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Part 3: Notch-sparing γ-secretase inhibitors: SAR studies of 2-substituted aminopyridopyrimidinones

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ABSTRACT

In search for novel lead compounds as γ-secretase inhibitors, analogs of aminopyrido[2,3-*d*]pyrimidin-7-ones (**I**) were synthesized and evaluated for inhibitory effects on amyloid-β-peptide production and cleavage of the Notch1 receptor mediated by γ-secretase. Selected pyridopyrimidines, such as **1**, **8**, **9**, **10**, **11** and **16** are γ-secretase inhibitors that did not have an effect on Notch1 receptor processing.

Alzheimer's disease (AD) is a progressive and fatal neurodegenerative disease that currently affects over 5 million Americans.[1] AD is characterized by slow but progressive impairment in cognition, behavior and daily-life function. The main pathological hallmarks of AD are the extracellular deposition of amyloid-β (Aβ) peptides and the formation of intracellular neurofibrillary tangles.[2] To date, treatment of AD involves drugs that can only delay the inevitable AD symptoms without tackling the main neuropathological causes of the disease. Scientific advances in understanding AD etiology has led to the identification of the oligomerization of Aβ peptides in the brain as a key causal factor for AD.[3] Accumulation of Aβ oligomers begins in the brain long before the onset of AD as a result of reduced Aβ clearance or increased Aβ production.[4,5] Aβ peptides are produced from the amyloid precursor protein (APP) through sequential proteolytic cleavages catalyzed by β- and γ-secretases.[6] Hence, suppressing Aβ production by inhibiting γ-secretase has been proposed as one of the disease-modifying approaches for AD therapy.[7]

γ-Secretase is a protein complex composed of nicastrin, Aph-1, Pen-2, and either presenilin-1 (PS-1) or presenilin-2 (PS-2).[8] Cleavage of Notch receptors, required for their cell signaling, is also catalyzed by γ-secretase.[9] Interference with Notch receptor signaling, particularly from Notch1, can lead to severe side effects, including intestinal goblet cell hyperplasia, thymus atrophy, decrease in lymphocytes, and alterations in hair color.[10] Our strategy for seeking AD therapeutics has been Notch-sparing gamma-secretase inhibitors. Greengard and colleagues discovered that Aβ production by γ-secretase in cells is dependent on adenosine triphosphate (ATP).[11] It was reported that compounds such as Gleevec™ (imatinib mesylate) and PD173955 (Fig. 1) compete with ATP for binding to target kinases and can also inhibit Aβ production by γ-secretase with selectivity over the cleavage of Notch1. Coincidentally, both Gleevec and PD173955 incorporate a phenyl aminopyrimidine pharmacophore.

Our early screening focus for this series of compounds was evaluating permutations at the C-2 pyrimidine position of the template of PD173955. In Table 1, when R^1 is hydrogen (as in PD173955) and R^2 is 4-OCH_3-phenyl (**1**, 47%) and 4-SCH_3-phenyl (**2**, 47%), overall better activity and selectivity was observed. There also was no change observed for Notch1 processing for either of these compounds. Interestingly, the 4-thiomethylphenyl analog (**2**) showed Aβ40 inhibition whereas the 3-thiomethylphenyl analog PD173955 (**1**) showed no Aβ40 inhibition. In addition, 4-CF_3-phenyl analog (**4**) showed a significant decrease in activity.

Phenyl and benzyl analogs (**3** and **7**) and small alkyl (R^2 = isopropyl, **5**) led to decreased or no activity (26%, 0%, 0%, respectively). However, a longer alkyl chain analog (R^2 = *n*-hexyl, **6**) and a methylpyridinyl analog (**8**) showed a significant increase in potency (53%, 59%, respectively), with (**6**) also showing inhibition of Notch1 processing. Additionally, activity increased substantially

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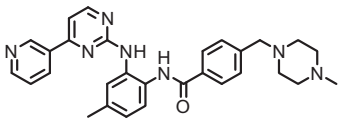

Imatinib

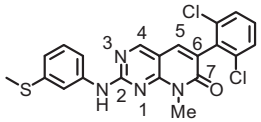

PD173955

Figure 1. Structure of Imatinib and PD173955.

when the hydrogen of (**7**) was replaced with isopropyl group to give **10** (0–90%). Good activity of substituted benzyl analogs **11** and **12** (3,4-diCl, 50% and 3,4-diF-, 73%, respectively) was maintained, although not as active as the unsubstituted benzyl analog **10** (90%).

Examples of replacing R^1 and R^2 of (**I**) with a 4-substituted piperazine ring are illustrated in Table 2. Activity was increased significantly with a 4-F-phenyl-substituted piperazine ring (**16**, 68%) and a 2,6-dichloro-phenyl group at C-6.

Similar to phenyl pyridopyrimidinone **10,** this piperazinyl pyridopyrimidinone **16** also had very good Aβ40 inhibition. Evaluation of selected drug-like properties (e.g., solubility, Log*D*, plasma protein binding, permeability, and human and rodent microsomal stability) of each of these two compounds was performed. These results indicated that **16** had a slightly better drug-like profile and thus, was further tested in an Aβ42 cellular assay along with other analogs. Pyridopyrimidinone **16** showed inhibition in cells (Aβ42) with an IC_{50} = 2.7 μM, (see Supplemental data).

Additional piperazinyl pyridopyrimidinone analogs were synthesized as illustrated in Table 2. An unsubstituted phenyl ring (**18**, 14%) or other substitutions such as 2,6-diF- (**15**, 20%), 4-Cl- (**14**, 0%), and 4-*t*-butyl- (**13**, 2%) on the phenyl ring at C-6 diminished activity. Additionally, replacing the 4-F-phenyl ring (R^1) of **16** with a simple methyl group (**17**) and with various heterocycles (**19**–**22**) all yielded 0% inhibition of Aβ40.

