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Purine derivatives as potent γ-secretase modulators

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ABSTRACT

The development of a novel series of purines as γ-secretase modulators for potential use in the treatment of Alzheimer's disease is disclosed herein. Optimization of a previously disclosed pyrimidine series afforded a series of potent purine-based γ-secretase modulators with 300- to 2000-fold in vitro selectivity over inhibition of Notch cleavage and that selectively reduces Aβ42 in an APP-YAC transgenic mouse model.

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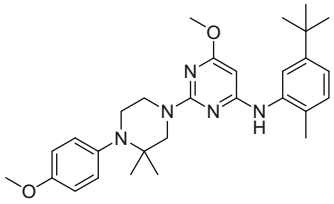
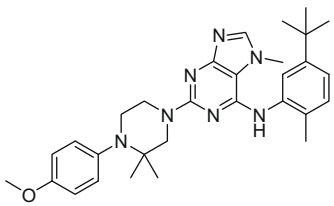
Alzheimer's disease (AD) is a debilitating illness with unmet medical needs.[1] The number of people afflicted with the disease worldwide is expected to triple by the year 2050.[2] While there are symptomatic treatments available, there is currently no treatment that is considered disease-modifying.[3] 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 have previously disclosed a novel series of piperazinyl pyrimidines, which selectively inhibits the production of Aβ42 over Aβ40 (e.g., **1**; IC_{50}-Aβ42 = 260 nM and IC_{50}-Aβ40 = 5700 nM) and shows >180-fold selectivity over inhibition of Notch cleavage (Fig. 1).[9] Herein, we describe the subsequent optimization of this series resulting in the identification of a novel purine series (e.g., **2**) possessing low nanomolar potency for the selective in vitro inhibition of Aβ42 and proof of activity in vivo.



1 (IC_{50}(nM) - Aβ42/40 = 260/ 5700 nM)



2 (IC_{50}(nM) - Aβ42/40 = 21/ 350 nM)

Figure 1. Novel class of purine-based γ-secretase modulators.

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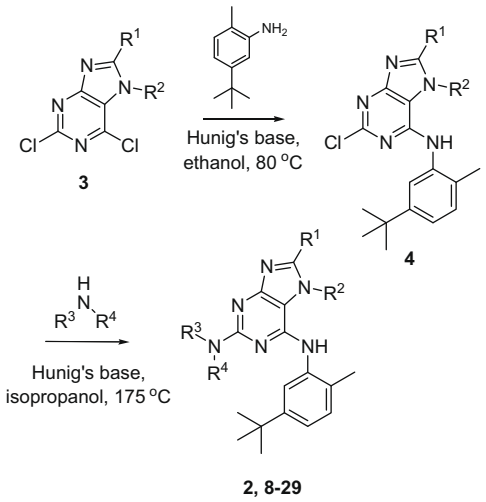
The general synthetic approach employed to prepare analogs of **2** is outlined in Scheme 1. Regioselective nucleophilic addition of 5-*tert*-butyl-2-methylaniline to 2,6-dichloropurine **3** in the presence of base afforded the corresponding 6-aminopurine **4**. A second addition of a variety of amines to **4** using microwave irradiation gave the corresponding purines **2**, **8**–**29**.

During the course of our SAR studies with the previously disclosed pyrimidine series (e.g., **1**), we discovered that polar groups were well tolerated in the 6-position of the pyrimidine ring with retained or enhanced potency and improved physical properties. The enhancement of potency in certain cases was thought to be partially attributed to a hydrogen bond interaction. With this hypothesis in mind, we continued to explore the SAR of the pyrimidine ring with the goal of enhancing this interaction in order to improve the potency. While exploring a variety of fused pyrimidine analogs, we discovered purine **2** that was 10-fold more potent than **1** and equally selective, thus supporting the hypothesis that a hydrogen bond interaction may be important (Table 1). This hypothesis is reinforced by the loss in potency of regioisomer **5**, pyrrolopyrimidine **6** (see also **6** vs **8**), and pyridopyrimidine **7**. Further SAR studies with **2** showed that the purine ring is tolerant of minor substitution, with subtle changes often leading to small fluctuations in potency (**8**–**12**).

