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The Discovery of Pyridone and Pyridazone Heterocycles as γ-Secretase Modulators

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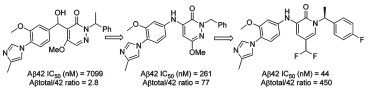
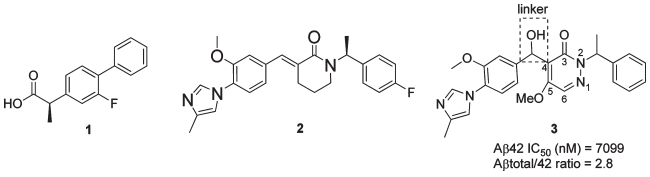
ABSTRACT A series of novel pyridazone and pyridone compounds as γ-secretase modulators were discovered. Starting from the initial lead, structure−activity relationship studies were carried out in which an internal hydrogen bond was introduced to conformationally fix the side chain, and compounds with improved in vitro Aβ42 inhibition activity and good Aβtotal/Aβ42 selectivity were quickly discovered. Compound **35** displayed very good in vitro activity and excellent selectivity with good in vivo efficacy in both CRND8 mouse and nontransgenic rat models. This compound displayed a good overall profile in terms of rat pharmacokinetics and ancillary profile. No abnormal behavior and side effects were observed in all of the studies.

KEYWORDS Pyridazone, pyridone, γ-secretase, modulator, Alzheimer's disease



Alzheimer's disease (AD) is an age-related neurodegenerative disorder (cognitive impairment, loss of memory, and language ability) that affects millions of older people in the United States.[1,2] To date, there is still an unmet medical need for treatments of this disease, and research laboratories across academia and pharmaceutical companies are working aggressively to identify new therapeutic agents to cure or, at a minimum, retard progression of it. Although the cause of the AD is still debated,[3−5] short Aβ peptides generated from the cleavage of Aβ peptides in the brain are believed to be one of the main contributors to the disease.[6] γ-Secretase is a biochemically complex aspartyl protease enzyme, which, when coupled with β-secretase, can process amyloid precursor protein (APP) to produce Aβ peptides. Soluble oligomeric forms of Aβ peptides have been proposed as the neurotoxic agents. Inhibition of these enzymes would reduce production of Aβ, which should slow or halt the progression of cell death and cognitive decline. γ-Secretase cleavage of CTFβ leads to Aβ peptides of 37−42 amino acids of which Aβ42, the more hydrophobic form, is most toxic.[7] γ-Secretase modulators shift the γ-secretase cleavage toward short peptides by selectively inhibiting Aβ42 without blocking overall γ-secretase function (Aβtotal). Modulation as opposed to outright inhibition should offer a potentially improved side effect profile—for example, versus notch processing.[8−10] Because of this advantage, research in this field has heated up in recent years in the pharmaceutical industry. There are two major classes of γ-secretase modulators in clinical trials. One is structure-related to nonsteroidal anti-inflammatory acids (NSAIDs),[11,12] such as **1** from Myriad. The other is the non-NSAIDs class, such as **2** from Eisai. In our effort in the AD area, we have recently identified an initial γ-secretase modulator compound (**3**) that was discovered based on the idea of mimicking the double bond in compound **2** with intramolecular hydrogen bonding between the hydroxyl and the pyridazone carbonyl groups. Herein, we report our structure−activity relationship (SAR) effort based on this lead to quickly identify compounds with good in vitro and in vivo activity with good Aβtotal/Aβ42 selectivity.



Initial SARs focusing on the pyridazone core and modification of the left-hand side imidazole suggested that the methyl group was important for in vitro activity. When there was no methyl substitution on the imidazole, the Aβ42 inhibition decreased by at least 2-fold (**4**, Table 1). We then turned our attention to the central linker modification. When the hydroxyl methyl group was converted to a carbonyl group, the activity increased by about 3-fold (**5**). Hypothesizing that an NH group as the linker might hydrogen bond with the pyridazone carbonyl group and lock the side chain in the same conformation as the double bond in the lead structure **2** (Figure 1, **3** to **3′**), we prepared compound **6**, which showed improved activity. Although the desired H-bond

