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Design, Synthesis, and Biological Evaluation of a Novel Class of γ-Secretase Modulators with PPARγ Activity

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We present a novel class of dual modulators of γ-secretase and peroxisome proliferator-activated receptor γ (PPARγ) based on the structure of 2-(bis(phenethoxy)pyrimidine-2-ylthio)hexanoic acid **8** (IC_{50}(Aβ42) = 22.8 μM, EC_{50}(PPARγ) = 8.3 μM). The modulation of both targets with approved drugs (i.e., amyloid-β 42 (Aβ42)-lowering NSAIDs for γ-secretase and glitazones for PPARγ) has demonstrated beneficial effects in in vitro and in vivo models of Alzheimer's disease (AD). However, although NSAIDs and PPARγ agonists share similar structural features, no druglike compounds with dual activities as γ-secretase modulators (GSMs) and PPARγ agonists have been designed so far. On the basis of our initial lead structure **8**, we present the structure−activity relationships (SARs) of broad structural variations. A significant improvement was reached by the introduction of *p*-trifluoromethyl substituents at the phenyl residues yielding compound **16** (IC_{50}(Aβ42) = 6.0 μM, EC_{50}(PPARγ) = 11.0 μM) and the replacement of the two phenyl residues of **8** by cyclohexyl yielding compound **22** (IC_{50}(Aβ42) = 5.1 μM, EC_{50}(PPARγ) = 6.6 μM).

Introduction

Alzheimer's disease (AD[a]) is the most common age-related cause of dementia with 26.6 million patients worldwide in 2006 and over a 100 million predicted cases by 2050.[1] The cardinal symptom of the disease is progressive memory loss due to the degeneration of neurons and synapses in the cerebral cortex and subcortical regions of the brain. The neuropathology of AD is characterized by the extracellular deposition of amyloid-β (Aβ) plaques, the formation of intracellular neurofibrillary tangles, chronic brain inflammation, and oxidative damage.[2] According to the modified amyloid hypothesis, small soluble oligomers of Aβ peptides, particularly those formed by the highly hydrophobic Aβ42 species, are the disease-initiating agents in AD.[3] Aβ42 is a proteolytic fragment resulting from sequential cleavage of the amyloid precursor protein (APP) by two aspartyl proteases, i.e., β-secretase and γ-secretase. Currently, a variety of potentially disease-modifying therapeutic approaches are under development that target the production, aggregation, or clearance of Aβ peptides in brain (Scheme 1).[4,5] Substantial advances have been made with respect to inhibitors of the γ-secretase enzyme, a multiprotein complex that catalyzes the final step in the cellular generation of Aβ peptides. However, because of its essential role in the NOTCH signaling pathway, mechanism-based toxicity with severe phenotypes in the gastrointestinal and hematopoietic systems has been observed in preclinical studies of γ-secretase inhibitors[6−8] and might be a limiting factor in clinical efficacy. Intriguingly, a subset of nonsteroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen and indomethacin were found to modulate the γ-secretase activity in a way that allowed selective inhibition of Aβ42 production (Figure 1).[9] These compounds, termed γ-secretase-modulators (GSMs), shifted the proteolytic processing of APP toward higher production of shorter Aβ species such as Aβ38 at the expense of the highly toxic Aβ42. Importantly, impairment of NOTCH processing and signaling was not observed.[9,10] Unfortunately, these NSAID-type GSMs suffer from low potency against γ-secretase (Figure 1), poor brain permeability, and side effects related to inhibition of cyclooxygenases (COX) such as gastrointestinal toxicity and increased cardiovascular mortality.[11] Consequently, NSAID-type GSMs and derived analogues, whose consumption appeared to confer a reduced risk for AD in epidemiological studies, have produced negative or inconclusive clinical results in patients suffering from established AD[11−14] or showed unacceptable side effects for long-term treatment. Recently, potent GSMs with favorable pharmacological properties have emerged.[15,16]

In addition, some NSAIDs including the GSMs indomethacin and ibuprofen function as agonists of the peroxisome

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[a] Abbreviations: Aβ, amyloid β peptide; AD, Alzheimer's disease; APP, amyloid precursor protein; COX, cyclooxygenase; GSM, γ-secretase modulator; NSAIDs, nonsteroidal anti-inflammatory drugs; PPAR, peroxisome proliferator-activated receptor; SAR structure−activity relationship.

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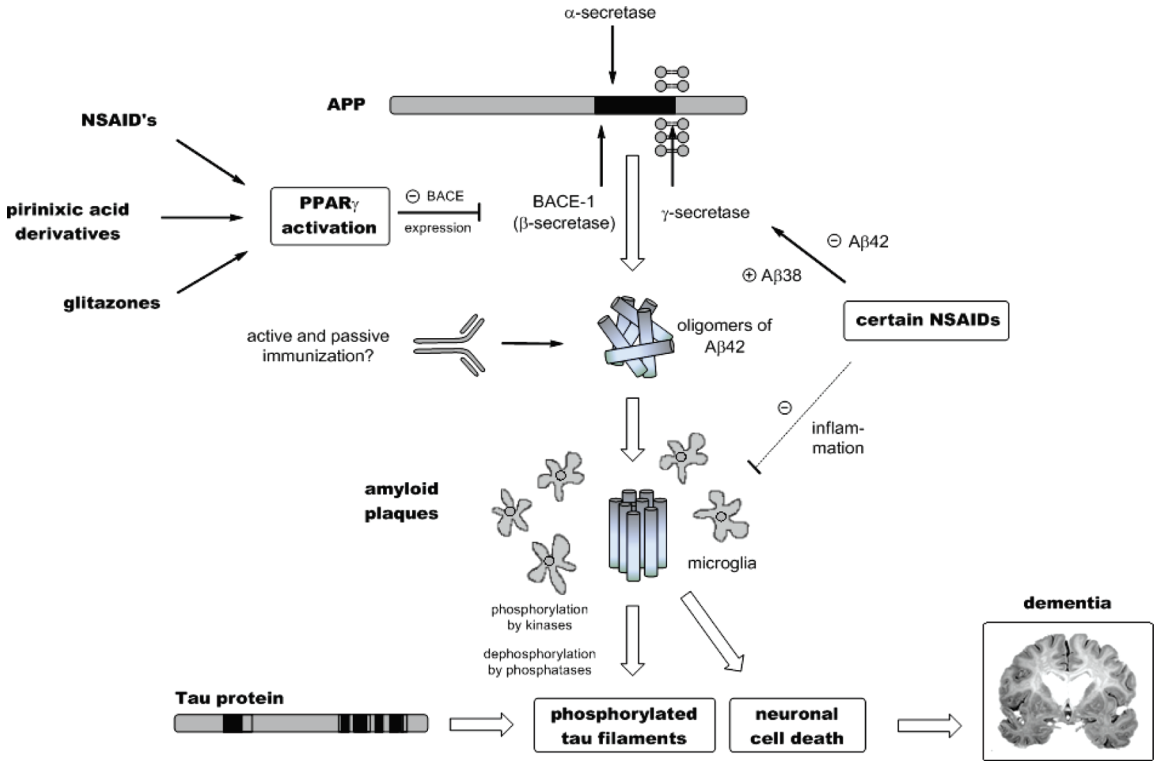
Scheme 1. Amyloid Cascade



