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SAR studies of acidic dual γ-secretase/PPARγ modulators

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ABSTRACT

A novel set of dual γ-secretase/PPARγ modulators characterized by a 2-benzyl hexanoic acid scaffold is presented. Synthetic efforts were focused on the variation of the substitution pattern of the central benzene. Finally, we obtained a new class of 2,5-disubstituted 2-benzylidene hexanoic acid derivatives, which act as dual γ-secretase/PPARγ modulators in the low micromolar range. We have explored broad SAR and successfully improved the dual pharmacological activity and the selectivity profile against potential off-targets such as NOTCH and COX. Compound **17** showed an IC_{50} Aβ42 = 2.4 μM and an EC_{50} PPARγ = 7.2 μM and could be a valuable tool to further evaluate the concept of dual γ-secretase/PPARγ modulators in animal models of Alzheimer's disease.

1. Introduction

The accumulation and aggregation of amyloid β-42 peptides (Aβ42) in brain is believed to be a crucial disease causing step in the pathology of Alzheimer's disease (AD). Based on the amyloid hypothesis of AD,[1,2] several pharmacological intervention strategies to lower Aβ levels in brain have been explored in in vitro and in vivo models of AD.[3,4] Among those, targeting γ-secretase appears to be one of the most promising, potentially disease modifying approaches for the treatment of AD. γ-Secretase, an aspartyl-protease, is responsible for the last step in the proteolytic release of Aβ42 peptides from a large type-I transmembrane protein, the amyloid precursor protein (APP). Besides APP, numerous other transmembrane proteins such as the NOTCH receptor are processed by γ-secretase.[4,5]

Initially, γ-secretase inhibitors (GSIs) have been advanced in preclinical and clinical development. Unfortunately, the use of GSIs is limited by severe toxicity resulting from the inhibition of NOTCH processing. For example, treatment with the GSI LY 411,575 resulted in gastrointestinal bleedings, and inhibition of thymocyte proliferation and B-cell differentiation in preclinical studies in mice.[6,7] As an appealing alternative to GSIs, so-called γ-secretase modulators (GSMs) have been identified.[8] GSMs are small molecules that selectively lower generation of the Aβ42 peptides while sparing other substrates of γ-secretase such as NOTCH. Characteristically, GSMs appear not to affect the overall enzymatic activity of γ-secretase, but shift the ratio of proteolytic APP products by increasing the generation of shorter and less aggregation-prone Aβ peptides such as Aβ38 at the expense of the amyloidogenic Aβ42 species.[9]

The first GSMs were discovered in the class of nonsteroidal anti-inflammatory drugs (NSAIDs; e.g., ibuprofen and indomethacin; Fig. 1).[8,10] However, these drugs display very low potency against γ-secretase and limited brain permeability. Furthermore, NSAID-type GSMs are potent inhibitors of cyclooxygenases (COX) and chronic COX-inhibition is associated with serious side effects.[11] With (*R*)-flurbiprofen (Fig. 1), a COX-inactive NSAID-type GSM has been evaluated in clinical trials. Unfortunately, the compound failed to meet any of the clinical end points in a phase III study.[12] A straightforward interpretation of the trial results and earlier clinical observations exposes the low GSM potency (IC_{50} = 305 μM) as a major weakness of (*R*)-flurbiprofen, indicating that more potent GSMs are required to achieve efficacy in the central nervous system and better clinical outcomes.[12,13]

Recent developments in the field include highly active acidic GSMs with some structural similarities to NSAIDs[14,15] and structurally different nonacidic GSMs.[16,17] Furthermore, some in vivo

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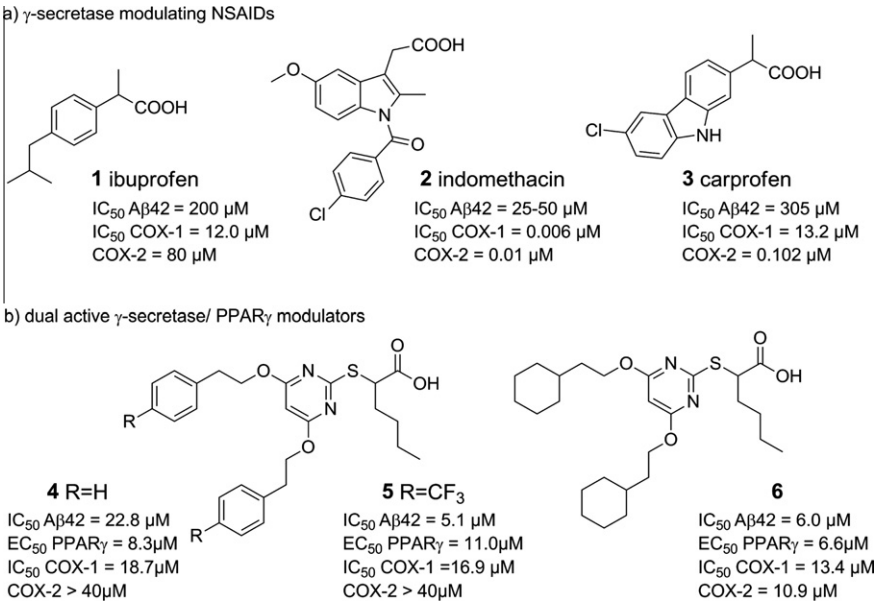


Figure 1. Activity profile of selected NSAIDs and dual active γ-secretase/PPARγ modulators.[23,46–48]

studies of potent GSMs in AD animal models were reported[18–22] and a few GSMs appear to have entered clinical trials.[17] A detailed discussion can be found in a recent review of Oehlrich et al.[17]

We have contributed to recent advances by the characterization of a novel class of GSMs that combines γ-secretase modulation with another pharmacological activity of therapeutic potential in AD, that is, modulation of peroxisome proliferator-activated receptor-γ (PPARγ).[23] PPARγ is a ligand-activated nuclear receptor that plays a central role in lipid and glucose homeostasis.[24] Evidence indicates that activation of PPARγ by small molecule agonists might exert beneficial effects in the AD brain by reducing Aβ levels, toxic pro-inflammatory molecules and cerebrovascular impairment.[25–29] In addition, the incidence rate of AD appears to be increased around twofold in individuals with type 2 diabetes,[30,31] and diabetes and insulin resistance exacerbated amyloid pathology and cognitive dysfunction in AD animal models.[32,33] Therefore, insulin-sensitizing effects of PPARγ agonists might also be beneficial in AD. Recently, both clinically used PPARγ agonists rosiglitazone (EC_{50} PPARγ = 0.043 μM, no brain permeability, phase III)[34] and pioglitazone (EC_{50} PPARγ = 0.58 μM, low brain permeability, phase II)[34] failed to meet clinical end points in larger trials.[35,36] However, this might be related to the low blood–brain barrier permeability of the glitazones, and the application of PPARγ agonists in the treatment of AD warrants further investigation.[37] Importantly, beneficial effects of PPARγ agonists and insulin-sensitizing drugs might manifest primarily in AD risk reduction in individuals with type 2 diabetes, and current treatment and prevention trials for type 2 diabetes include cognition ancillary studies to address this possibility.[38]