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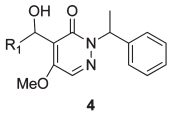
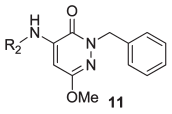
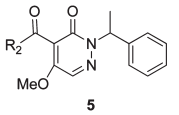
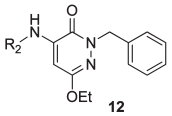
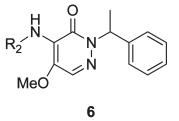
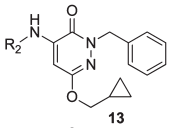
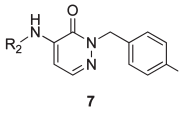
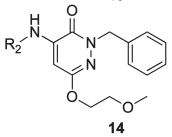
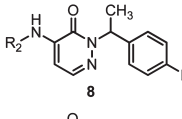
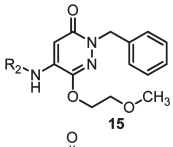
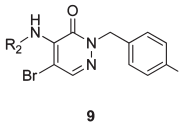
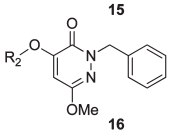
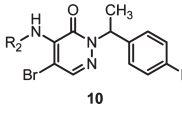
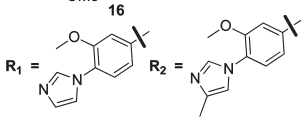
Table 1. SAR Studies of the Pyridazone Series

Compound	Aβ42 IC_{50} (nM)[a]	Aβtotal/42 ratio	Compound	Aβ42 IC_{50} (nM)[a]	Aβtotal/42 ratio
 **4**	20626	1.0	 **11**	261	77
 **5**	2505	n.d.[b]	 **12**	402	50
 **6**	3103	n.d.	 **13**	310	64
 **7**	345	58	 **14**	303	66
 **8**	503	40	 **15**	4911	4.1
 **9**	2019	18	 **16**	884	23
 **10**	2883	6.9			

[a] Each IC_{50} value is an average of at least two determinations. [b] n.d. = not determined.

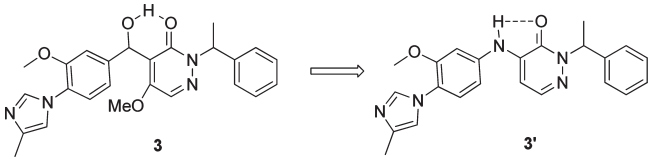
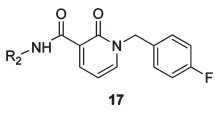
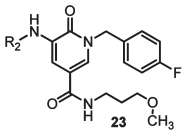
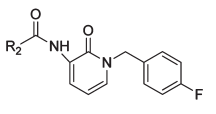
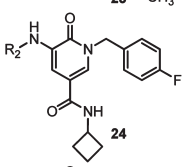
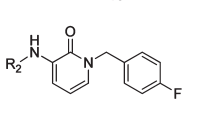
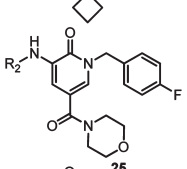
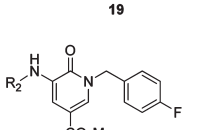
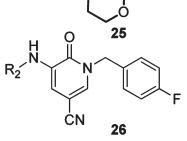
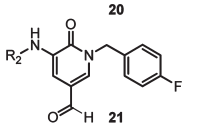
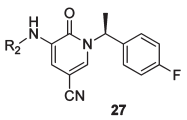
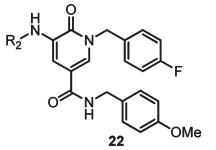
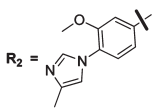


Figure 1. Employing intramolecular hydrogen bonding to lock the confirmation.

effect was not observed, we thought that the C5 methoxy group might diminish the effect because of steric repulsion. Further improvement of the activity was indeed achieved by removing the C5 methoxy group, and the IC_{50} value had a dramatic improvement to 345 nM (**7**), a 10-fold increase. This result suggests that the bulkyl group at C5 is not amenable to improving the in vitro activity, and intramolecular hydrogen bonding may indeed have a beneficial effect. This observation was further confirmed with the preparation of **9** and **10**. A 6-fold loss of activity was observed when the bromine atom was introduced at C5. When the methyl group at the right-hand benzylic position (N2) was installed, the in vitro activity did not change significantly (**8**). To further improve the activity, we next turned our attention to modification of the C6

Table 2. Initial SAR Studies of the Pyridone Series

Compound	Aβ42 IC_{50} (nM)[a]	Aβtotal/42 ratio	Compound	Aβ42 IC_{50} (nM)[a]	Aβtotal/42 ratio
 **17**	562	38	 **23**	404	20
 **18**	2405	8.3	 **24**	334	60
 **19**	148	135	 **25**	326	61
 **20**	118	158	 **26**	159	126
 **21**	161	124	 **27**	148	135
 **22**	203	98			

[a] Each IC_{50} value is an average of at least two determinations.

position. As summarized in Table 1, introduction of alkyl ethers at C6 was tolerated and gave reasonably good in vitro activity (**11**−**13**). Bulky substituents did not affect the activity too much and gave comparable Aβ42 inhibition and Aβtotal/42 selectivity (**14**). This suggests that there is space available for further modification at this position. C4 substitution with an amine linker proved to be important for in vitro activity. When the biaryl aniline was moved from the C4 to the C5 position, we saw more than a 10-fold decrease of activity (**15**). When an oxygen linker was introduced (**16**), the activity was 4-fold less potent as compared to its amino analogue (**11**).