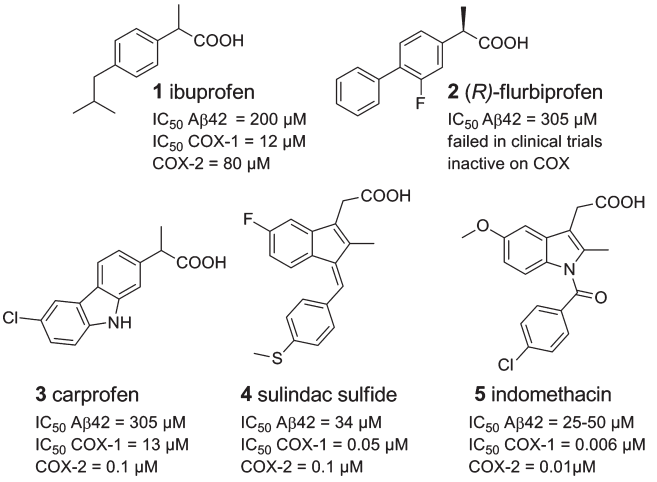


Figure 1. In vitro pharmacology of γ-secretase-modulating NSAIDs.[15,47−49]

proliferator-activated receptor γ (PPARγ).[17,18] PPARγ belongs to a family of ligand-activated nuclear receptors that act as lipid sensors, and synthetic ligands for PPARγ include the widely prescribed antidiabetic drugs rosiglitazone and pioglitazone.[19] Evidence indicates that PPARγ agonists might have multiple beneficial effects in AD both on core pathological processes in brain and on peripheral factors such as serum glucose levels and insulin sensitivity that constitute potential risk factors for AD.[20] Several studies have demonstrated that AD is associated with perturbations in insulin metabolism, and diet-induced insulin resistance caused increased brain Aβ levels and plaque formation in a mouse model of AD.[21] In addition, a recent cross of an AD mouse model to leptin-deficient diabetic mice revealed accelerated learning deficits and severe cerebrovascular amyloid deposition.[22] Importantly, individuals with type 2 diabetes appear to have a 2-fold increased risk of developing AD.[23,24] Potential direct effects of PPARγ agonists on brain pathology in AD include lowering of Aβ levels, suppression of toxic proinflammatory mediators, and reduction of cerebrovascular impairment.[25−30] Recently, a phase III clinical study with the brain-impermeable PPARγ agonist rosiglitazone failed to demonstrate efficacy in patients with established AD.[31] However, PPARγ agonists might be able to counteract the increased AD risk in patients with type 2 diabetes, and in fact, ongoing treatment and prevention trials for type 2 diabetes include cognition ancillary studies.[24] In this respect, a pilot study with the PPARγ agonist pioglitazone has provided evidence for cognitive stabilization in AD patients with concurrent type 2 diabetes.[32] Notably, pioglitazone has been shown to have at least limited blood−brain barrier permeability.[33]

In summary, small molecules with dual GSM and PPARγ agonist activity could be a novel strategy for prevention of AD, particularly in patients with type 2 diabetes. In an initial screening of our in-house compound library, we have identified compound **8** (2-(bis(phenethoxy)pyrimidine-2-ylthio)-hexanoic acid) that displayed a typical GSM profile and concentration-dependently and selectively decreased Aβ42 levels with a concomitant increase in Aβ38 production (IC_{50}(Aβ42) = 22.8 μM, EC_{50}(Aβ38) = 11.3 μM, Figure 2). Additionally, compound **8** is a subtype-selective PPARγ modulator (EC_{50} = 8.3 μM, 60% max activation). Here, we describe the structure−activity relationships (SARs) of a novel class of dual γ-secretase/PPARγ modulators based on the scaffold of compound **8**. Our strategy aimed at improving the GSM activity while maintaining PPARγ agonism. Characteristic structural features of compound **8** are a carboxylic acid headgroup and two phenethyl residues forming a lipophilic backbone. As conventional NSAIDs have similar structural features, we further determined the inhibitory activity of all compounds for COX-1 and COX-2.

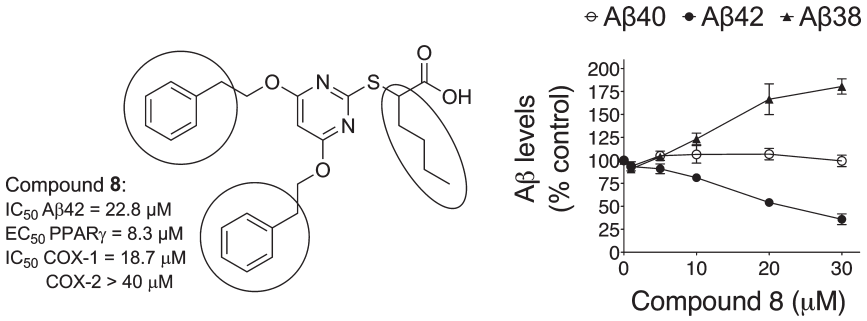
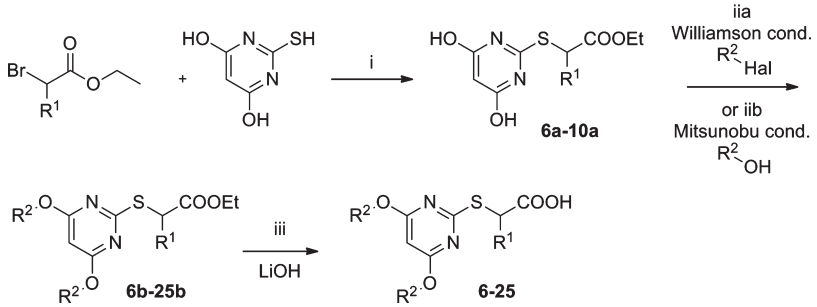


Figure 2. Lead compound **8** and its impact on Aβ38, Aβ40, and Aβ42 formation. SARs based on **8** were obtained by structural modifications of the encircled residues.

Scheme 2. Synthetic Route of Compounds **6**−**25**[a]



[a] Reagents and conditions: (i) 2-bromo-(R^1)-ethyl acetate (1.5 equiv), thiobarbituric acid (1 equiv), TEA (1.5 equiv), DMF, 80 °C, 4 h; (iia) **6a**−**10a** (1 equiv), R^2-Hal (2.1 equiv), K_2CO_3 (2.3 equiv), DMF, 80 °C, 3−28 h or (iib) **6a**−**10a** (1 equiv), R^2-OH (2 equiv), DEAD (2.5 equiv), TPP (2.5 equiv), THF, room temp, 1−3 h; (iii) LiOH (5 equiv), THF/MeOH, H_2O, 25−50 °C, 2−24 h.