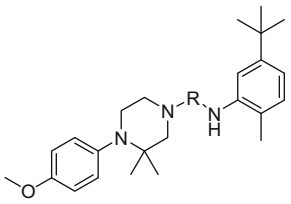
We then turned our attention to exploring the SAR profile of the 4-methoxyphenyl piperazine of **2** with the goal of improving the potency and replacing this moiety with something less prone to metabolism (Table 2). Survey of the SAR showed that this region is tolerant to a wide variety of substitutions, which is in stark contrast to the lack of tolerability that this same region showed in the previously disclosed pyrimidine series (e.g., **1**).[9] Interestingly, the enhancement of selectivity by the *gem*-dimethyl substitution, which we observed in the pyrimidine series, is not present in the purine series (**2** vs **14**). These differences in SAR may be attributed to a shift in the binding mode of the purine series where the 4-methoxyphenyl is situated in a less constrained location. The replacement of the oxygen atom with a carbon atom (**14** vs **15**) led to a 20-fold loss in potency, which may be partially due to the loss of a hydrogen bond interaction between the 4-methoxy group and the enzyme. As a result, we continued our SAR studies while attempting to preserve this presumable hydrogen bond interaction. Variation of the phenyl or piperazine rings led to a substantial loss in potency (**16**–**18**). Introduction of an imidazolyl or pyridyl piperidine led to loss of potency that could be recovered in the pyridyl piperidine case by addition of a carbon spacer (**19**–**21**). SAR studies of 4-(piperidin-4-ylmethyl)pyridine of **21** led to further improvement of potency (**22**–**24**). Interestingly, replacement of the piperidine ring with a phenyl ring led not only to a loss of potency but also to a reduction in selectivity (**21** vs **25**). Introduction of a tetrahydropyran in place of the pyridine ring led to loss of potency as well but without a substantial impact on selectivity (**22** vs **26**). While introduction of pyridyl piperidine led to a 17-fold loss in potency relative to **2**, the incorporation of a pyridyl pyrrolidine led only to a threefold loss (**20** vs **27**). Maintaining the pyrrolidine ring system was not required for retention of potency (**28**, **29**), which is in contrast to what was observed in the SAR of 4-methoxyphenyl piperazine (**14** vs **17**).

Several lead compounds were further profiled in a Notch cleavage assay[11] and found to have at least a 300-fold selectivity ratio between inhibition of Aβ42 and Notch cleavage (Table 3).



Scheme 1. Synthetic strategy for the preparation of the purine series.

Table 1
In vitro activity of the purine series against Aβ42 and Aβ40



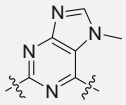
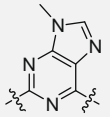
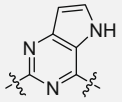
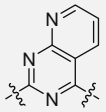
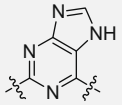
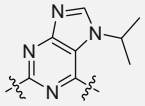
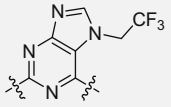
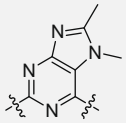
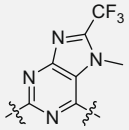
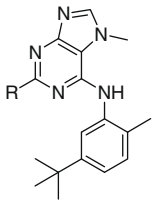
Compound	R	Aβ42 IC_{50}[10] (nM)	Aβ40 IC_{50}[10] (nM)
2		21	350
5		130	2600
6		750	3000
7		210	3100
8		68	1000
9		50	350
10		55	420
11		22	160
12		50	1000