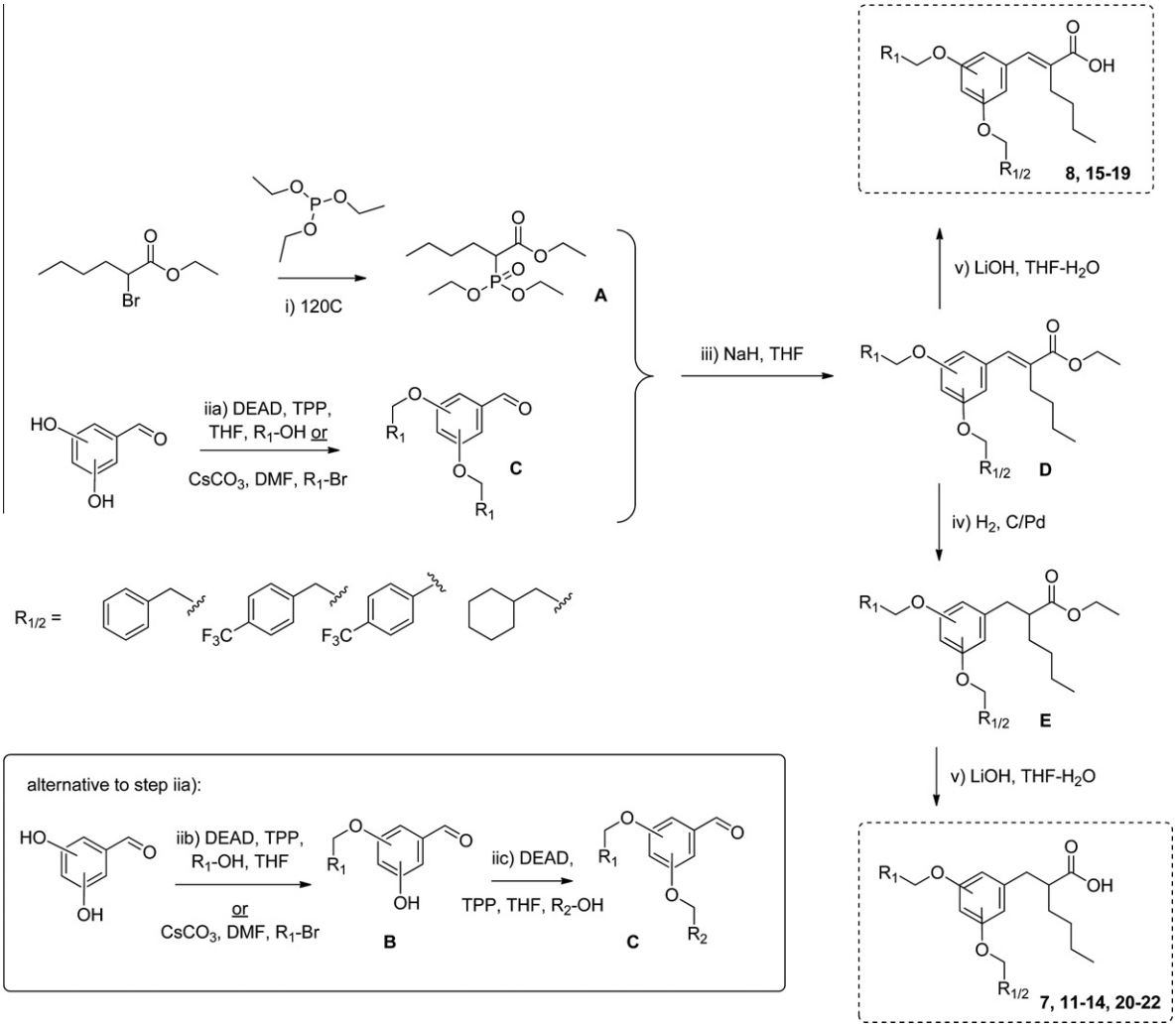
Our previously reported class of dual γ-secretase/PPARγ modulators is based on 2-[(4,6-diphenethoxypyrimidine-2-yl)thio]hexanoic acid (compound **4**: IC_{50} Aβ42 = 22.8 μM, EC_{50} PPARγ = 8.3 μM; Fig. 1), which was first identified as a GSM through screening of an in-house library of PPARγ activators.[23] Based on compound **4**, we have shown structure–activity relationships (SAR) of broad structural variations with a focus on the optimization of the α-alkyl chain and the terminal phenylalkoxy residues.

In this Letter, we describe the modification of the central aromatic scaffold and of the spacer to the acidic function in order to explore the SAR of the substitution pattern of the central aromatic and to further improve the potency. Initially, we replaced the central pyrimidine as well as the thioether moiety of **4** by a pure carbon-based scaffold leading to 2-(3,5-diphenethoxybenzyl)hexanoic acid **7**. This new central scaffold enabled the synthetic accessibility of systematic structural variations of the diphenylethoxy substitution pattern at the central aromatic. In addition, the phenylethoxy residues were replaced by previously identified superior substituents, and SAR of the thioether moiety were explored. The in vitro pharmacology of the presented compounds includes the determination of γ-secretase and PPARγ activity as well as effects on other PPAR subtypes (α and δ) and COX-1 and -2 as potential off-targets. Potential cytotoxic effects were determined for all compounds, and the influence on NOTCH processing was investigated for the lead compounds.

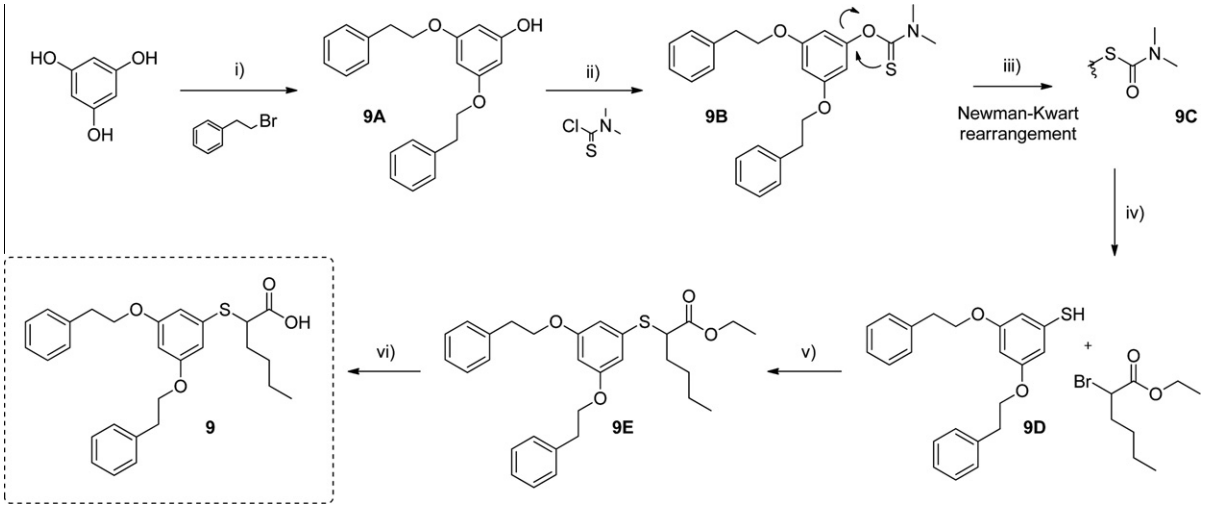
2. Results and discussion

2.1. Chemistry

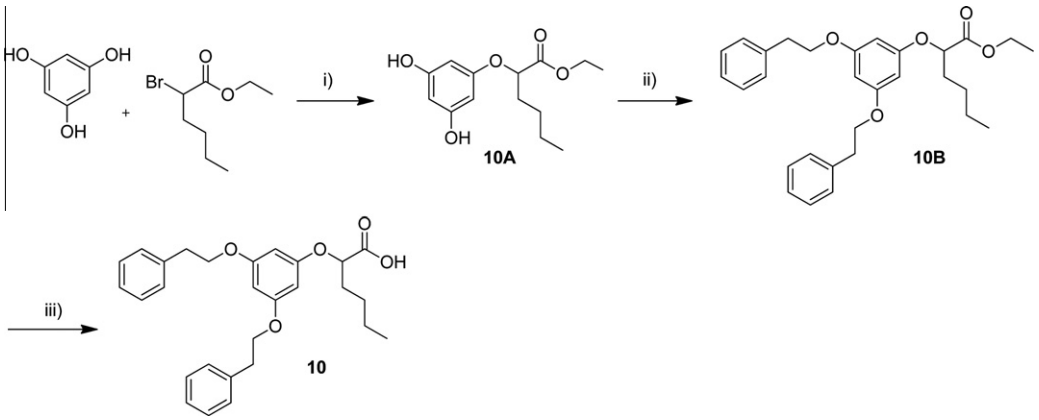
The synthetic procedure leading to compounds **7**–**19** was previously described in Hieke et al.[39] Of note, most of the presented compounds also show anti-inflammatory activity by inhibition of microsomal Prostaglandin E2 synthase-1 (mPGES-1) and/or 5-lipoxygenase (5-LO). A detailed discussion together with the pharmacological data can be found elsewhere.[39] Preparation of compounds **7, 8**, and **11–22** is presented in Scheme 1 and was carried out in 4–6 steps. Synthesis starts with an Arbuzov reaction (step i) to yield the generally used phosphonate precursor **A**. Next, the two hydroxyl groups of the respective dihydroxybenzaldehydes were etherified to intermediates **C** (step ii). Introduction of two equal moieties (step iia) was done under Mitsunobu or Williamson-like conditions using 2 eq of the alcohol or alkylhalogenide as reactants, respectively. The introduction of two different lipophilic residues (final compounds **19**, **21**, and **22**) was carried out in two steps via iib and c. The first lipophilic moiety was introduced under Mitsunobu or Williamson–like conditions using 1 eq alcohol/alkylhalogenide followed by a second Mitsunobu reaction. All intermediates **C** were converted to the respective ethyl 2-(benzylidene)hexanoates **D** in a Wittig-Horner-reaction (step iii) together with the phosphonate precursor **A**. The resulting ester derivatives were hydrolyzed (step v) to the respective disubstituted 2-(benzylidene)hexanoic acids **8** and **15–19**. Alternatively,



Scheme 1. Synthesis of compounds **7**, **8** and **11**–**22**.[39,49] Reagents and conditions: (i) triethylphosphite (1.0 eq), α-bromo ethylhexanoate (1.0 eq), 120 °C, 12 h. (iia) Dihydroxybenzaldehyde (1 eq), R_1-OH (2.1 eq), TPP (2.5 eq), DEAD (2.5 eq), THF, rt, 1.4–24 h or dihydroxybenzaldehyde (1 eq), R_1-bromide (1.2 eq), $CsCO_3$ (1.2 eq), DMF, 50 °C, 5 h. (iib) Dihydroxybenzaldehyde (1 eq), R_1-OH (1.0 eq), TPP (1.2 eq), DEAD (1.2 eq), THF, rt, 1.5–3 h or Dihydroxybenzaldehyde (1 eq), R_1-bromide (1.2 eq), $CsCO_3$ (1.2 eq), DMF, 50 °C, 5 h. (iic) **B** (1 eq), R_2-OH (1.1 eq), TPP (1.2 eq), DEAD (1.2 eq), THF, rt. (iii) **A** (1.3 eq), **C** (1 eq), NaH (1.3 eq), THF, room temperature, 2-22 h. (iv) **D**, Pd/C (10%), ethanol, 24 h, rt. (v) **D** or **E** (1 eq), LiOH × H_2O (5.0 eq), THF/H_2O, 50–60 °C, 6–40 h.