Chemistry

The general synthetic procedure of the compounds is illustrated in Scheme 2. Presented compounds were prepared in a three-step reaction, which has been described previously by Koeberle et al.[34] First, a nucleophilic substitution between thiobarbituric acid and the respective α-bromoethyl ester using dimethylformamide (DMF) as solvent and triethylamine (TEA) as corresponding base was carried out. This step was followed by the etherification of the two hydroxyl groups, either by a Mitsunobu reaction or by a Williamson ether synthesis. In the case of the Mitsunobu reaction, we used the precursor, the respective alcohol, triphenylphosphine (TPP), and diethylazodicarboxylate (DEAD) and stirred the solution at room temperature under argon atmosphere.[35] The Williamson ether synthesis was carried out with the respective alkyl halogenide, K_2CO_3, and DMF at 80 °C. Finally, the ester group was hydrolyzed with LiOH in tetrahydrofuran (THF) and H_2O to yield final compounds **6**−**25**.

Biological Assays

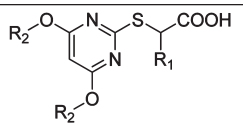
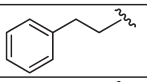
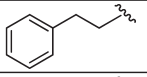
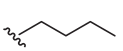
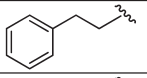
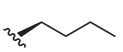
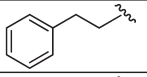
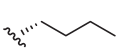
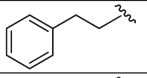
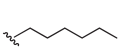
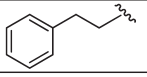
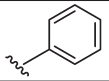
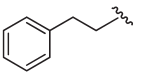
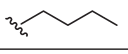
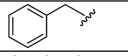
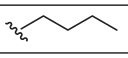
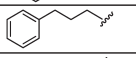
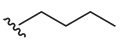
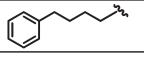
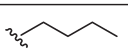
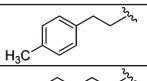
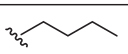
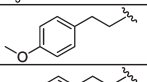
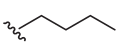
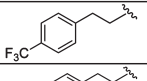
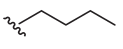
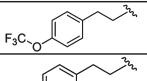
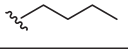
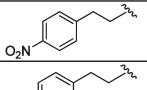
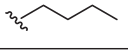
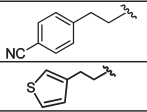
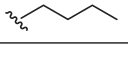
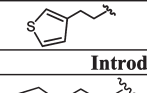
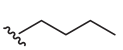
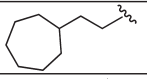
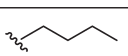
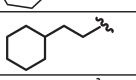
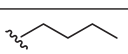
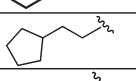
PPAR activity of the final compounds was tested in a cellular luciferase-based PPAR transactivation assay, which has been described previously.[36] Final compounds **6**−**25** were fully characterized on all PPAR subtypes (α, γ, and δ). Since every derivative described in this paper was inactive on PPARα and PPARδ (tested concentration of 10 μM), the following discussion is only focused on PPARγ activity. For biological characterization of GSM activity, we measured the levels of Aβ38, Aβ40, and Aβ42 peptides in cell supernatants with an ELISA assay as described with some modifications.[37] Inhibition of COX was tested in a cell-free assay using isolated ovine COX-1 and human recombinant COX-2 enzymes.[34] Potential effects on proteolytic processing of NOTCH were investigated for the two compounds with the highest GSM activity using a previously described reporter assay.[38] Cytotoxicity of all compounds was determined using alamar blue reagent.

Results

In an initial screening of our in-house-library we have identified 2-(bis(phenethoxy)pyrimidine-2-ylthio)hexanoic acid **8**, a selective PPARγ modulator that displayed the characteristic GSM activity with IC_{50}(Aβ42) = 22.8 μM (Figure 2). As the first hit, compound **8** served as the structural template for all presented derivatives. Important structural features of **8** are two 2-phenethyl residues forming a lipophilic backbone and a carboxylic acid function as headgroup. This acidic headgroup is an essential structural element required for PPARγ activation[39] and of NSAID-type GSMs.[15,40] Therefore, we have not modified the carboxylic acid group and focused our efforts on the remaining parts of lead structure **8**.

In previous studies, we have observed that the length of the alkyl chain of α-substituted pirinixic acid derivatives has a high impact on PPAR activity.[41,42] Hence, we systematically varied this position by elongation and shortening of the α-*n*-butyl residue as well as by introduction of a α-phenyl residue (compounds **6**−**10**; see Table 1). The inhibitory activity on Aβ42 production showed clear SAR depending on the length of the α-alkyl chain. Removal of the α-chain (compound **6**, R^1 = H) caused a complete loss of GSM activity. In line with this observation, compound **7** with the shorter ethyl residue showed only weak GSM activity (IC_{50}(Aβ42) = 41.6 μM). A slight decrease of GSM activity was also observed after elongation to *n*-hexyl (compound **9**). In regard to PPARγ

Table 1. PPARγ Activation and γ-Secretase Modulation (Determined by Aβ42 Inhibition and Aβ38 Activation) of Compounds **6**–**25**[a]

Compound			PPARγ activation		γ-secretase modulation	
	R_1	R_2	EC_{50} in μM ± SD	Maximal activation in [%]± SD	Inhibition of Aβ42 (IC_{50} in μM)	Activation of Aβ38 (EC_{50} in μM)
Variation of α-substitution						
6	-H		3.7 ± 1.0	111 ± 22	> 40	> 40
7			6.1 ± 0.2	57 ± 2	41.6	31.1
8			8.3 ± 0.1	61 ± 1	22.8	11.3
8c			22% @10 μM		21.5	8.4
8d			10.4 ± 0.4	62 ± 3	32.6	13.5
9			8.8 ± 0.4	59 ± 5	30.4	24.2
10			Inactive		29.3	12.8
Variation of alkyl spacer						
11			31% @ 20 μM		25.2	24.2
12			8.9 ± 2.1	71 ± 14	30.9	16.4
13			10.0 ± 1.2	55 ± 7	22.9	16.4
Introduction of para-substituents						
14			7.5 ± 1.4	63 ± 17	13.0	6.4
15			23% @10 μM		19.3	6.2
16			11.0 ± 0.3	112 ± 2	6.0	1.8
17			10.3 ± 1.6	115 ± 21	8.6	5.5
18			33% @ 6 μM		31.2	Not determined
19			7.0 ± 0.9	65 ± 7	> 40	Not determined
20			32% @10 μM		37.7	17.5
Introduction of aliphatic residues						
21			Inactive		11.3	1.9
22			6.6 ± 0.4	71 ± 5	5.1	4.6
23			12.1 ± 0.2	127 ± 2	17.2	9.0
24			4.3 ± 0.9	79 ± 8	> 40	24.0
25			11.0 ± 0.1	69 ± 1	28.2	10.9

