Bioorganic & Medicinal Chemistry Letters 26 (2016) 2129–2132

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Part 1: Notch-sparing γ-secretase inhibitors: The identification of novel naphthyl and benzofuranyl amide analogs

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ARTICLE INFO

Article history:
Received 2 January 2016
Revised 10 March 2016
Accepted 11 March 2016
Available online 12 March 2016

Keywords:
Alzheimer's disease
γ-Secretase inhibitors
Naphthyl amides
Notch processing
Aβ production

ABSTRACT

γ-Secretase is one of two proteases directly involved in the production of the amyloid β-peptide (Aβ), which is pathogenic in Alzheimer's disease. Inhibition of γ-secretase to suppress the production of Aβ should not block processing of one of its alternative substrates, Notch1 receptors, as interference with Notch1 signaling leads to severe toxic effects. In the course of our studies to identify γ-secretase inhibitors with selectivity for APP over Notch, **1** [3-(benzyl(isopropyl)amino)-1-(naphthalen-2-yl)propan-1-one] was found to inhibit γ-secretase-mediated Aβ production without interfering with γ-secretase-mediated Notch processing in purified enzyme assays. As **1** is chemically unstable, efforts to increase the stability of this compound led to the identification of **2** [naphthalene-2-carboxylic acid benzyl-isopropyl-amide] which showed similar biological activity to compound **1**. Synthesis and evaluation of a series of amide analogs resulted in benzofuranyl amide analogs that showed promising Notch-sparing γ-secretase inhibitory effects. This class of compounds may serve as a novel lead series for further study in the development of γ-secretase inhibitors.

Alzheimer's disease (AD) is characterized by neurodegeneration and progressive deterioration of memory and cognitive abilities. At present, there is no cure or effective treatment for this disease, only several approved drugs for alleviating certain AD symptoms. Over the past 20 years, advances in deciphering AD pathology have revealed that AD is a protein-misfolding disorder,[1] as aggregation of the amyloid β-peptide (Aβ) in the brain is a central event in AD pathology.[2] Aβ proteins are produced naturally inside the human brain as proteolytic products of the amyloid precursor protein (APP) through sequential cleavages mediated by β- and γ-secretases. Secreted Aβ varies in length ranging from 37 to 43 amino acids,[3] of which the 42 amino acid peptide (Aβ42) is especially prone to aggregation when over-produced or normal clearance is interrupted.[2] Aβ dimers and higher order assemblies can initiate a series of cellular events that can ultimately cause neuronal dysfunctions and the onset and progression of AD.[4] Based on the Aβ hypothesis of AD-pathogenesis, several disease-modifying approaches have been proposed, including suppression of Aβ production, prevention of Aβ aggregation and promotion of Aβ clearance.[5]

Suppressing Aβ production through inhibition of γ-secretase has been aggressively pursued as a potential disease-modifying approach. However, γ-secretase is a multifunctional protease with many substrates including the essential cell-signaling Notch receptors.[6] Hence, identification of γ-secretase inhibitors or modulators that can lower Aβ production in general or Aβ42 in particular with minimal effects on Notch signaling (particularly that of Notch1) has become one of the most prominent challenges in the pursuit of AD therapeutics.[3] While much progress has been made toward Aβ42-lowering γ-secretase modulators,[7–9] various 'Notch-sparing' γ-secretase inhibitors have been reported,[8,10–13] and even for these the degree of selectivity for APP versus Notch1 is often unclear. Thus, there is a keen need to identify new structures with this important substrate-selective inhibitory property as demonstrated in comparable biochemical assays.

In search of such compounds, one of our earliest hits was naphthylaminoalkyl ketone **1**.[14] Unfortunately, **1** is unstable and subject to degradation by a retro-Michael-addition to give the corresponding naphthyl vinyl ketone and *N*-benzylisopropylamine. One attempt to seek more stable analogs of **1** was to generate its amide counterpart **2** (Fig. 1), with the ethylene linker between the carbonyl and amine nitrogen removed. This compound **2** showed

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http://dx.doi.org/10.1016/j.bmcl.2016.03.040
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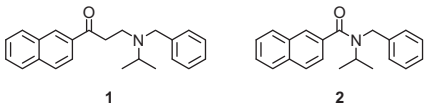
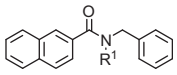


Figure 1. Naphthyl aminoalkyl ketone **1** and naphthyl amide **2**.