Scheme 2. Synthesis of 2-[(3,5-diphenethoxyphenyl)thio]hexanoic acid **9**.[39–42] Reagents and conditions: (i) Phloroglucinol (1.0 eq), phenylethyl bromide (2.1 eq), NaH (3 eq), DMF, room temperature, 20 h. (ii) **9A** (1.0 eq), dimethylthiocarbamoyl chloride (1.0 eq), NaH (1.2 eq), DMF, 60 °C, 5 h. (iii) **9B**, solvent-free, 240 °C, 5 h. (iv) **9C** (1.0 eq), NaOH (1 mol/l; 10 ml), THF–MeOH, 80 °C, 3 h. (v) **9D** (1.0 eq), α-bromo ethylhexanoate (1.2 eq), NaH (1.2 eq), DMF, room temperature, 3 h. (vi) **9E** (1.0 eq), LiOH × H_2O (5.0 eq), THF/H_2O, 50 °C, 3 h.



Scheme 3. Synthesis of 2-(3,5-diphenethoxyphenoxy)hexanoic acid **10**.[39,40] Reagents and conditions: (i) Phloroglucinol (1.0 eq), NaH (1.6 eq), α-bromo ethylhexanoate (0.8 eq), DMF, room temperature, 8 h. (ii) **10A** (1.0 eq), phenylethanol (2.1 eq), DEAD (2.5 eq), TPP (2.5 eq), THF, room temperature, 4 h. (iii) **10B** (1.0 eq), LiOH × H_2O (5.0 eq), THF/H_2O, 50 °C, 24 h.

they were first hydrogenated to the corresponding ethyl 2-(benzyl)hexanoate **E** (step iv: Pd/C, H_2) and subsequently hydrolyzed (step v) with LiOH and H_2O to yield the substituted benzylidene hexanoic acids **7** and **11–14**, **20–22**.

2-[(3,5-Diphenethoxyphenyl)thio]hexanoic acid **9** was prepared in six steps as shown in Scheme 2. First, two of the three hydroxyl groups of phloroglucinol were etherified (step i).[40] The product **9A** was isolated from a mixture of mono-, di-, and trisubstituted phloroglucinol ethers in low yields. The remaining hydroxyl group was activated with dimethylthio-carbamoylchloride and sodium hydride (step ii) to yield **9B**. Heating at 240 °C led to a Newman-Kwart-Rearrangement (step iii),[41] which yielded the protected thiol derivative. Deprotection with NaOH resulted in the free thiol derivative **9D** (step iv),[42] which reacted in a nucleophilic substitution with α-bromo ethylhexanoate to **9E** (step v). Finally, hydrolysis (step vi) yielded compound **9**.

Synthesis of 2-(3,5-diphenethoxyphenoxy)hexanoic acid **10** is presented in Scheme 3. The compound was prepared in three steps. First, one hydroxyl group of phloroglucinol was etherified with ethyl α-bromohexanoate and sodium hydride to **10A** (step i).[40] Second, the two remaining hydroxyl groups were etherified with 2-phenylethanol under Mitsunobu conditions (step ii) to **10B**, which was followed by hydrolysis (step iii) to the carboxylic acid **10**.

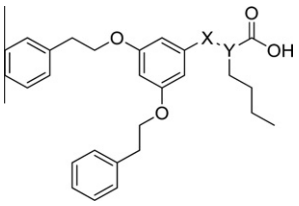
2.2. Biology

Biological characterization was carried out with established cell-based assays for PPAR and γ-secretase activity. A detailed description of all experimental procedures has been published recently.[23] In brief, PPAR activity of the final compounds **7–22** was tested in a cellular luciferase-based PPAR transactivation assay for all three PPAR subtypes (α, γ, and δ). Within this set of compounds, we did not observe significant activity on PPARα and PPARδ (tested concentration: 10 μM). Consequently, the following discussion is restricted to PPARγ activity. For characterization of γ-secretase activity, the levels of Aβ38, Aβ40, and Aβ42 peptides were measured in cell supernatants by ELISA. Inhibition of COX was tested in a cell-free assay using isolated ovine COX-1 and human recombinant COX-2 enzymes. Potential effects on proteolytic processing of NOTCH-1 were investigated for lead compounds (**16 and 17**) using a previously described reporter assay.[23] Cytotoxicity of all compounds was determined using alamarBlue reagent.[23]

2.3. SAR

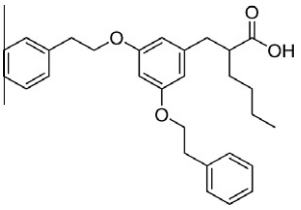
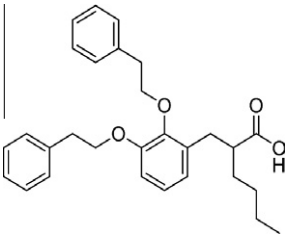
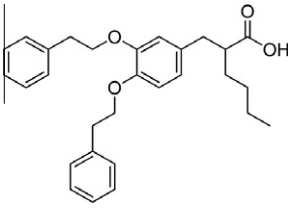
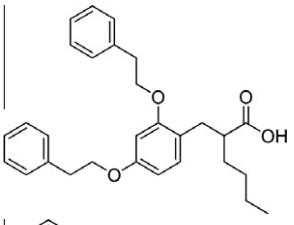
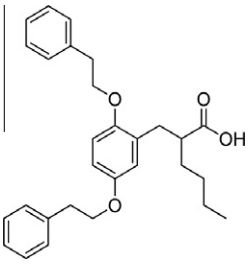
Previously, we identified compound **4**, a subtype-selective modulator of PPARγ (EC_{50} = 8.3 μM, maximal activation 60%) and NSAID-like GSM with an IC_{50} Aβ42 = 22.8 μM; EC_{50} Aβ38 = 11.3 μM (Fig. 1), and investigated SAR of the α-alkyl substituent and the phenylalkyl residues.[23] In this study, we focused our efforts on the central part of **4** and replaced the pyrimidine ring and the thioether substructure by a pure carbon-based scaffold (compound **7**). This initial exchange (2-(3,5-diphenethoxybenzyl)hexanoic acid **7**) led to a weaker γ-secretase (IC_{50} Aβ42 = 38.4 μM) and slightly higher PPARγ modulating activity (EC_{50} = 4.7 μM; see Table 1).

Table 1
In vitro pharmacological characterization of compounds **7**–**10**; variation of exocyclic thioether moiety



Compound	X	Y	PPARγ activation		Aβ42 inhibition (IC_{50} in μM)	Aβ38 activation (EC_{50} in μM)
			EC_{50} in μM	Maximal activation in (%)		
7	CH_2–	CH	4.7 ± 1.8	71 ± 10	38.4 ± 1.1	28.1 ± 3.3
8	CH=	C	7.8 ± 2.7	95 ± 15	37.9 ± 10.9	27.3 ± 10.2
9	S	CH	3.4 ± 0.1	73 ± 1	32.7 ± 0.4	22.2 ± 3.2
10	O	CH	4.8 ± 0.9	46 ± 4	33.7 ± 0.8	26.4 ± 1.4

Table 2
In vitro pharmacological characterization of compounds **11–14**; variation of the substitution pattern of the central benzene

Compound	Structure	PPARγ activation		γ-secretase modulation	
		Activation (EC_{50} in μM)	Maximal activation in (%)	Aβ42 inhibition (IC_{50} in μM)	Aβ38 activation (EC_{50} in μM)
7		4.7 ± 1.8	71 ± 10	38.4 ± 1.1	28.1 ± 3.3
11		3.7 ± 0.8	79 ± 7	>40	>40
12		2.2 ± 0.7	100 ± 9	>40	>40
13		Inactive @ 10 μM		Inverse modulation; IC_{50} Aβ38 ~5 μM	
14		10.8 ± 0.8	97 ± 5	19.1 ± 5.2	14.5 ± 5.4

Resulting from a synthetic precursor of **7**, we have also prepared the corresponding 2-(3,5-diphenethoxybenzylidene)hexanoic acid **8** with a double bond connecting the acidic head group with the benzene core. This compound showed similar γ-secretase (IC_{50} Aβ42 = 37.9 μM) and PPARγ (EC_{50} = 7.8 μM) modulating activity as compared to **7**.