[a] Inactive: tested concentration of 10 μM.

activation, the length of the α-alkyl chain did not considerably influence the activity (EC_{50} values of **6**–**9** between 3 and 9 μM, with a tendency for decreased activity with longer chains). Phenyl-substituted **10** showed no PPARγ activity and can

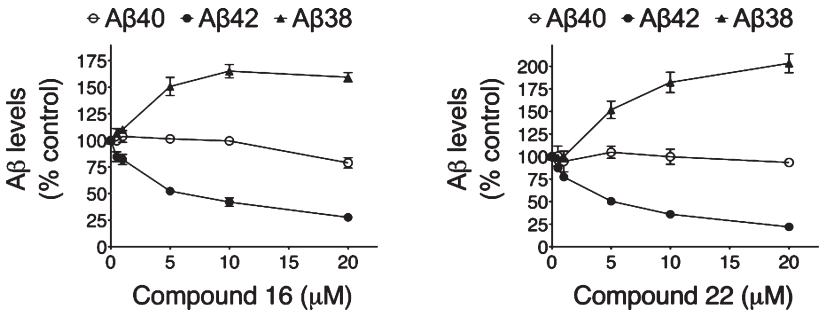


Figure 3. GSM activity of compounds **16** and **22**: dose−response curves for Aβ40, Aβ42, and Aβ38 formation.

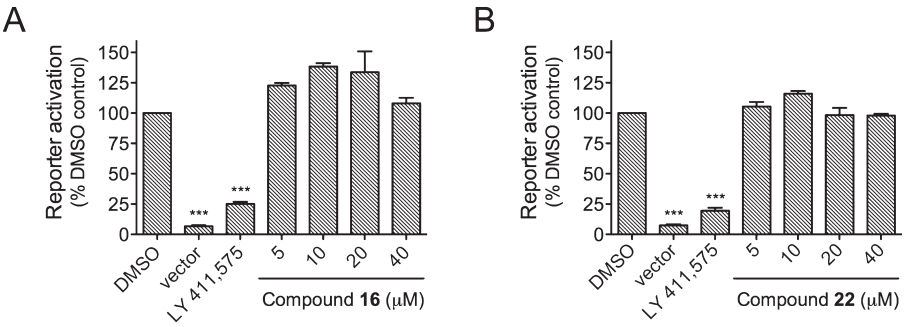


Figure 4. Lack of effect of compounds **16** and **22** in the NOTCH reporter gene assay.

thus be described as a conventional GSM. With an IC_{50} of 29.3 μM for Aβ42 inhibition, compound **10** is somewhat less potent compared to the lead structure. Together, the initial *n*-butyl chain (**8**) turned out to have the optimal chain length in α-position for dual modulation of γ-secretase and PPARγ. Interestingly, it was possible to modify the activity profile toward a conventional GSM by the introduction of a bulky phenyl moiety in α-position to the carboxylic acid.

On the basis of previous studies where we have assessed the influence of the stereochemical configuration of some α-alkyl substituted pirinixic acid derivatives on PPAR activity,[43] we decided to investigate the stereochemical impact on the biological activity of **8**. We have separated **8** into its single enantiomers (**8c** and **8d**) using enantioselective preparative HPLC.[44] While the (*R*)-enantiomer **8c** (IC_{50}(Aβ42) = 21.5 μM) showed slightly superior activity compared to the (*S*)-enantiomer (**8d**) (IC_{50}(Aβ42) = 32.6 μM) on γ-secretase, the results on PPARγ were vice versa (**8c**, 22% activation at 10 μM; **8d**, EC_{50} = 10.4 μM). In summary, we were not able to identify any superior biological activity of one enantiomer. Thus, the stereocenter in the α-position of the carboxylic acid does not cause a significant impact on γ-secretase and PPARγ activity.

Most space for structural modifications is provided by the two ether substituents of the central pyrimidine core of lead structure **8**. First, we elongated and shortened the aliphatic spacer between the two ether moieties and the phenyl residues (compounds **11**−**13**). Compound **11** (IC_{50}(Aβ42) = 25.2 μM) with a shorter methylene and compound **13** with a longer butylene spacer were almost equipotent on γ-secretase compared to **8**, whereas the elongation to a propylene spacer (**12**) led to a decreased potency. In regard to PPARγ activation, the methylene analogue **11** showed a substantially decreased activity. The elongation to propylene and butylene spacers (**12** and **13**) did not cause considerable changes in PPARγ activity.

Second, we introduced different substituents in para-position of both phenyl moieties based on lead structure **8**. Methyl (compound **14**) and methoxy (**15**) substituents increased GSM activity with methyl (IC_{50}(Aβ42) = 13.0 μM) being slightly superior to methoxy (IC_{50}(Aβ42) = 19.3 μM). Further improvement of Aβ42 inhibition was finally achieved by introduction of trifluoromethyl (**16**) (IC_{50}(Aβ42) = 6.0 μM, Figure 3) and trifluoromethoxy moieties (**17**) (IC_{50}(Aβ42) = 8.6 μM), which belong to the most potent GSMs from this series. Compounds **16** and **17** modulate PPARγ with an EC_{50} of about 10 μM. In contrast to these four derivatives, introduction of nitro (**18**), cyano (**19**), and the bioisosteric replacement of both benzenes by thiophene (**20**) caused a clear decrease of GSM activity with IC_{50} values above 30 μM.

Finally, we replaced the phenyl moieties by aliphatic rings of various sizes. Substitution with cycloheptyl (**21**), cyclohexyl (**22**), and cyclopentyl (**23**) moieties improved the potency regarding inhibition of GSM activity with IC_{50} values for Aβ42 in the low micromolar range. The cyclopentyl (IC_{50}(Aβ42) = 17.2 μM) and cycloheptyl (IC_{50}(Aβ42) = 11.3 μM) derivatives showed considerable activity. However, the most active compound is the cyclohexyl derivative **22** (IC_{50}(Aβ42) = 5.1 μM), which represents the most potent GSM presented in this study (Figure 3). Further size restriction to cyclopropyl (**24**) as well as ring-opening of the cyclopropyl (isopentyl-substituted **25**) was detrimental with IC_{50} > 28 μM. Within this series, PPARγ activity remained in the range between 4 and 11 μM (**21**−**25**). The largest cycloheptyl substituent (**21**) caused a complete loss of PPARγ activity, thereby representing the second conventional GSM besides α-phenyl substituted **10**.