To further improve the in vitro activity, we decided to turn our attention to core modifications and began by adjusting the electron density of the core. We quickly identified that the pyridone core was a suitable substitution of the pyridazone core. We first introduced the amide linker (**17** and **18**, Table 2) in this series. Compound **17** showed better activity than **18**, which had a reversed amide linker, but both were not as good as the pyridazone analogues. On the other hand, when the amino group was used as the linker, compound **19** displayed obviously improved in vitro activity and selectivity. We then decided to focus on modification of the C5 position with the amino group as the linker. Ester (**20**) and aldehyde (**21**) groups were tolerable at this position and gave good Aβ42 inhibition and Aβtotal/42 selectivity. When a series of amide groups were introduced (**22**−**25**), only moderate in vitro activity was

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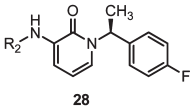
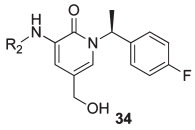
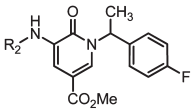
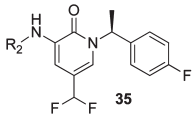
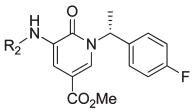
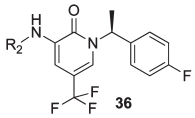
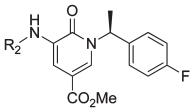
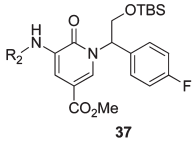
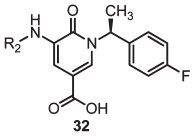
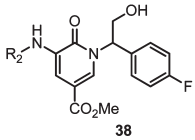
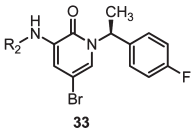
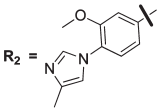
Table 3. SAR Studies of the Pyridone Series Focusing on the C5 Modification

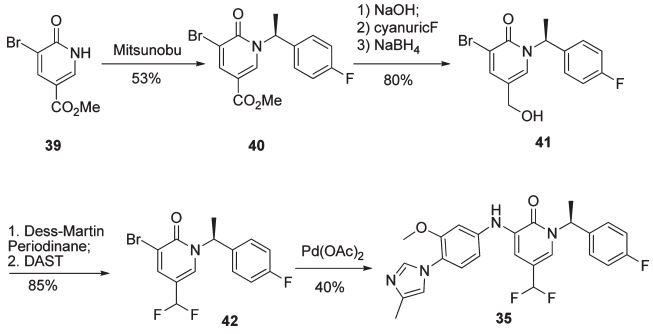
Compound	Aβ42 IC_{50} (nM)[a]	Aβtotal/42 ratio	Compound	Aβ42 IC_{50} (nM)[a]	Aβtotal/42 ratio
 **28**	131	152	 **34**	48	377
 **29**	163	96	 **35**	44	450
 **30**	363	46	 **36**	101	155
 **31**	136	148	 **37**	66	259
 **32**	3120	6.4	 **38**	114	113
 **33**	80	251			

[a] Each IC_{50} value is an average of at least two determinations.

observed; an aromatic ring (**22**) and alkyl group (**23**) did not change the IC_{50} value significantly. In comparison, when a smaller group such as a cyano group was introduced, compound **26** showed improved activity and selectivity even though it had similar potency and selectivity as the unsubstituted compound **19**. A methyl group at the right-hand side benzylic position (N1) again did not affect the activity too much, and compound **27** showed similar in vitro activity to **26**. However, the methyl group did have some impact on the pharmacokinetic (PK) profile of these compounds. The rat oral PK[13] at 10 mpk (0−6 h) showed compound **27** had a better brain concentration (917.3 ng/g) at the 6 h time point than compound **26** (391.3 ng/g), which is desirable in the current project.