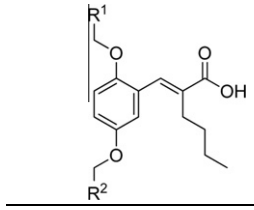
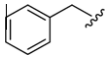
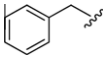
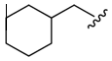
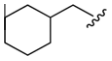
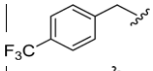
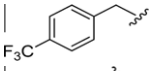
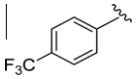
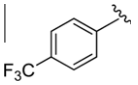
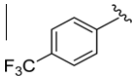
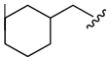
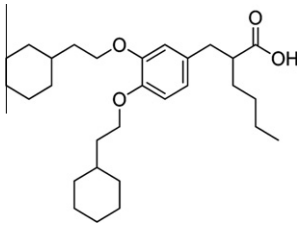
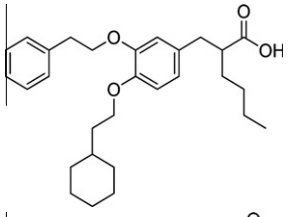
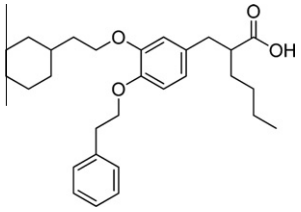
Next, we have investigated the role of the exocyclic methylene group of **7** by synthesizing the respective thioether (**9**) and ether (**10**) analogs. Both exocyclic heteroatoms led to a slight improvement in GSM activity with almost identical pharmacological data for the thioether analog **9** (IC_{50} Aβ42 = 32.7 μM, EC_{50} PPARγ 3.4 μM) and the ether analog **10** (IC_{50} Aβ42 = 33.7 μM, EC_{50} PPARγ = 4.8 μM; Table 1).

Our particular focus in this study was the substitution pattern of the central benzene of **7**. Starting with commercially available dihydroxybenzaldehydes, we have systematically varied the positions of the phenylethoxy residues at the benzene ring. Based on initially introduced 3,5-diphenethoxy-substituted **7**, we have prepared the 2,3-, 2,4-, 2,5- and 3,4- disubstituted analogs (compounds **11–14**). This subset of compounds yielded clear SAR for both γ-secretase and PPARγ modulation (see Table 2). Introduction of the phenylethoxy moieties in 2,3- and 3,4-position (compound **11** and **12**) diminished GSM activity (IC_{50} Aβ42 >40 μM), but led to two of the most potent PPARγ modulators presented in this study (EC_{50} PPARγ = **11**: 3.7 μM, **12**: 2.2 μM).

The 2,4-diphenethoxy benzene derivative **13** displayed an interesting and for this series unique pharmacological profile: inverse modulation of γ-secretase (i.e., reduction of Aβ38 and elevation of Aβ42 levels). Inverse modulation of γ-secretase was previously observed for some drugs including fenofibrate and celecoxib, and certain isoprenoids (GGPP, FPP).[43] Interestingly, **13** is the first reported inverse GSM containing a carboxylic acid as structural characteristic.

The most favorable pharmacological profile showed the 2,5-substitution pattern (compound **14**). γ-Secretase modulation was improved to an Aβ42 IC_{50} of 19.1 μM. With its low micromolar PPARγ activity (EC_{50} = 10.8 μM), compound **14** was an ideal starting point for further structural optimization.

Table 3
In vitro pharmacological characterization of compounds **15–22**; structural modifications based on compounds **14** and **12**

Compound			PPARγ activation		γ-secretase modulation	
	R^1	R^2	EC_{50} in μM	Maximal activation in (%)	Aβ42 inhibition (IC_{50} in μM)	Aβ38 activation (EC_{50} in μM)
*Modifications based on compound **14***						
15			1.8	47 ± 6	15.4 ± 5.8	6.7 ± 2.1
16			75 ± 8% @ 10 μM		3.9 ± 0.9	3.1 ± 1.2
17			7.2 ± 1.3	86 ± 12	2.4 ± 0.4	1.6 ± 0.4
18			28 ± 8% @ 10 μM		1.2 ± 0.3	1.1 ± 0.1
19			27 ± 5% @ 10 μM		2.7 ± 0.2	2.5 ± 0.7
*Modifications based on compound **12***						
20			1.1 ± 0.1	69 ± 3	>40	>40
21			2.5 ± 0.4	61 ± 5	>40	>40
22			1.5 ± 0.5	128 ± 18	>40	>40

Next, we synthesized the corresponding 2-(2,5-diphenethoxybenzylidene)hexanoic acid **15**. Interestingly, **15** was superior to its hydrogenated analog **14** for both GSM and PPARγ activity (IC_{50} Aβ42 = 15.4 μM, EC_{50} PPARγ = 1.8 μM). Therefore, we have used compound **15** as template for further structural optimization and introduced previously identified privileged substructures for GSM activity.[23] Compounds **5** and **6** bearing cyclohexylethoxy- and *p*-(trifluoromethyl)phenylethoxy moieties (Fig. 1), respectively, represented the most potent dual γ-secretase/PPARγ modulators of our previous study. Consequently, we transferred these substructures to 2-(2,5-diphenethoxybenzylidene)hexanoic acid **15**.

The obtained compounds **16** (cyclohexyl) and **17** (*p*-trifluoromethyl) showed further increased GSM activity (IC_{50} Aβ42 = **16**: 3.9 μM, **17**: 2.4 μM) combined with low micromolar PPARγ modulation (EC_{50} <10 μM; see Table 3). Additionally, a derivative with shorter *p*-(trifluoromethyl)phenylmethoxy residues was synthesized (compound **18**), which is the most potent GSM presented in this study (IC_{50} Aβ42 = 1.2 μM). However, PPARγ activity was clearly diminished by this modification (28% activation at 10 μM). Finally, a 2-(*p*-trifluormethyl)phenylmethoxy and a 5-cyclohexylethoxy moiety were combined in compound **19**, which showed similar potency as **18** (IC_{50}Aβ42 = 2.7 μM, PPARγ 27% activation at 10 μM).

For further investigation of the SAR of PPARγ-selective compound **12**, three additional compounds with a 3,4-disubstitution pattern were synthesized (Table 3). In **20**, both phenylethoxy residues were replaced by cyclohexylethoxy residues. Furthermore, the 3,4-disubstitution pattern allowed the stepwise introduction of two different substituents. This was realized in compounds **21** and **22**, where one of each phenylethoxy residues was replaced by a cyclohexylethoxy in 3- and 4-position, respectively. In summary, 3,4-dicyclohexylethoxy substitution (**20**) showed the highest activity on PPARγ (EC_{50} = 1.1 μM).

2.4. Selectivity profile

Most of the acidic NSAID-type GSMs show considerable COX inhibition with micromolar or even nanomolar activity (Fig. 1). We screened our set of compounds for their COX inhibitory activity in order to facilitate the design of COX-sparing dual γ-secretase/PPARγ modulators. COX-1 and -2 inhibition was determined for every presented compound and is summarized in Table 4. Lead compound **4** is a weak COX inhibitor with an IC_{50} COX-1 of 16.7 μM and an IC_{50} COX-2 >40 μM. We determined the remaining COX-activity at a test concentration of 10 μM (Table 4). None of the presented compounds reduced the remaining activity lower than 60% for both COX-1 and -2. The most active GSMs **16**–**19** were even weaker COX inhibitors with a remaining activity above 75% for COX-1 and no inhibition of COX-2 at 10 μM (>100%).

In addition, potential effects on NOTCH processing and signaling were determined for the two most potent dual modulators **16** and **17** (Fig. 2). NOTCH is a substrate of γ-secretase and its inhibition (e.g., by nonselective γ-secretase inhibitors) causes severe side effects.[9] Inhibition of NOTCH-1 processing was tested in a cell-based reporter assay in a concentration range of 0–30 μM.[44] Importantly, NOTCH-1 reporter activity was not significantly affected by the selected compounds indicating at least an 8- to 12-fold window of selectivity.

Cytotoxicity was determined in the concentration range 10–100 μM using alamarBlue reagent (Fig. 3). None of the presented compounds displayed detectable cytotoxicity up to a concentration of 40 μM.

2.5. Discussion

In this study, we have investigated the SAR of the central part of our previously identified dual γ-secretase/PPARγ modulator **4**.[23] By replacement of the central pyrimidine with benzene, we were able to systematically vary the substitution pattern of the central aromatic ring. Shifting the ether moieties around the benzene led to a set of molecules with diverse biological activities: While compound **7** (3,5-substituted) and **14** (2,5-substituted) displayed a micromolar active dual γ-secretase/PPARγ modulation profile, the 2,3- and 3,4-substituted compounds (**11** and **12**) were the most potent PPARγ agonists, but inactive on γ-secretase. Interestingly, we obtained the first inverse GSM (**13**) containing a carboxylic acid group by introduction of a 2,4-substitution pattern.

The most potent GSM **18** with an IC_{50} of 1.2 μM is characterized by a 2,5-di-*p*-trifluoromethylbenzyl substitution pattern, whereas the most potent dual γ-secretase/PPARγ modulators contain a 2,5-dicyclohexylethoxy-substitution (**16**) and a 2,5-di-*p*-trifluoromethylphenethoxy-substitution (**17**).