NOTCH Processing. Potential effects on NOTCH processing were examined for compounds **16** and **22** that displayed the highest GSM activity with an established reporter assay.[38] Treatment of cells with the γ-secretase inhibitor L-685,458 or replacement of the plasmid encoding NOTCH with an empty vector caused a dramatic decrease in reporter activity as expected (Figure 4). However, treatment of cells with 5−40 μM compounds **16** (Figure 4A) and **22** (Figure 4B)

Table 2. COX Inhibition of Compounds **6**−**25**[a]

	remaining activity ± SD at 10 μM (%)			remaining activity ± SD at 10 μM (%)	
compd	COX-1	COX-2	compd	COX-1	COX-2
6	91.8 ± 5.5	91.7 ± 15.6	**15**	72.8 ± 29.1	94.6 ± 33.0
7	84.6 ± 5.5	56.9 ± 1.4	**16**	54.4 ± 23.6 IC_{50} = 16.9 μM	69.0 ± 27.7 IC_{50} > 40 μM
8	64.2 ± 24.8 IC_{50} = 18.7 μM	90.9 ± 28.8 IC_{50} > 40 μM	**17**	73.0 ± 11.5	75.8 ± 8.7
8c	73.9 ± 15.8	98.5 ± 11.1	**18**	82.1 ± 12.2	91.6 ± 14.6
8d	83.5 ± 12.6	92.8 ± 2.1	**19**	88.8 ± 16.6	82.6 ± 24.3
9	61.8 ± 17.0	63.8 ± 16.8	**20**	76.6 ± 6.9	102.4 ± 16.9
10	72.2 ± 13.3	86.0 ± 21.8	**21**	56.1 ± 8.8	50.1 ± 9.3
11	88.9 ± 21.3	61.6 ± 2.4	**22**	52.7 ± 11.1 IC_{50} = 13.4 μM	49.5 ± 18.5 IC_{50} = 10.9 μM
12	78.8 ± 14.9	51.9 ± 11.3	**23**	68.2 ± 6.9	84.7 ± 3.5
13	78.8 ± 14.0	53.9 ± 14.2	**24**	97.7 ± 17.4	87.9 ± 5.8
14	57.2 ± 12.7	93.3 ± 20.0	**25**	72.4 ± 31.3	80.5 ± 25.5

[a] IC_{50} values were determined for compounds **8**, **16**, and **22**.

did not reduce reporter activity, indicating that these potent dual γ-secretase/PPARγ modulators did not affect NOTCH processing or signaling in this concentration range (Figure 4).

Cytotoxicity. Cytotoxicity of all novel analogues was determined in the concentration range between 10 and 100 μM using Alamar blue reagent and is summarized in the Supporting Information. With one exception (i.e., compound **16**), none of the compounds displayed detectable cytotoxicity up to 60 μM. Compound **16** showed 25% cytotoxicity at 60 μM without significant effects at 40 μM, which is 6- to 7-fold above its IC_{50} value for Aβ42 inhibition (6.0 μM).

COX Inhibition. Most NSAIDs-type GSMs such as indomethacin inhibit COX-1 and COX-2 with IC_{50} values in the nanomolar range (Figure 1). Consequently, COX-related side effects are a major obstacle for their long-term clinical use. Thus, one of the main objectives of this study was to develop a potent dual γ-secretase/PPARγ modulator with substantially weaker COX inhibitory activity compared to NSAID-type GSMs. Inhibition of isolated ovine COX-1 and human recombinant COX-2 is summarized in Table 2 and presented as remaining activity at 10 μM. Importantly, all novel compounds synthesized within this study were weak COX-1 and COX-2 inhibitors with at least half-maximal remaining activities at 10 μM. Furthermore, we have determined IC_{50} values for lead compound **8** as well as for the most active compounds **16** and **22**. Compound **8** displays moderate COX-1 inhibition with an IC_{50} of 18.7 μM and very weak COX-2 inhibition (IC_{50} > 40 μM). The introduction of cyclohexyl residues (compound **22**: IC_{50}(Aβ42) = 5.1 μM, EC_{50}(PPARγ) = 6.6 μM) improved dual γ-secretase/PPARγ activity but also caused slightly increased COX-1 inhibition and COX-2 inhibition (IC_{50}(COX-1) = 13.4 μM, IC_{50}(COX-2) = 10.9 μM). *p*-Trifluoromethylphenyl-substituted **16** (IC_{50}(Aβ42) = 6.0 μM, EC_{50}(PPARγ) = 11 μM) shows weaker COX-1 inhibitory activity (IC_{50} = 16.9 μM) while sparing inhibition of COX-2. These results suggest an advantage of *p*-trifluorormethylphenyl over cyclohexyl substitution in regard to COX selectivity. In summary, the introduction of *p*-trifluoromethylphenyl residues (**16**) resulted in the most favorable pharmacological profile with superiority of GSM activity versus COX-inhibition.

Discussion

This study presents the successful establishment of a novel and robust scaffold for potent dual γ-secretase/PPARγ modulators. The structural starting point was our initial hit **8**, which was used as a template for structural variations with a special focus on the lipophilic parts of the molecule. Our SAR studies revealed clear SAR in α-position to the carboxylic acid, where the introduction of a *n*-butyl chain led to the highest GSM activity. In contrast, the lipophilic backbone of the molecule showed high tolerance toward structural modification. Introduction of different aromatic and aliphatic moieties linked by aliphatic spacers was well tolerated and showed activity on both γ-secretase and PPARγ. Substantial improvement was finally achieved by the replacement of the two phenyl residues with cyclohexyl (**22**) or by the attachment of *p*-trifluoromethyl substituents (**16**). Cyclohexyl-substituted **22** showed nearly equipotent activities in the low micromolar range with an Aβ42 IC_{50} of 5.1 μM and a PPARγ EC_{50} of 6.6 μM. The *p*-trifluoromethyl derivative had comparable GSM activity with an Aβ42 IC_{50} of 6.0 μM, slightly weaker PPARγ activity with an EC_{50} of 11 μM but the more promising selectivity profile (IC_{50}(COX-1) = 16.9 μM, IC_{50}(COX-2) > 40 μM). Importantly, these compounds did not impair NOTCH processing in the noncytotoxic concentration range.

Taken together, this series represents the first class of compounds described as dual γ-secretase/PPARγ modulators. By structural modifications we were able to cover the whole range from selective GSMs to equipotent and low micromolar active dual γ-secretase/PPARγ modulators. A major liability of the initially discovered GSMs in the class of NSAIDs are COX-associated gastrointestinal and cardiovascular side effects. These side effects most likely preclude the long-term use of Aβ42-lowering NSAIDs to prevent AD.[45] However, the here presented compounds show substantially lower COX inhibition compared to Aβ42-lowering NSAIDs such as ibuprofen (Figure 1). We propose that dual γ-secretase/PPARγ modulators could provide a promising strategy to address the increased dementia risk in patients with insulin resistance and type 2 diabetes. In these individuals, dual-active molecules might ameliorate the deleterious effects associated with insulin resistance by activation of PPARγ and further confront the elevated risk for AD by modulating γ-secretase activity and lowering Aβ42 levels in brain.

