinyl pyrimidines was discovered which demonstrates potent and selective in vitro inhibition of Aβ42 over Aβ40 production and at least 180-fold selectivity over inhibition of Notch cleavage. The SAR studies are highlighted by the significant improvement in Aβ42/Aβ40 selectivity via introduction of the *gem*-dimethyl group. These results support that modulating the cleavage site of γ-secretase substrates with small molecules may prove to be an effective anti-amyloid therapeutic strategy while avoiding Notch-related toxicities.

References and notes

1. (a) Selkoe, D. *Physiol. Rev.* **2001**, *81*, 741; Hardy, J. A.; Higgins, G. A. *Science* **1992**, *256*, 184; Golde, T. E. *J. Clin. Invest.* **2003**, *111*, 1.
2. (a) Herbert, L. E.; Beckett, L. A.; Scherr, P. A.; Evans, D. A. *Alzheimer Dis. Assoc. Disord.* **2001**, *15*, 169; (b) Griffiths, H. H.; Morten, I.; Hooper, N. M. *Expert Opin. Ther.* **2008**, *12*, 704.
3. Cummings, J. L. *N. Eng. J. Med.* **2004**, *351*, 56.
4. (a) Dickson, D. W. *J. Neuropathol. Exp. Neurol.* **1997**, *56*, 321; (b) Hardy, J.; Selkoe, D. J. *Science* **2002**, *297*, 353.
5. Imbimbo, B. P. *Curr. Top. Med. Chem.* **2008**, *8*, 54.
6. (a) Maillard, I.; Adler, S. H.; Pear, W. S. *Immunity* **2003**, *19*, 781; (b) Stanger, B. Z.; Datar, R.; Murtaught, L. C.; Melton, D. A. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 12443; (c) Behr, D. *Curr. Top. Med. Chem.* **2008**, *8*, 34.
7. (a) In't Veld, B. A.; Ruitenberg, A.; Hofman, A.; Launer, L. J.; Van Duijn, C. M.; Stijnen, T.; Breteler, M. M. B.; Stricker, B. H. C. *N. Eng. J. Med.* **2001**, *345*, 1515; (b) Weggen, S.; Eriksen, J. L.; Das, P.; Sagi, S. A.; Wang, R.; Pietrzik, C. U.; Findlay, K. A.; Smith, T. E.; Murphy, M. P.; Butler, T.; Kang, D. E.; Marquez-Sterling, N.; Golde, T. E.; Koo, E. H. *Nature* **2001**, *414*, 212; (c) Lim, G. P.; Yang, F.; Chu, T.; Chem, P.; Beech, W.; Teter, B.; Tran, T.; Ubeda, O.; Hsaio Ahse, K.; Reautschy, S. A.; Cole, G. M. *J. Neurosci.* **2000**, *20*, 5709.
8. (a) Peretto, I.; La Porta, E. *Curr. Top. Med. Chem.* **2008**, *8*, 38; (b) Pissarnitski, D. *Curr. Opin. Drug Disc. Dev.* **2007**, *10*, 392.
9. Blurton, P.; Fletcher, S.; Teall, M.; Harrison, T.; Munoz, B.; Rivkin, A.; Hamblett, C.; Siliphaivanh, P.; Otte, K. WO 099210, 2008.
10. IC_{50} measurements for Aβ40 and Aβ42 were determined using electrochemiluminescent detection of peptides secreted by SH-SY5Y cells stably overexpressing the β-APP C-terminal fragment SPA4CT. Consistent with the profiles of γ-secretase modulators, total Aβ peptide levels were constant (a) Best, J. D.; Jay, M. T.; Otu, F.; Ma, J.; Nadin, A.; Ellis, S.; Lewis, H. D.; Pattison, C.; Reilly, M.; Harrison, T.; Shearman, M. S.; Williamson, T. L.; Atack, J. R. J. *Pharm. Exp. Ther.* **2005**, *313*, 902; (b) Clarke, E. E.; Shearman, M. S. *J. Neurosci. Methods* **2000**, *102*, 61; (c) Dyrks, T.; Dyrks, E.; Monning, U.; Urmoneit, B.; Turner, J.; Beyreuther, K. *FEBS Lett.* **1993**, *335*, 89.
11. Compound **29** was prepared using methods reported by Li, C.; Rosenau, A. *Tetrahedron Lett.* **2009**, 5888.
12. Conformational analysis was performed with the implicit Generalized–Born solvent model with 8 kcal/mol energy cutoff. Clustering of conformers based on the atomic RMS dissimilarity was used to generate the 10 most diverse representative conformers. Two of the most diverse conformers for **1** and **2** are shown in Figure 2.
13. HeLa cells were made to co-express nonfunctional halves of luciferase, one fused to NotchΔE and the other to RBP-Jκ. γ-Secretase mediated cleavage of NotchΔE results in release of Notch intracellular domain (NICD)/N-terminal luciferase, which translocates to the nucleus and binds the RBP-Jκ/C-terminal luciferase to form a functional luciferase enzyme. This split-luciferase complementation system is used to detect NICD levels by measuring total luminescence upon addition of luciferin to lysed cells Paulmurugan, R.; Gambhir, S. S. *Anal. Chem.* **2005**, *77*, 1295.