Table 4
COX inhibition of compounds **7**–**22** (remaining activity at 10 μM ± SE) as described in Hieke et al.[39]

Compound	COX-1 inhibition (r.a.@10 μM)	COX-2 inhibition (r.a.@10 μM)	Compound	COX-1 inhibition (r.a.@10 μM)	COX-2 inhibition (r.a.@10 μM)
7	78.7 ± 12.9	78.4 ± 11.9	**15**	68.0 ± 12.1	94.4 ± 10.7
8	81.4 ± 8.5	81.4 ± 8.9	**16**	76.4 ± 7.3	123.4 ± 19.5
9	71.3 ± 2.1	109.7 ± 4.9	**17**	81.0 ± 11.8	102.4 ± 7.3
10	71.6 ± 3.7	92.5 ± 2.7	**18**	82.6 ± 4.7	102.0 ± 5.5
11	66.1 ± 9.8	89.6 ± 13.4	**19**	92.3 ± 9.2	106.1 ± 11.9
12	95.8 ± 21.7	86.4 ± 11.8	**20**	73.8 ± 15.1	79.1 ± 13.2
13	99.1 ± 12.1	110.3 ± 5.8	**21**	69.1 ± 6.9	91.5 ± 5.7
14	63.8 ± 16.6	66.5 ± 11.0	**22**	82.0 ± 11.7	96.6 ± 16.4

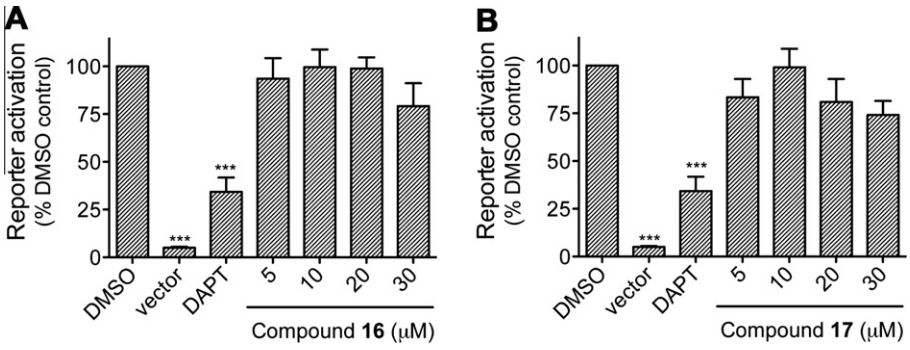


Figure 2. Impact on NOTCH-processing.

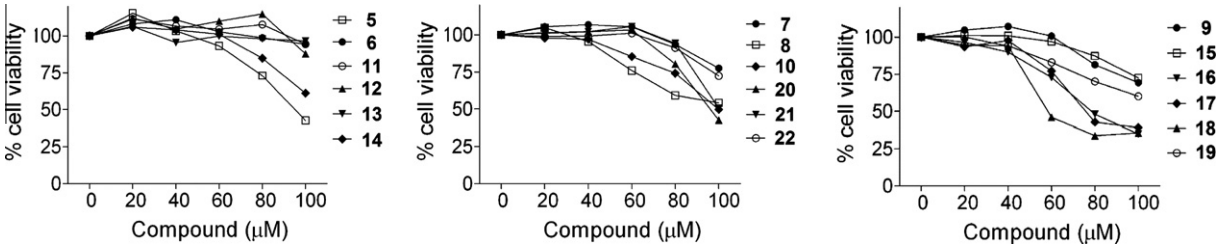


Figure 3. Cytotoxicity.

In summary, we have explored broad SAR and obtained selective GSMs (such as **18**) as well as dual γ-secretase/PPARγ modulators (such as **16** and **17**), both with low micromolar activity. Compared to our previously reported compounds, the off-target selectivity towards COX was clearly improved. With their balanced low micromolar dual γ-secretase/PPARγ activity, compounds such as **16** and **17** could be valuable tools to further investigate the concept of dual γ-secretase/PPARγ modulators in AD animal models.

3. Experimental

3.1. Compounds and chemistry

The structures of all tested compounds were confirmed by ^{1}H NMR, ^{13}C NMR and mass spectrometry (ESI). Compounds **7–19** were described previously,[39] analytical data of compounds **20–22** (and their intermediates) are presented herein. The purities of the tested compounds were determined by combustion analysis and are 98% 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 or an AV 200 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 an Elemental Vario Micro Cube. The synthetic procedure for compounds **7–22** is illustrated in Schemes 1–3.

3.1.1. Analytical characterization of compounds 20–22

3.1.1.1. Intermediates of 20. *Characterization of 3,4-bis(2-cyclohexylethoxy)benzaldehyde* (**20C**): ^{1}H NMR (300.13 MHz, $(CD_3)_2SO$): δ = 0.87–0.96 (m, 4H, Cyclohex-H), 1.13–1.20 (m, 6H, Cyclohex-H), 1.25–1.34 (m, 2H, Cyclohex-H), 1.58–1.77 (m, 16H, Cyclohex-H + –CH_2), 4.01–4.11 (m, 4H, Ph-O–CH_2), 7.13–7.16 (d, 1H, Ph-C_5), 7.36–7.37 (d, 1H, Ph-C_2), 7.48–7.52 (dd, 1H, Ph-C_6), 9.80 (s, 1H, CHO). ^{13}C NMR (75.44 MHz, $(CD_3)_2SO$): δ = 25.74 (Cyclohex-$C_{3/5}$), 26.02 (Cyclohex-C_4), 32.65 (Cyclohex-$C_{2/6}$), 34.09 (Cyclohex-CH_2), 66.45 + 66.54 (Ph-O–CH_2), 111.37 (Ph-C_5), 112.40 (Ph-C_2), 125.76 (Ph-C_6), 129.52 (Ph-C_1), 148.90 (Ph-C_3), 154.01 (Ph-C_4), 191.25 (CHO). MS (ESI^+) = *m*/*e* = 359.0 $[M+1]^+$.

*Characterization of ethyl 2-(3,4-bis(2-cyclohexylethoxy)benzylidene)hexanoate (***20D***):* ^{1}H NMR (300.13 MHz, $(CD_3)_2SO$): δ = 0.84–1.70 (m, 38H, Cyclohex-H + –CH_2, Bu-CH_3, Bu-CH_2 + Et-CH_3), 2.47–2.50 (m, 2H, Bu-CH_2), 3.95–4.02 (m, 4H, Ph-O–CH_2), 4.06–4.20 (m, 4H, O–CH_2), 6.96–7.02 (m, 3H, Ph-$C_{2/5/6}$), 7.49 (s, 1H, Ph-CH). ^{13}C NMR (75.44 MHz, $(CD_3)_2SO$): δ = 13.68 (Bu-CH_3), 21.46 (Bu-CH_2), 25.77 (4C, Cyclohex-$C_{3/5}$), 26.04 (2C, Cyclohex-C_4), 26.89 (Bu-CH_2), 30.77 (Bu-CH_2), 32.70 (4C, Cyclohex-$C_{2/6}$), 34.76 (2C, Cyclohex-CH_2), 36.16 (2C, Cyclohex-C_1), 60.23 (O–CH_2), 66.36 + 66.49 (2C, Ph-O–CH_2), 113.36 (Ph-C_2), 114.69 (Ph-C_5), 121.63 (Ph-C_6), 127.63 (Ph-C_1), 130.72 (C═C–COO), 138.07 (Ph-C═C), 148.22 (Ph-C_3), 149.21 (Ph-C_4), 167.68 (COO–). MS (ESI^+) = *m*/*e* = 485.8 $[M+1]^+$.

*Characterization of ethyl 2-(3,4-bis(2-cyclohexylethoxy)benzyl)hexanoate (***20E***):* ^{1}H NMR (300.13 MHz, $(CD_3)_2SO$): δ = 0.79–0.84 (t, 3H, *J* = 7.1 Hz, Bu-CH_3), 0.89–1.24 (m, 15H, Et-CH_3, Bu-CH_2, Cyclohex-H), 1.38–1.77 (m, 20H, Cyclohex-H + –CH_2, Bu-CH_2), 2.53–2.73 (m, 2H, Prop-C_2H + C_3H), 3.39–3.46 (m, 1H, Prop-C_3H), 3.87–4.00 (m, 6H, Ph-O–CH_2 + O–CH_2), 6.59–6.62 (dd, 1H, *J* = 1.6; 8.1 Hz, Ph-C_6), 6.72–6.73 (d, 1H, *J* = 1.6 Hz, Ph-C_2), 6.79–6.81 (d, 1H, *J* = 8.1 Hz, Ph-C_5). ^{13}C NMR (75.44 MHz, $(CD_3)_2SO$): δ = 13.73 (Bu-CH_3), 14.04 (Et-CH_3), 21.96 (Bu-CH_2), 25.76 (4C, Cyclohex-$C_{3/5}H_2$), 26.04 (2C, Cyclohex-C_4H_2), 28.83 (Bu-CH_2), 31.27 (Bu-CH_2), 32.71 (4C, Cyclohex-$C_{2/6}H_2$), 34.03 (2C, Cyclohex-CH_2), 36.20 (2C, Cyclohex-C_1H), 37.44 (Prop-C_3), 46.98 (Prop-C_2), 59.47 (O–CH_2), 66.27 + 66.43 (2C, Ph-O–CH_2), 113.85 (Ph-C_2), 114.57 (Ph-C_5), 120.91 (Ph-C_6), 131.86 (Ph-C_1), 146.97 (Ph-C_4), 148.29 (Ph-C_3), 174.78 (COO–). MS (ESI^+) = *m*/*e* = 487.7 $[M+1]^+$.