Because of the improved PK properties provided by the methyl group, we next focused our SAR efforts on modification of the C5 position with the α-methylbenzyl group installed at the right side N1 position in the hope to identify compounds with a good overall profile (Table 3). When there was no substitution at C5, compound **28** retained good in vitro activity and selectivity. The configuration of the substituent on N1 had a clear impact on activity. Racemic compound **29** had an IC_{50} value of 163 nM. Its enantiomers, however, displayed different activity. The *R* isomer (**30**) was 3-fold less active than the *S* isomer (**31**). Thus, our further

Scheme 1. Synthesis of Pyridone Analogue **35**



SAR effort was focused on the compounds with the *S* configuration. A carboxylic acid group at the C5 position (**32**) was not a good choice in terms of improving the Aβ42 inhibition. Smaller electron-withdrawing groups seemed to improve activity. With the introduction of a bromine atom, the compound showed much improved Aβ42 IC_{50} value (**33**, 60 nM) and excellent selectivity over Aβtotal (251 fold). A hydroxy methyl group was tolerated and further improved the in vitro activity (**34**, 48 nM). When a difluoromethyl group was introduced (**35**), further improvement in selectively was observed. A stronger electron-withdrawing group such as the trifluoromethyl group seemed not very helpful at improving the activity (**36**). At this point, we wanted to find out whether the C5 and N1 substitutions had a synergistic effect on the in vitro potency. We therefore introduced polar groups on the methyl side chain at the N1 position. Even with a bulky TBDMS group at the right-hand side, racemic compound **37** showed 3-fold better activity than compound **29**. This suggests that a large cavity may be available at the N1 position for further SAR modification. Interestingly, the more polar, and smaller, hydroxy methyl benzyl group at the N1 position was tolerated, and compound **38** had an Aβ42 IC_{50} value at 114 nM as a racemic mixture.

As summarized in Table 3, compound **35** showed one of the best in vitro profiles in terms of enzyme activity and Aβ42 selectivity over Aβtotal. Therefore, it was further profiled in in vivo studies. This compound showed very good in vivo efficacy in a CRND8 mouse model, giving over 85% reduction of Aβ42 in plasma at 30 mpk with little effect on the Aβtotal. In the nontransgenic rat in vivo model,[14] this compound displayed a 40% reduction of Aβ42 in the CSF at 100 mpk and a 26% reduction of Aβ42 in brain, while the Aβtotal only had a 7% reduction in the CSF. Compound **35** had good rat PK with an AUC_{1-6h} of 7.5 μM.h at 10 mpk and favorable brain concentration (347.3 ng/g) at the 6 h time point. No abnormal behavior or side effects were observed in those studies.

To demonstrate the synthesis of the pyridone analogues, the synthetic route to compound **35** is illustrated in Scheme 1. Starting from commercially available compound **39**, a Mitsunobu reaction with (*R*)-1-hydroxy-1-(4-fluorophenyl)-ethane gave enantiomerically pure **40**. The ester group was converted to alcohol **41** in three steps[15] since direct reduction with $LiAlH_4$ resulted in a complex mixture. Compound **41** was converted to difluoro compound **42** in two steps via Dess−Martin oxidation and fluorination. A final coupling

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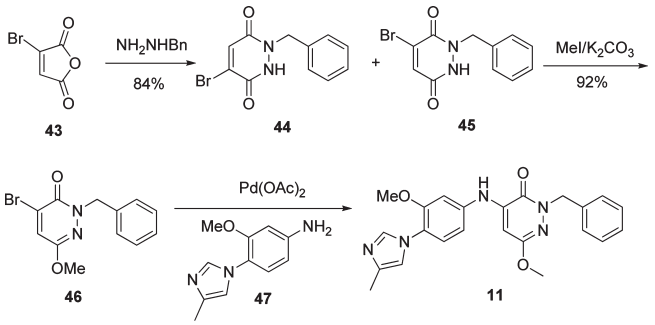
Scheme 2. Synthesis of Pyridazone Analogues



reaction using $Pd(OAc)_2$ gave the desired product in moderate yield. Other pyridone compounds were prepared in a similar fashion. The synthesis of pyridazone compounds was straightforward and is shown in Scheme 2. Bromides **44** and **45** were obtained from compound **43** by treatment with NH_2NHBn. Methylation of compound **45** furnished **46**, which was coupled with aniline **47** using a catalytic amount of $Pd(OAc)_2$ to give the final product **11**.

In summary, we have indentified a series of novel pyridazone and pyridone compounds as γ-secretase modulators. Starting from the initial lead, we have carried out SAR studies employing a strategy that utilized an internal hydrogen bond to lock the conformation of the side chain present in the lead structure. The new analogues displayed an improved in vitro Aβ42 activity and good Aβtotal/Aβ42 selectivity. Compound **35** displayed very good in vitro activity and excellent selectivity with good in vivo efficacy in both the CRND8 mouse and the nontransgenic rat models. This compound had a good overall profile in terms of rat PK and ancillary profile such as clean hERG, clean hPXR, acceptable P450 inhibition profile, and good human hepatocyte clearance data (2.9 μL/m/M cell). Further profiling is in progress, and the result will be reported in due course.

SUPPORTING INFORMATION AVAILABLE Experimental procedures and spectral data for compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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