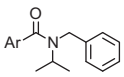
Table 1
Naphthyl amides

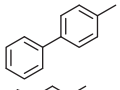
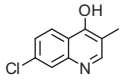
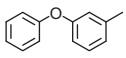


Entry	R^1	Aβ40 (%) inhibition[a]	Notch1 processing[b]
2	*i*-Pr	58	No change
3	H	0	n.t.
4	CH_3	15	n.t.
5	*t*-Bu	52	No change
6	Cyclic-Pr	37	No change

n.t. = not tested; see References and notes sections for assay descriptions.[19]
[a] Compounds were tested at 100 μM. Inhibitory effects on Aβ40 production were recorded as a percentage in comparison with DMSO control.
[b] Compounds were tested at 100 μM. Effects on Notch processing were recorded (Western blot) in comparison with DMSO control.

Table 2
Aryl amide analogs replacing the 2-naphthyl group

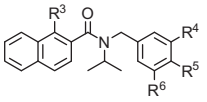


Entry	Ar	Aβ40 (%) inhibition[a]	Notch1 processing[b]
2		58	No change
7		67	No change
8		10	n.t.
9		85	Inhibition
10		27	n.t.
11		14	n.t.
12		40	No change
13		0	n.t.
14		50	Inhibition
15		33	No change
16		50	Inhibition

[a,b] See Table 1 notes. n.t. = not tested.

similar biological activity to **1**. Evaluation of various drug-like properties (e.g., solubility, Log*D*, plasma protein binding, permeability, and human and rodent microsomal stability—see Supplemental data) of **2** was completed and the results indicated

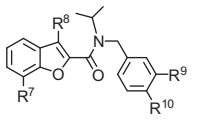
Table 3
Naphthyl amide analogs with substituted phenyl rings



Entry	R^3	R^4	R^5	R^6	Aβ40 (%) inhibition[a]	Notch1 processing[b]
17	H	H	Cl	Cl	58	No change
18	OH	H	Cl	Cl	41	No change
19	H	H	F	F	34	No change
20	OH	H	F	F	49	No change
21	H	F	H	F	21	n.t.
22	OH	F	H	F	27	n.t.
23	H	H	OMe	H	47	Inhibition
24	OH	H	OMe	H	33	No change
25	H	CN	H	H	31	n.t.
26	H	H	CN	H	31	n.t.
27	H	OMe	H	OMe	35	No change
28	H	OMe	H	H	19	No change
29	H	H		H	38	Inhibition

[a,b] See Table 1 notes. n.t. = not tested.

Table 4
Benzofuranoyl amide analogs



Entry	R^7	R^8	R^9	R^{10}	Aβ40 (%) inhibition[a]	Notch1 processing[b]
30	H	H	H	H	40	No change
31	H	H	Cl	Cl	51	No change
32	H	Me	Cl	Cl	0	n.t.
33	OMe	H	Cl	Cl	75	No change
34	OMe	H	Cl	F	49	No change
35	OMe	H	F	Cl	23	No change
36	OEt	H	Cl	Cl	58	No change

[a,b] See Table 1 notes. n.t. = not tested.

a reasonable profile to begin synthesizing analogs. A series of these amide analogs were synthesized as illustrated in Tables 1–4.

These aryl amides were synthesized by the methods illustrated in Scheme 1. Alkylation of readily available amines (i.e., isopropyl or cyclopropyl amine) with various substituted benzyl halides was carried out at 0 °C to room temperature for 6–8 h. Excess amine was then removed in vacuo. The crude product contained ~10–15% of the undesired dialkylated product which was easily removed by an aqueous–organic partitioning at pH 8–8.5. The desired mono-alkylated amine (**II**) was obtained by subsequent extraction at pH 11–12. Coupling of amine (**II**) or commercially available amine with either acyl chlorides or aryl acids yielded the desired target amide analogs (**III**).

These amides were then evaluated for their inhibitory effects on Aβ40 production from purified human γ-secretase[15] and a recombinant APP-based substrate using a specific ELISA.[16–18] Effects on γ-secretase processing of a comparable recombinant Notch1-based substrate were examined by Western blot.[14] Compounds with >50% inhibition in the Aβ40 ELISA were typically evaluated for their effects on Notch processing. Since our goal in the early stages of our program was to identify viable chemical leads, testing our new analogs at a high concentration in search for at least 50% inhibition of Aβ production without effect on Notch1 cleavage led to some novel compounds and chemical series that are now being presented here and in subsequent manuscripts.