Characterization of final product 2-(3,4-bis(2-cyclohexylethoxy)benzyl)hexanoic acid **20**: ^{1}H NMR (300.13 MHz, $(CD_3)_2SO$): δ = 0.79–0.99 (m, 7H, Bu-CH_3 + Bu-CH_2), 1.05–1.75 (m, 28H, Bu-CH_2 + Cyclohex-H + –CH_2), 2.44–2.75 (m, 3H, Prop-C_3H_2 + C_2H), 3.88–3.98 (q, 4H, *J* = 6.2 Hz, Ph-O–CH_2), 6.61–6.64 (dd, 1H, *J* = 1.8; 8.1 Hz, Ph-C_6), 6.75–6.81 (m, 2H, Ph-$C_{2/5}$), 12.05 (br s, 1H, COOH). ^{13}C NMR (75.44 MHz, $(CD_3)_2SO$): δ = 13.78 (Bu-CH_3), 22.04 (Bu-CH_2), 25.77 (4C, Cyclohex-$C_{3/5}$), 26.05 (2C, Cyclohex-C_4), 28.92 (Bu-CH_2), 31.16 (Bu-CH_2), 32.72 (4C, Cyclohex-$C_{2/6}$), 34.04 (2C, Cyclohex-CH_2), 36.24 (2C, Cyclohex-C_1), 37.31 (Prop-C_3), 46.90 (Prop-C_2), 66.28 + 66.42 (2C, Ph-O–CH_2), 113.77 (Ph-C_2), 114.63 (Ph-C_5), 120.91 (Ph-C_6), 146.92 (Ph-C_4) 148.27 (Ph-C_3), 176.43 (COO–). MS (ESI^+) = *m*/*e* = 457.6 $[M+1]^+$. Combustion Anal. Calcd $C_{29}H_{46}O_4$ [458.67]: C, 75.94; H, 10.11. Found: C, 76.07; H, 10.26. Difference: C, 0.13; H, 0.15.

3.1.1.2. Intermediates of compound 21. *Characterization of 3-hydroxy-4-(2-cyclohexylethoxy)benzaldehyde* (**21B**): ^{1}H NMR (300.13 MHz, $(CD_3)_2SO$): δ = 0.92–0.96 (m, 2H, Cyclohex-H), 1.13–1.20 (m, 3H, Cyclohex-H), 1.25–1.34 (m, 1H, Cyclohex-H), 1.58–1.77 (m, 8H, Cyclohex-H + –CH_2), 4.00–4.11 (m, 4H, Ph-O–CH_2), 7.10–7.12 (d, 1H, Ph-C_5), 7.23–7.24 (d, 1H, Ph-C_2), 7.37–7.38 (dd, 1H, Ph-C_6), 9.44 (s, 1H, –OH), 9.85 (s, 1H, CHO). ^{13}C NMR (75.44 MHz, $(CD_3)_2SO$): δ = 25.67 (Cyclohex-$C_{3/5}$), 26.01 (Cyclohex-C_4), 32.61 (Cyclohex-$C_{2/6}$), 33.80 (Cyclohex-C_1), 35.84 (Cyclohex-CH_2), 66.41 + 66.54 (Ph-O–CH_2), 112.44 (Ph-C_5), 113.55 (Ph-C_2), 124.29 (Ph-C_6), 129.65 (Ph-C_1), 147.08 (Ph-C_3), 152.73 (Ph-C_4), 191.33 (CHO). MS (ESI^-) = *m*/*e* = 246.9 $[M-1]^-$.

Characterization of 4-(2-Cyclohexylethoxy)-3-phenethoxybenzaldehyde (**21C**): ^{1}H NMR (300.13 MHz, $(CD_3)_2SO$): δ = 0.92–1.75 (m, 13H, Cyclohex-H + –CH_2), 3.02–3.06 (t, 2H, *J* = 6.5 Hz, Ph′-CH_2), 4.05–4.09 (t, 2H, *J* = 6.5 Hz, Ph-C_4–O–CH_2), 4.19–4.23 (t, 2H, *J* = 6.6 Hz, Ph-C_3–O–CH_2), 7.14–7.38 (m, 7H, Ph-$C_{2/5}$H + Ph′-H), 7.49–7.52 (dd, 1H, *J* = 1.8; 8.3 Hz, Ph-C_6), 9.79 (s, 1H, CHO). ^{13}C NMR (75,44 MHz, $(CD_3)_2SO$): δ = 25.74 (Cyclohex-$C_{3/5}$), 26.01 (Cyclohex-C_4), 32.64 (Cyclohex-$C_{2/6}$), 33.96 (Cyclohex-C_1), 34.92 (Cyclohex-CH_2), 35.90 (Ph′-CH_2), 66.49 (Ph-C_4–O–CH_2), 68.94 (Ph-C_3–O–CH_2), 111.29 (Ph-C_5), 112.37 (Ph-C_2), 125.87 (Ph-C_6), 126.22 (Ph′-C_4), 128.13 (Ph′-$C_{2/6}$), 129.07 (Ph′-$C_{3/5}$), 129.43 (Ph-C_1), 138.42 (Ph′-C_1), 148.34 (Ph-C_3), 153.86 (Ph-C_4), 191.31 (CHO). MS (ESI^+) = *m*/*e* = 353.2 $[M+1]^+$.

Characterization of ethyl 2-(4-(2-cyclohexylethoxy)-3-phenethoxybenzylidene)hexanoate (**21D**): ^{1}H NMR (300.13 MHz, $(CD_3)_2SO$): δ = 0.80–0.85 (t, 3H, *J* = 7.0 Hz, Bu-CH_3), 0.91–1.32 (m, 10H, Et-CH_3 + Bu-CH_2 + Cyclohex-H), 1.41–1.75 (m, 10H, Cyclohex-H + –CH_2), 2.43–2.50 (m, 1H, Bu-CH_2), 3.00–3.04 (t, 2H, *J* = 6.7 Hz, Ph′-CH_2), 3.97–4.01 (m, 2H, Ph-C_4–O–CH_2), 4.13–4.20 (m, 4H, O–CH_2 + Ph-C_3–O–CH_2), 6.96–7.03 (m, 3H, Ph-$C_{2/5/6}$), 7.17–7.34 (m, 5H, Ph′-H), 7.48 (s, 1H, C═CH). ^{13}C NMR (75.44 MHz, $(CD_3)_2SO$): δ = 13.64 (Bu-CH_3), 14.14 (Et-CH_3), 22.36 (Bu-CH_2), 25.73 (2C, Cyclohex-$C_{3/5}$), 26.03 (Cyclohex-C_4), 26.86 (Bu-CH_2), 30.72 (Bu-CH_2), 32.67 (2C, Cyclohex-$C_{2/6}$), 33.99 (Cyclohex-CH_2), 34.76 (Ph′-CH_2), 36.09 (Cyclohex-C_1), 60.22 (O–CH_2), 66.26 (Ph-C_4–O–CH_2), 69.02 (Ph-C_3–O–CH_2), 113.25 (Ph-C_2), 114.64 (Ph-C_5), 122.93 (Ph-C_6), 126.19 (Ph-C_1), 127.55 (Ph′-C_4), 128.13 (2C, Ph′-$C_{2/6}$), 128.99 (2C, Ph′-$C_{3/5}$), 130.69 (C═C–COO), 138.02 (Ph-C═C), 138.44 (Ph′-C_1), 147.66 (Ph-C_3), 149.05 (Ph-C_4), 169.01 (COO–). MS (ESI^+) = *m*/*e* = 479.5 $[M+1]^+$.