Experimental Section

Compounds and Chemistry. The structures of compounds **6**−**25** were confirmed by ^{1}H NMR, ^{13}C NMR, and mass spectrometry (ESI). The purities of the final compounds described here were determined by combustion analysis and are 95% or

higher. Commercial chemicals and solvents were reagent grade and used without further purification. ^{1}H and ^{13}C NMR spectra were measured in DMSO-d_6 or $CDCl_3$ on a Bruker ARX 300 (^{1}H NMR) and AC 200 E (^{13}C NMR) spectrometer. Chemical shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as internal standard. Mass spectra were obtained on a Fissous Instruments VG Platform 2 spectrometer measuring in the positive- or negative-ion mode (ESI-MS system). Combustion analysis was performed by the Microanalytical Laboratory of the Institute of Organic Chemistry and Chemical Biology, Goethe University Frankfurt, on a Foss Heraeus CHNO rapid elemental analyzer (for details see the Supporting Information).

The general synthesis of compounds (Scheme 2) follows the routes described recently.[34] The synthetic procedure is described representatively for compounds **8** and **16**. Detailed synthesis and analytical data of all compounds are provided in the Supporting Information.

Step i. 2-Thiobarbituric acid (4.33 g/30 mmol, 1 equiv) was suspended in anhydrous DMF (~30 mL, quantum satis), and triethylamine (4.56 g/45 mmol, 1.5 equiv) was added. Heating to 80−90 °C yielded a clear solution, to which ethyl 2-bromohexanoate (10.05 g/45 mmol, 1.5 equiv) was added dropwise. After being stirred for 4 h at 80 °C (TLC control), the reaction mixture was quenched with at least four parts of water and extracted three times with ethyl acetate. Solvent evaporation of the organic phase yielded the crude product, which was purified by column chromatography using hexane/ethyl acetate to obtain **8a** as a white solid. Yield: 55.9% (4.8 g).

Step iia. Compound **8b** was prepared under Williamson conditions. The precursor from step i (1.2 g/4.19 mmol, 1 equiv) and (2-bromoethyl)benzene (1.63 g/8.8 mmol, 2.1 equiv) were suspended in anhydrous DMF (quantum satis) and heated to 80−90 °C. K_2CO_3 (1.34 g/9.72 mmol, 2.32 equiv) was added, and the resulting suspension was stirred for 9 h. After completion of the reaction (TLC control), DMF was evaporated in vacuo at 60 °C and the remaining solid was diluted with water. After neutralization to pH 7, the aqueous phase was extracted two times with ethyl acetate. The combined organic fractions were washed with brine and dried over $MgSO_4$. Solvent evaporation gave the crude product, which was purified by column chromatography using hexane/ethyl acetate to yield **8b** as clear oil. Yield: 63.7% (1.32 g).

Step iib. Compound **16b** was synthesized under Mitsunobu conditions. Thereby, **8a** (0.45 g/1.6 mmol, 1 equiv), 4-trifluormethylphenylethanol (0.6 g/3.2 mmol, 2.1 equiv), and triphenylphosphine (1.2 g/4.57 mmol, 2.5 equiv) were dissolved in anhydrous THF and stirred under argon atmosphere with ice bath cooling. Diethylazodicarboxylate (DEAD, 0.78 g/4.57 mmol, 2.5 equiv) diluted in 5 mL of THF was added dropwise via a syringe, and the solution was stirred for 1 h until the reaction was completed (TLC control). Subsequently, THF was evaporated and the remaining residue was purified by column chromatography using hexane/ethyl acetate to yield **16b** as clear oil in 64.3% (0.64 g) yield.

Step iii. The corresponding ester (**8b** (0.84 g/1.7 mmol, 1 equiv) or **16b** (0.4 g/0.6 mmol, 0.6 equiv)) was dissolved in a mixture of 5 mL of THF/10 mL of MeOH, and a solution of $LiOH \cdot H_2O$ (3 equiv) in 3 mL of H_2O was added. After the mixture was stirred at 50 °C until saponification was completed, the solvent was removed and the residue was dissolved in water (under heating; if necessary, low amounts of MeOH were added). The solution was acidified with diluted hydrochloric acid. The formed precipitate (**8**) was filtered, washed to neutrality with water, and then washed with *n*-hexane. Recrystallization from *n*-hexane/ethyl acetate yielded compound **8** as a white solid. Yield: 78.4% (0.62 g).

In the case of compound **16**, the precipitate formed after addition of diluted hydrochloric acid was oily. To purify the crude product, the residue was extracted with ethyl acetate and separated by column chromatography using *n*-hexane/ethyl acetate. Yield: 92.2% (0.35 g).

Ethyl 2-(4,6-Dihydroxypyrimidin-2-ylthio)hexanoate (8a). White solid, mp 173 °C. ^{1}H NMR (300.13 MHz, $(CD_3)_2SO$): δ = 0.82−0.87 (t, 3H, J = 6.9 Hz, CH_3-Bu), 1.14−1.19 (t, 3H, J = 7.1 Hz, -CH_3), 1.28−1.31 (m, 4H, CH_2-Bu), 1.72−1.92 (m, 2H, CH_2-Bu), 4.08−4.15 (q, 2H, J = 7.1 Hz, OCH_2), 4.47−4.52 (t, 1H, J = 7.1 Hz, S-CH), 5.22 (s, 1H, Pyr-5H), 11.75 (s, br, 2H, Pyr-OH). ^{13}C NMR (75.44 MHz, $(CD_3)_2SO$): δ = 13.63 (-CH$_3$), 13.89 (CH$_3$-Bu), 21.59 (CH$_2$-Bu), 28.49 (CH$_2$-Bu), 31.27 (CH$_2$-Bu), 46.39 (S-CH), 61.06 (OCH$_2$), 85.62 (Pyr-C_5), 167.79 (Pyr-C_2), 170.86 (COO). MS (ESI−): m/e = 284.9 [M − 1]$^-$.