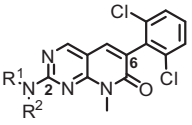
The general synthetic routes to obtain 2-aminopyrido[2,3-*d*] pyrimidin-7-one analog (**I**) are described by several research teams.[12–14] According to these reported methods, some of our compounds were prepared as shown in Scheme 1. The preparation of these pyridopyrimidinones typically requires an eight-step synthesis beginning with commercially available 4-chloro-2-thiomethyl-5-pyrimidine-carboxylate ethyl ester (**II**) which is first converted to the corresponding methylamine and then subsequently reduced with lithium aluminum hydride to yield alcohol (**III**). Oxidation of (**III**) with manganese oxide gave intermediate 2-thiomethyl-pyrimidine-5-carboxaldehyde (**IV**). Condensation of aldehyde (**IV**) with 2,6-dichlorophenyl-acetonitrile provided pyrido[2,3-*d*]pyrimidin-7-ylideneamine (**V**). Acylation of (**V**) gave 7-*N*-acetylimine (**VI**) which was readily hydrolyzed to yield methylsulfide intermediate (**VII**). Subsequent oxidation of (**VII**) with *m*-chloro-perbenzoic acid provided sulfonyl pyridopyrimidinone (**VIII**). Refluxing (**VIII**) with selected amines in DMF or neat led to the production of the desired 2-amino pyridopyrimidinones (**I**) in good yields, ranging from 50% (**6**) to 95% (**4**).

This original eight-step reported synthesis illustrated in Scheme 1 worked well, but the route was inconvenient for further variation on the C-6 position. To simplify the synthesis, we developed a novel method to prepare key intermediate pyridopyrimidinone (**VII**) in four steps rather than 5 steps. Methylsulfide intermediate (**VII**) was easily obtained by the condensation of 5-pyrimidinecarboxyaldehyde (**IV**) with readily available substituted phenylacetates in DMF in the presence of Cs_2CO_3 at room temperature or under refluxing conditions. This improved synthetic method, a total of 6 steps rather than eight steps, was used to prepare compounds **3** and **4** (Table 1) and **13**–**22** (Table 2).

As a means of reducing the molecular weight of the target pyridopyrimidinone compounds as well as to explore the role of the C-6 aryl group, compound **23** was synthesized from 8-methyl-2-(methylsulfonyl)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (**IX**, Scheme 2). Pyridopyrimidinone (**IX**) was prepared from intermediate (**IV**) according to a reported method.[14]

This series of pyridopyrimidinones were evaluated for their inhibition of γ-secretase-mediated Aβ40 production by ELISA using purified human γ-secretase complexes and recombinant APP substrate C100Flag.[15–21] Effects of these compounds on γ-secretase-mediated processing of Notch1 were examined by Western blot analysis using recombinant substrate N100Flag as previously reported.[21] Compounds with at least a 50% inhibition in the Aβ40 ELISA were chosen for evaluation of Notch1 processing.

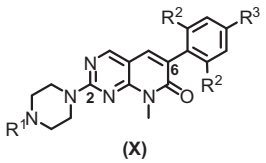
Table 1
2-Amino-6-(2,6-dichlorophenyl)-pyrido[2,3-*d*]-pyrimidin-7-ones


(I)

Entry	R^1	R^2	Aβ40 (%) inhibition[a]	Notch1 processing[b]
PD173955	H	3-(CH_3S)Ph-	0	No change
1	H	4-(CH_3O)Ph-	47	No change
2	H	4-(CH_3S)Ph-	47	No change
3	H	Ph-	26	n.t.
4	H	4-(CF_3)Ph-	12	n.t.
5	H	*i*-Pr	0	No change
6	H	*n*-Hexyl	53	Inhibition
7	H	$PhCH_2$-	0	n.t.
8	H	(2-Pyridinyl) CH_2-	59	No change
9	CH_3	$Ph(CH_2)_2$-	48	No change
10	*i*-Pr	$PhCH_2$-	90	No change
11	*i*-Pr	(3,4-Cl_2Ph)CH_2-	50	No change
12	*i*-Pr	(3,5-F_2Ph)CH_2-	73	Inhibition

n.t. = not tested.
[a,b]See Refs. 15,20.
[a] Compounds were tested at the concentration of 100 μM. The inhibition of Aβ40 production is a percentage of DMSO control.
[b] Compounds were tested at the concentration of 100 μM. The effects on Notch1 processing are compared to DMSO control; No change: Notch1 processing was not affected by the tested compounds at the concentration of 100 μM.

Table 2
2-Piperazinyl-pyridopyrimidin-7-ones


(X)

Entry	R^1	R^2	R^3	Aβ40 (%) inhibition[a]	Notch1 processing[b]
13	4-F-Ph-	H	*t*-Bu	2	n.t.
14	4-F-Ph-	H	Cl	0	n.t
15	4-F-Ph-	F	H	20	Inhibition
16	4-F-Ph-	Cl	H	68	No change
17	CH_3	Cl	H	0	No change
18	4-F-Ph	H	H	14	Inhibition
19	2-Tetrahydrofuran-methyl	Cl	H	0	n.t.
20	2-Pyridinyl	Cl	H	0	n.t.
21	2-Pyrimindyl	Cl	H	0	n.t.
22	3-Pyridinyloxyl	Cl	H	0	n.t.

[a,b]See Table 1 and Refs. 15,20.
n.t. = not tested.