Table 2
In vitro activity of the purine series against Aβ42 and Aβ40



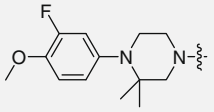
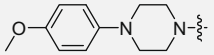
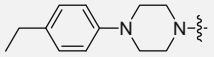
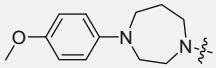
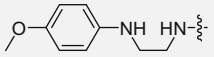
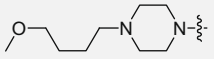
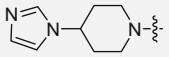
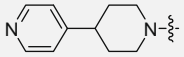
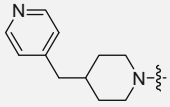
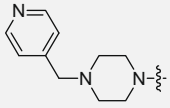
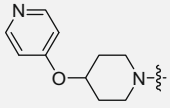
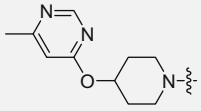
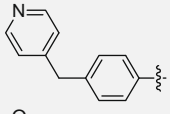
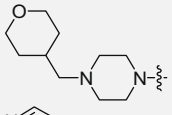
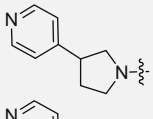
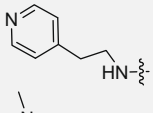
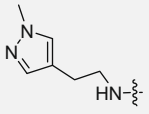
Compound	R	Aβ42 IC_{50}[10] (nM)	Aβ40 IC_{50}[10] (nM)
13		21	270
14		39	730
15		810	3900
16		380	4100
17		500	1100
18		870	4700
19		340	1600
20		350	1800
21		30	310
22		12	130
23		9.4	57
24		16	120
25		800	1100
26		910	8000
27		54	630
28		36	250
29		67	400

Table 3
In vitro activity of select purine analogs against Notch cleavage

Compound	Notch IC_{50}[11] (nM)	Notch IC_{50}/Aβ42 IC_{50}
2	>50,000	>2400
11	9200	420
12	>50,000	>1000
14	>50,000	>1300
22	9100	760
24	13,000	810
29	22,000	330

Table 4
Relative levels of Aβ42 and Aβ40 inhibition in APP-YAC transgenic mouse model for **2** and **24**

	Aβ42 (%)	Aβ40 (%)	Brain (μM)	Plasma (μM)
2	−73	N.S.	7.8	23
24	−69	−26	7.8	27

N.S. = not significant.

The in vivo properties of compounds **2** and **24** were evaluated using the APP-YAC transgenic mouse model.[12] Levels of Aβ42/40 in the brain at 6 h post-acute oral dosing, relative to vehicle and a positive control γ-secretase inhibitor, together with plasma and brain drug concentrations were determined. Both compounds demonstrated selective inhibition of brain Aβ42 production relative to Aβ40 at 100 mg/kg po (Table 4). Exposure levels of **2** and **24** in mouse brain and plasma were similar.

In summary, a novel class of purines was discovered which demonstrates potent and selective in vitro inhibition of Aβ42 over Aβ40 production and 300- to 2000-fold selectivity over inhibition of Notch cleavage. The Aβ42/Aβ40 in vitro selectivity was consistent in the in vivo model with lead compounds showing selective reduction of Aβ42 in an APP-YAC transgenic mouse model. 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.

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8. (a) Peretto, I.; La Porta, E. *Curr. Top. Med. Chem.* **2008**, *8*, 38; (b) Pissarnitski, D. *Curr. Opin. Drug Discovery Dev.* **2007**, *10*, 392.
9. (a) Rivkin, A.; Ahearn, S. P.; Chichetti, S. M.; Kim, Y. R.; Li, C.; Rosenau, A.; Kattar, S. D.; Jung, J.; Shah, S.; Hughes, B. L.; Crispino, J. L.; Middleton, R. E.; Szewczak, A. A.; Munoz, B.; Shearman, M. S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1269; (b) Blurton, P.; Fletcher, S.; Teall, M.; Harrison, T.; Munoz, B.; Rivkin, A.; Hamblett, C.; Siliphaivanh, P.; Otte, K. WO 099210, 2008.; (c) Rivkin, A.; Ahearn, S. P.; Chichetti, S. M.; Hamblett, C. L.; Garcia, Y.; Martinez, M.; Munoz, B. WO 019392, 2010.
10. IC_{50} measurements for Aβ40 and Aβ42 were determined using AlphaLISA detection of peptides secreted by SH-SY5Y cells stably overexpressing the β-

APP C-terminal fragment SPA4CT: (a) Bielefeld-Sevigny, M. *Assay Drug Dev. Technol.* **2009**, 90; (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. 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.
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