Ethyl 2-(4,6-Diphenethoxypyrimidin-2-ylthio)hexanoate (8b). Clear oil. ^{1}H NMR (250.13 MHz, $(CD_3)_2SO$): δ = 0.82 (t, 3H, J = 7.1 Hz, CH_3-Bu), 1.05 (t, 3H, J = 7.1 Hz, -CH_3), 1.26−1.34 (m, 4H, CH_2-Bu), 1.74−1.91 (m, 2H, CH_2-Bu), 2.98 (t, 4H, J = 7.1 Hz, Ph-CH_2), 4.11 (q, 2H, J = 7.1 Hz, OCH_2), 4.32−4.48 (m, 5H, S-CH_2 + Pyr-O-CH_2), 5.89 (s, 1H, Pyr-5H), 7.19−7.32 (m, 10H, Ph-H). ^{13}C NMR (75.44 MHz, $(CD_3)_2SO$): δ = 13.63 (CH$_3$-Bu), 13.83 (−CH$_3$), 21.60 (CH$_2$-Bu), 28.79 (CH$_2$-Bu), 30.72 (CH$_2$-Bu), 34.43 (2C, Ph-CH$_2$), 47.13 (S-CH$_2$), 60.90 (OCH$_2$), 67.12 (2C, Pyr-O-CH$_2$), 86.01 (Pyr-C5), 126.34 (2C, Ph-C4), 128,12 (4C, Ph-C_2 + -C_6), 128.61 (4C, Ph-C_3 + -C_5), 137.80 (2C, Ph-C1), 168.54 (Pyr-C_2), 170.07 (2C, Pyr-C_4 + -C_6), 171.35 (COO). MS (ESI+): m/e = 495.2 [M + H]$^+$.

Ethyl 2-(4,6-Bis(4-(trifluoromethyl)phenethoxy)pyrimidin-2-ylthio)hexanoate (16b). Clear oil. ^{1}H NMR (300.13 MHz, $(CD_3)_2SO$): δ = 0.79−0.83 (t, 3H, J = 7.1 Hz, CH_3-Bu), 1.02−1.06 (t, 3H, J = 7.0 Hz, -CH_3), 1.29−1.40 (m, 4H, CH_2-Bu), 1.84−1.92 (m, 2H, CH_2-Bu), 3.07−3.11 (t, 4H, J = 6.6 Hz, Ph-CH_2), 3.97−4.08 (m, 2H, OCH_2), 4.29−4.35 (t, 1H, J = 7.2 Hz, S-CH), 4.47−4.53 (m, 4H, Pyr-O-CH_2), 5.89 (s, 1H, Pyr-5H), 7.49−7.52 (d, 4H, J = 8.0 Hz, Ph-C$_2$ + -C$_6H$), 7.63−7.66 (d, 4H, J = 8.1 Hz, Ph-C$_3$ + -C$_5H$). ^{13}C NMR (75.44 MHz, $(CD_3)_2SO$): δ = 13.60 (CH$_3$-Bu), 14.07 (-CH$_3$), 21.60 (CH$_2$-Bu), 28.79 (CH$_2$-Bu), 30.64 (CH$_2$-Bu), 34.15 (2C, Ph-CH$_2$), 47.16 (S-CH), 60.88 (OCH$_2$), 66.59 (2C, Pyr-O-CH$_2$), 86.06 (Pyr-C_5), 125.11 (4C, Ph-C_3 + -C_5), 126.13−127.35 (2C, Ph-CF$_3$), 129.71 (4C, Ph-C_2 + -C_6), 143.01 (2C, Ph-C_1), 168.59 (Pyr-C_2), 169.99 (2C, Pyr-C_4 + -C_6), 171.31 (COO). MS (ESI+): m/e = 631.6 [M + 1]$^+$.

2-(4,6-Diphenethoxypyrimidin-2-ylthio)hexanoic Acid (8). White solid, mp 99 °C. ^{1}H NMR (300.13 MHz, $(CD_3)_2SO$): δ = 0.81 (t, 3H, J = 7.1 Hz, CH_3-Bu), 1.23−1.38 (m, 4H, CH_2-Bu), 1.78−1.90 (m, 2H, CH_2-Bu), 2.98 (t, 4H, J = 6.9 Hz, Ph-CH_2), 4.29 (t, 1H, J = 7.2 Hz, S-CH), 4.46 (t, 4H, J = 6.9 Hz, Pyr−O-CH_2), 5.87 (s, 1H, Pyr-5H), 7.18−7.32 (m, 10H, Ph-H). ^{13}C NMR (75.44 MHz, $(CD_3)_2SO$): δ = 13.70 (CH$_3$-Bu), 21.71 (CH$_2$-Bu), 28.92 (CH$_2$-Bu), 31.09 (CH$_2$-Bu), 34.44 (2C, Ph-CH$_2$), 47.61 (S-CH$_2$), 67.08 (2C, Pyr-O-CH$_2$), 85.87 (Pyr-C5), 126.31 (2C, Ph-C4), 128.30 (4C, Ph-C2 + -C6), 128.84 (4C, Ph-C3 + -C5), 137.88 (2C, Ph-C1), 169.01 (Pyr-C2), 170.03 (2C, Pyr-C4 + -C6), 172.70 (COOH). MS (ESI+): m/e = 467.0 [M + H]$^+$.

2-(4,6-Bis(4-(trifluoromethyl)phenethoxy)pyrimidin-2-ylthio)-hexanoic Acid (16). Yellow oil. ^{1}H NMR (300.13 MHz, $(CD_3)_2SO$): δ = 0.78−0.83 (t, 3H, J = 7.1 Hz, CH_3-Bu), 1.22−1.37 (m, 4H, CH_2-Bu), 1.74−1.92 (m, 2H, CH_2-Bu), 3.06−3.11 (t, 4H, J = 6.6 Hz, Ph-CH_2), 4.27−4.30 (t, 1H, J = 7.1 Hz, S-CH), 4.48−4.53 (t, 4H, J = 6.6 Hz, Pyr-O-CH_2), 5.87 (s, 1H, Pyr-5H), 7.49−7.52 (d, 4H, J = 8.0 Hz, Ph-C$_2$ + -C$_6H$), 7.63−7.66 (d, 4H, J = 8.0 Hz, Ph-C$_3$ + -C$_5H$), 12.82 (s, br, 1H, COOH). ^{13}C NMR (75.44 MHz, $(CD_3)_2SO$): δ = 13.64 (CH$_3$-Bu), 21.67 (CH$_2$-Bu), 28.92 (CH$_2$-Bu), 30.91 (CH$_2$-Bu), 34.16 (2C, Ph-CH$_2$), 47.46 (S-CH), 66.54 (2C, Pyr-O-CH$_2$), 85.97 (Pyr-C_5), 124.99 (4C, Ph-C_3 + -C_5), 125.14−127.74 (2C, Ph-CF$_3$), 129.69 (4C, Ph-C_2 + -C_6), 143.06 (2C, Ph-C_1), 168.95 (Pyr-C_2), 169.97 (2C, Pyr-C_4 + -C_6), 172.23 (COOH). MS (ESI-): m/e = 601.4 [M − 1]$^-$.