Characterization of ethyl 2-(4-(2-cyclohexylethoxy)-3-phenethoxybenzyl)hexanoate (**21E**): ^{1}H NMR (300.13 MHz, $(CD_3)_2SO$): δ = 0.78–0.83 (t, 3H, J = 7.1 Hz, Bu-CH_3), 0.86–0.97 (m, 2H, Bu-CH_2), 1.01–1.06 (t, 3H, J = 7.0 Hz, Et-CH_3), 1.15–1.77 (m, 17H, Bu-CH_2 + Cyclohex-H + –CH_2), 2.50–2.72 (m, 3H, Prop-C_3H_2 + –C_2H), 2.97–3.01 (t, 2H, J = 6.7 Hz, Ph′-CH_2), 3.85–3.98 (m, 4H, Ph-C_4–O–CH_2 + OCH_2), 4.07–4.12 (t, 2H, J = 6.8 Hz, Ph-C_3–O–CH_2), 6.60–6.63 (dd, 1H, J = 1.9; 8.1 Hz, Ph-C_6), 6.74 (d, 1H, J = 1.9 Hz, Ph-C_2), 6.80–6.82 (d, 1H, J = 8.1 Hz, Ph-C_5), 7.17–7.34 (m, 5H, Ph′-H). ^{13}C NMR (75.44 MHz, $(CD_3)_2SO$): δ = 13.73 (Bu-CH_3), 14.04 (Et-CH_3), 21.96 (Bu-CH_2), 25.73 (4C, Cyclohex-$C_{3/5}$), 26.04 (2C, Cyclohex-C_4), 28.82 (Bu-CH_2), 31.30 (Bu-CH_2), 32.70 (4C, Cyclohex-$C_{2/6}$), 33.96 (2C, Cyclohex-C_1), 35.13 (2C, Cyclohex-CH_2), 36.27 (Ph′-CH_2), 37.41 (Prop-C_3), 46.97 (Prop-C_2), 59.47 (O–CH_2), 66.40 (Ph-C_4–O–CH_2), 69.04 (Ph-C_3-O–CH_2), 113.80 (Ph-C_2), 114.52 (Ph-C_5), 121.09 (Ph-C_6), 126.16 (Ph′-C_4), 128.13 (2C, Ph′-$C_{2/6}$), 128.90 (2C, Ph′-$C_{3/5}$), 131.86 (Ph-C_1) 138.53 (Ph′-C_1), 146.88 (Ph-C_4), 147.97 (Ph-C_3), 174.79 (COO–). MS (ESI$^+$) = m/e = 481.5 [M+1]$^+$.

Characterization of final product 2-(4-(2-cyclohexylethoxy)-3-phenethoxybenzyl) hexanoic acid **21**: ^{1}H NMR (300.13 MHz, $(CD_3)_2SO$): δ = 0.79–0.83 (t, 3H, J = 7.0 Hz, Bu-CH_3), 0.85–1.30 (m, 9H, Bu-CH_2 + Cyclohex-H), 1.34–1.73 (m, 10H, Cyclohex-H + CH_2 + Bu-CH_3), 2.45–2.59 (m, 2H, Prop-C_2H + C_3H), 2.66–2.74 (dd, 1H, J = 5.0; 8.4 Hz, Prop-C_3H), 2.96–3.01 (t, 2H, J = 6.7 Hz, Ph′-CH_2), 3.86–3.90 (t, 2H, J = 6.4 Hz, Ph-C_4–O–CH_2), 4.08–4.12 (t, 2H, J = 6.8 Hz, Ph-C_3–O–CH_2), 6.62–6.66 (dd, 1H, J = 1.8; 8.1 Hz, Ph-C_6), 6.76 (d, 1H, J = 1.8 Hz, Ph-C_2), 6.79–6.82 (d, 1H, J = 8.2 Hz, Ph-C_5), 7.18–7.34 (m, 5H, Ph′-H), 12.05 (br s, 1H, COOH). ^{13}C NMR (75.44 MHz, $(CD_3)_2SO$): δ = 13.77 (Bu-CH_3), 22.03 (Bu-CH_2), 25.74 (2C, Cyclohex-$C_{3/5}$), 26.04 (Cyclohex-C_4), 28.92 (Bu-CH_2), 31.20 (Bu-CH_2), 32.71 (2C, Cyclohex-$C_{2/6}$), 33.97 (Cyclohex-CH_2), 35.16 (Ph′-CH_2), 36.31 (Cyclohex-C_1), 37.31 (Prop-C_3), 46.94 (Prop-C_2), 66.39 (Ph-C_4–O–CH_2), 69.02 (Ph-C_3–O–CH_2), 113.75 (Ph-C_2), 114.58 (Ph-C_5), 121.10 (Ph-C_6), 126.16 (Ph′-C_4), 128.13 (2C, Ph′-$C_{2/6}$), 129.02 (2C, Ph′-$C_{3/5}$), 132.26 (Ph-C_1), 138.54 (Ph′-C_1), 146.83 (Ph-C_4) 147.93 (Ph-C_3), 176.42 (COO–). MS (ESI$^-$) = m/e = 451.3 [M−1]$^-$. Combustion Anal. Calcd $C_{29}H_{40}O_4$ [452.29]: C, 76.95; H, 8.91. Found: C, 77.01; H, 8.95. Difference: C, 0.06; H, 0.04.

3.1.1.3. Intermediates of compound 22. *Characterization of 3-hydroxy-4-phenethoxybenzaldehyde* (**22B**): ^{1}H NMR (300.13 MHz, $(CD_3)_2SO$): δ = 3.05–3.10 (t, 2H, J = 7.0 Hz, Ph′-CH_2), 4.24–4.29 (t, 2H, J = 7.0 Hz, Ph-O–CH_2), 7.12–7.38 (m, 8H, Ph′-H + Ph-$C_{2/5/6}$H), 9.55 (br s, 1H, Ph-OH), 9.79 (s, 1H, CHO). ^{13}C NMR (75.44 MHz, $(CD_3)_2SO$): δ = 34.83 (Ph′-CH_2), 68.99 (Ph-O–CH_2), 112.58 (Ph-C_5), 113.54 (Ph-C_2), 124.34 (Ph-C_6), 126.31 (Ph′-C_4), 128.27 (Ph′-$C_{2/6}$), 129.02 (Ph′-$C_{3/5}$), 129.79 (Ph-C_1), 138.31 (Ph′-C_1), 147.07 (Ph-C_3), 152.45 (Ph-C_4), 191.42 (CHO). MS (ESI$^-$) = m/e = 240.9 [M−1]$^-$.

Characterization of 3-(2-cyclohexylethoxy)-4-phenethoxybenzaldehyde (**22C**): ^{1}H NMR (300.13 MHz, $(CD_3)_2SO$): δ = 0.92–1.76 (m, 13H, Cyclohex-H + –CH_2), 3.03–3.07 (t, 2H, J = 6.6 Hz, Ph′-CH_2), 3.99–4.03 (t, 2H, J = 6.5 Hz, Ph-C_3–O–CH_2), 4.24–4.29 (t, 2H, J = 6.6 Hz, Ph-C_4–O–CH_2), 7.14–7.38 (m, 7H, Ph-$C_{2/5}$H + Ph′-H), 7.48–7.51 (dd, 1H, J = 1.8, 8.3 Hz, Ph-C_6), 9.80 (s, 1H, CHO). ^{13}C NMR (75.44 MHz, $(CD_3)_2SO$): δ = 25.72 (Cyclohex-$C_{3/5}$), 26.02 (Cyclohex-C_4), 32.68 (Cyclohex-$C_{2/6}$), 33.98 (Cyclohex-C_1), 34.82 (Ph′-CH_2), 36.03 (Cyclohex-C_1H), 66.37 (Ph-C_3–O–CH_2), 69.03 (Ph-C_4–O–CH_2), 111.26 (Ph-C_5), 112.39 (Ph-C_2), 125.75 (Ph-C_6), 126.28 (Ph′-C_4), 128.15 (Ph′-$C_{2/6}$), 129.10 (Ph′-$C_{3/5}$), 129.63 (Ph-C_1), 138.25 (Ph′-C_1), 148.60 (Ph-C_3), 153.63 (Ph-C_4), 191.34 (CHO). MS (ESI$^+$) = m/e = 353.3 [M+1].

Characterization of ethyl 2-(3-(2-cyclohexylethoxy)-4-phenethoxybenzylidene)hexanoate (**22D**): ^{1}H NMR (300.13 MHz, $(CD_3)_2SO$): δ = 0.84–0.90 (t, 3H, J = 7.1 Hz, Bu-CH_3), 0.94–1.74 (m, 20H, Et-CH_3 + Bu-CH_2 + Cyclohex-CH + –CH_2), 2.47 (m, 2H, Bu-CH_2), 2.99–3.04 (t, 2H, J = 6.5 Hz, Ph′-CH_2), 3.92–3.97 (t, 2H, J = 6.6 Hz, Ph-C_3-O–CH_2), 4.13–4.20 (m, 4H, O–CH_2 + Ph-C_4-O–CH_2), 6.95–7.04 (m, 3H, Ph-$C_{2/5/6}$), 7.18–7.35 (m, 5H, Ph′-H), 7.49 (s, 1H, C=CH). ^{13}C NMR (75.44 MHz, $(CD_3)_2SO$): δ = 13.66 (Bu-CH_3), 13.88 (Et-CH_3), 21.44 (Bu-CH_2), 25.72 (2C, Cyclohex-$C_{3/5}$), 26.29 (Cyclohex-C_4), 26.77 (Bu-CH_2), 30.75 (Bu-CH_2), 32.67 (2C, Cyclohex-$C_{2/6}$), 34.05 (Cyclohex-CH_2), 34.73 (Ph′-CH_2), 36.15 (Cyclohex-C_1), 60.21 (O–CH_2), 66.47 (Ph-C_3–O–CH_2), 68.91 (Ph-C_4–O–CH_2), 113.36 (Ph-C_2), 114.67 (Ph-C_5), 122.84 (Ph-C_6), 126.18 (Ph-C_1), 127.80 (Ph′-C_4), 128.11 (2C, Ph′-$C_{2/6}$), 129.01 (2C, Ph′-$C_{3/5}$), 130.81 (C=C–COO), 137.99 (Ph-C=C), 138.43 (Ph′-C_1), 148.85 (Ph-C_4), 148.85 (Ph-C_3), 167.64 (COO−). MS (ESI$^+$) = m/e = 479.4 [M+1]$^+$.