PPAR Transactivation Assay. COS7 cells were grown in DMEM supplemented with FCS, sodium pyruvate, and penicillin/streptomycine at 37 °C and 5% CO_2. The day before transfection, cells were seeded in 96-well plates at a density of 30 000 cells

per well. Transient transfection was carried out by Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's protocol with pFR-Luc (Stratagene), pRL-SV40 (Promega), and the Gal4-fusion receptor plasmids (pFA-CMV-hPPAR-LBD) of the respective subtype. At 5 h after transfection, the medium was changed to DMEM without phenol red and FCS, containing 0.1% DMSO and the respective concentrations of the test compounds.

Following overnight incubation with the test compounds, cells were assayed for reporter gene activity using Dual-Glo luciferase assay system (Promega) according to the manufacturer's protocol. Luminescence was measured with a GENios Pro luminometer (Tecan Deutschland GmbH, Crailsheim, Germany). Each concentration of the compounds was tested in triplicate wells, and each experiment was repeated independently at least three times. Normalization for transfection efficacy and cell growth was done by division of the firefly luciferase data by renilla luciferase data resulting in relative light units. Activation factors were obtained by dividing by DMSO control. EC_{50} and standard deviation values were calculated by mean values of at least three determinations by SigmaPlot 2001 (Systat Software GmbH, Erkrath, Germany) using a four-parameter logistic regression. All compounds were evaluated by comparison of the achieved maximum effect to that of the reference compound (pioglitazone for PPARγ, GW 7647 for PPARα, and L165,041 for PPARδ each with 1 μM).

Determination of γ-Secretase Modulator Activity. To characterize the GSM activity of novel analogues, their effects on the generation of Aβ40, Aβ42, and Aβ38 peptides were determined in a previously described cell-based ELISA assay with modifications.[37] CHO cells with stable overexpression of wild type human amyloid precursor protein and wild type human presenilin-1[37] were maintained in DMEM supplemented with 10% FBS and treated in 96-well plates for 24 h with increasing concentrations of the respective compounds or DMSO vehicle. Culture media were collected and analyzed by ELISA as follows: monoclonal antibody IC16 raised against amino acids 1−15 of the Aβ sequence was used as a capture antibody.[46] To distinguish different Aβ species, C-terminal antibodies specific for Aβ40, Aβ42, and Aβ38 labeled with horseradish peroxidase (HRP) using the Pierce EZ-Link Plus activated peroxidase kit (Thermo Fisher Scientific) were used for detection. The 96-well high-binding microtiter plates were coated overnight at 4 °C with capture antibody IC16 diluted 1:250 in PBS, pH 7.2. Capture antibody was removed, and conditioned media samples (10 μL for detection of Aβ40, 100 μL for Aβ42, 50 μL for Aβ38) or standard peptides were loaded. HRP-coupled detection antibodies diluted in assay buffer (PBS containing 0.05% Tween-20, 1% BSA) were added to each well and incubated overnight at 4 °C. Plates were washed 3 times with PBS containing 0.05% Tween-20 and once with PBS. Then 50 μL of TMB ultrasubstrate (Thermo Fisher Scientific) was added and incubated for 1−10 min at room temperature in the dark, and the reaction was stopped by adding 50 μL of 2 M H_2SO_4. Absorbance at 450 nm was recorded with a Paradigm microplate reader (Beckman-Coulter). Synthetic Aβ40, Aβ42, and Aβ38 peptides (Bachem AG) were used to generate standard curves. These Aβ peptides were solubilized in DMSO at 1 mg/mL, aliquoted, and stored frozen at −80 °C. Immediately before use, peptides were diluted in assay buffer to 250−3000 pg/mL. Triplicate measurements from each drug concentration were averaged and normalized to DMSO control condition. For calculation of IC_{50} values, cells were treated with eight increasing concentrations of each compound, and a nonlinear curve fit with variable slope model was applied to the results from two to four independent experiments. Statistics were performed using GraphPad Prism (GraphPad Software).

NOTCH Reporter Assay. The NOTCH reporter assay was performed as described.[38] In brief, subconfluent CHO cells were transiently transfected in 96-well plates with plasmid pCDNA3-Notch-ΔE-GVP encoding truncated NOTCH fused to a Gal4 DNA-binding/VP16 transactivation domain and the MH100 reporter plasmid encoding firefly luciferase under the UAS promotor (50 ng each) using GeneJuice transfection reagent (Merck Chemicals Ltd.). Co-transfection of the MH100 reporter with empty pcDNA3 plasmid served as a negative control. Then 5 ng of plasmid pRL-TK encoding renilla luciferase was added to the plasmid mix to control for transfection efficiency. At 24 h after the transfection, cells were treated for an additional 24 h with increasing concentrations of the compounds **16** and **22**, 0.5 μM γ-secretase inhibitor LY-411575, or DMSO vehicle. The cells were then lysed, and firefly and renilla luciferase activities were quantified using the Dual Glo luciferase assay system (Promega) and a PARADIGM microplate reader (Beckman-Coulter). Normalization for transfection efficacy and cell growth was achieved by dividing the firefly luciferase values by the renilla luciferase values. Percent activation of the reporter was then calculated by normalization of triplicate measurements from each condition to the DMSO control values. Unpaired t test analysis was performed from two independent experiments using GraphPad Prism: ***, $p < 0.001$.

Cytotoxicity Assay. CHO cells with stable overexpression of wild type human amyloid precursor protein and wild type human presenilin-1 were seeded at low density in 96-well plates (4000 cells/well) and cultured for 24 h. The cells were then treated in duplicate with increasing concentrations (0−100 μM) of the respective compounds or DMSO vehicle for an additional 24 h. Cell viability was assessed using the Alamar blue reagent (Invitrogen). Then 20 μL of Alamar blue was added to cells cultured in 200 μL of medium and incubated overnight. Absorbance was measured with a Paradigm microplate reader (Beckman-Coulter) at 570 nm, using 600 nm as the reference wavelength. The values expressed as percent viability of vehicle control represent averages of two independent experiments.

Determination of COX Inhibition. Inhibition of the activities of isolated ovine COX-1 and human recombinant COX-2 was performed as described.[34] Briefly, purified COX-1 (ovine, 50 units) or COX-2 (human recombinant, 20 units) were diluted in 1 mL of reaction mixture containing 100 mM Tris buffer, pH 8, 5 mM glutathione, 5 μM hemoglobin, and 100 μM EDTA at 4 °C and preincubated with the test compounds for 5 min. Samples were prewarmed for 60 s at 37 °C, and arachidonic acid (5 μM for COX-1, 2 μM for COX-2) was added to start the reaction. After 5 min at 37 °C, the COX product 12-HHT was extracted and then analyzed by HPLC as described.[41,47]

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Supporting Information Available: Chemical synthesis, ^1H and ^{13}C NMR data of intermediates and final compounds, mass spectrometry, combustion analysis, and cytotoxicity data. This material is available free of charge via the Internet at http://pubs.acs.org.

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