Characterization of ethyl 2-(3-(2-cyclohexylethoxy)-4-phenethoxybenzyl)hexanoate (**22E**): ^{1}H NMR (300.13 MHz, $(CD_3)_2SO$): δ = 0.79–0.83 (t, 3H, J = 7.1 Hz, Bu-CH_3), 0.87–1.73 (m, 23H, Bu-CH_2, Et-CH_3, Cyclohex-H + –CH_2), 2.52–2.73 (m, 3H, Prop-C_3H_2 + –C_2H), 2.95–2.99 (t, 2H, J = 6.7 Hz, Ph′-CH_2), 3.87–3.99 (m, 4H, Ph-C_3–O–CH_2 + OCH_2), 4.06–4.11 (t, 2H, J = 6.8 Hz, Ph-C_4–O–CH_2), 6.59–6.62 (dd, 1H, J = 1.8; 8.2 Hz, Ph-C_6), 6.73 (d, 1H, J = 1.8 Hz, Ph-C_2), 6.80–6.83 (d, 1H, J = 8.1 Hz, Ph-C_5), 7.17–7.33 (m, 5H, Ph′-H). ^{13}C NMR (75.44 MHz, $(CD_3)_2SO$): δ = 13.73 (Bu-CH_3), 14.05 (Et-CH_3), 21.96 (Bu-CH_2), 25.73 (2C, Cyclohex-$C_{3/5}$), 26.04 (Cyclohex-C_4), 28.82 (Bu-CH_2), 31.27 (Bu-CH_2), 32.69 (2C, Cyclohex-$C_{2/6}$), 33.96 (Cyclohex-C_1), 35.17 (Cyclohex-CH_2), 36.21 (Ph′-CH_2), 37.42 (Prop-C_3), 46.95 (Prop-C_2), 59.48 (O–CH_2), 66.25 (Ph-C_3–O–CH_2), 69.11 (Ph-C_4–O–CH_2), 113.77 (Ph-C_2), 114.53 (Ph-C_5), 120.89 (Ph-C_6), 126.13 (Ph′-C_4), 128.11 (2C, Ph′-$C_{2/6}$), 128.99 (2C, Ph′-$C_{3/5}$), 132.05 (Ph-C_1), 138.58 (Ph′-C_1), 146.65 (Ph-C_4), 148.20 (Ph-C_3), 174.78 (COO–). MS (ESI$^+$) = m/e = 481.5 [M+1]$^+$.

Characterization of 2-(3-(2-cyclohexylethoxy)-4-phenethoxybenzyl)hexanoic acid **22**: ^{1}H NMR (300.13 MHz, $(CD_3)_2SO$): δ = 0.79–0.85 (t, 3H, J = 7.0 Hz, Bu-CH_3), 0.91–1.74 (m, 20H, Bu-CH_2, Cyclohex-H + –CH_2), 2.47–2.71 (m, 3H, Prop-C_3H_2 + –C_2H), 2.96–3.00 (t, 2H, J = 6.6 Hz, Ph′-CH_2), 3.87–3.91 (t, 2H, Ph-C_3–O–CH_2), 4.07–4.11 (t, 2H, J = 6.7 Hz, Ph-C_4–O–CH_2), 6.61–6.64 (dd, 1H, J = 1.8; 8.1 Hz, Ph-C_6), 6.76 (d, 1H, J = 1.8 Hz, Ph-C_2), 6.80–6.83 (d, 1H, J = 8.2 Hz, Ph-C_5), 7.20–7.34 (m, 5H, Ph′-H), 12.06 (br s, 1H, COOH). ^{13}C NMR (75.44 MHz, $(CD_3)_2SO$): δ = 13.77 (Bu-CH_3), 22.04 (Bu-CH_2), 25.74 (2C, Cyclohex-$C_{3/5}$), 26.06 (Cyclohex-C_4), 28.91 (Bu-CH_2), 31.15 (Bu-CH_2), 32.72 (2C, Cyclohex-$C_{2/6}$), 34.00 (Cyclohex-C_1), 35.23 (Cyclohex-CH_2), 36.27 (Ph′-CH_2), 37.31 (Prop-C_3), 46.88 (Prop-C_2), 66.36 (Ph-C_3–O–CH_2), 69.19 (Ph-C_4–O–CH_2), 113.87 (Ph-C_2), 114.73 (Ph-C_5), 120.94 (Ph-C_6), 126.14 (Ph′-C_4), 128.13 (2C, Ph′-$C_{2/6}$), 128.99 (2C, Ph′-$C_{3/5}$), 132.53 (Ph-C_1) 138.62 (Ph′-C_1), 146.66 (Ph-C_4), 148.26 (Ph-C_3), 176.40 (COOH). MS (ESI$^+$) = m/e = 453.5 [M+1]$^+$. Combustion Anal. Calcd $C_{29}H_{40}O_4$ [452.29]: C, 76.95; H, 8.91. Found: C, 76.69; H, 8.87. Difference: C, 0.26; H, 0.04.

3.2. Determination of γ-secretase modulation

To characterize the GSM activity of novel analogs, their effects on the generation of Aβ40, Aβ42, and Aβ38 peptides were determined in a previously described cell-based ELISA.[23,45] In brief, CHO cells stably overexpressing wild type human amyloid precursor protein and wild type human presenilin-1 were maintained in DMEM supplemented with 10% FBS, and treated in 96-well plates for 24 h with increasing concentrations of respective compounds or DMSO as 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. 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. 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 three times with PBS containing 0.05% Tween-20 and once with PBS. Fifty microlitre TMB ultra substrate (Thermo Fisher Scientific) was added, incubated for 1–10 min at RT in the dark, and the reaction was stopped by adding 50 μl 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 a concentration of 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 2 to 4 independent experiments. Statistics were performed using GraphPad Prism Version 5 (GraphPad Software).

3.3. 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 LipofectamineTM 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. Five hours after transfection, 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, cells were assayed for reporter gene activity using the Dual-GloTM 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 through division of the firefly luciferase data by the corresponding renilla luciferase values resulting in relative light units. Activation factors were obtained by dividing through the DMSO control values. EC_{50} and standard deviation values were calculated with the mean values of at least three determinations by SigmaPlot 2001 (Systat Software GmbH) using 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). Pioglitazone showed a 6.2 ± 1-fold activation at 1 μM and its EC_{50} was determined as 0.30 ± 0.05 μM.

3.4. NOTCH reporter assay

The NOTCH reporter assay was performed as described.[23,44] In brief, subconfluent CHO cells were transiently transfected in 96-well plates with plasmid pCDNA3-Notch-ΔE-GVP encoding truncated NOTCH-1 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. Five nanogram of plasmid pRL-TK encoding renilla luciferase was added to the plasmid mix to control for transfection efficiency. Twenty-four hours after the transfection, cells were treated for an additional 24 h with increasing concentrations of the compounds **16** and **17**, 5 μM of the γ-secretase inhibitor DAPT or DMSO as 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. Normalization for transfection efficacy and cell growth was achieved through division of the firefly luciferase values by the corresponding renilla luciferase values. Percent activation of the reporter was then calculated by normalization of triplicate measurements from each condition to the DMSO control values. Results from three independent experiments were analyzed by one-way ANOVA with Dunnett's posttests using GraphPad Prism. $^{***}p$ <0.001.

3.5. Determination of COX inhibition

Inhibition of the activities of isolated ovine COX-1 and human recombinant COX-2 was performed as described in Hieke et al..[23,39] Briefly, purified COX-1 (ovine, 50 units) or COX-2 (human recombinant, 20 units) were diluted in 1 ml reaction mixture containing 100 mM Tris buffer pH 8, 5 mM glutathione, 5 μM hemoglobin, and 100 μM EDTA at 4 °C and pre-incubated with the test compounds for 5 min. Samples were pre-warmed 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.

3.6. Cytotoxicity assay

CHO cells stably overexpressing 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 duplicates with increasing concentrations (0–100 μM) of the respective compounds or DMSO as vehicle for an additional 24 h. Cell viability was assessed using the alamarBlue® reagent (Invitrogen). Twenty microlitre alamarBlue® was added to cells cultured in 200 μl medium and incubated overnight. Absorbance was measured with a Paradigm™ microplate reader at 570 nm, using 600 nm as the reference wavelength. Percent viability of vehicle control was calculated from two independent experiments.

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