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NSAID-derived γ-secretase modulation requires an acidic moiety on the carbazole scaffold

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ABSTRACT

Modulation of γ-secretase activity holds potential for the treatment of Alzheimer's disease. Most NSAID-derived γ-secretase modulators feature a carboxylic acid, which may impair blood–brain barrier permeation. The structure activity relationship of 33 carbazoles featuring diverse carboxylic acid isosteres or metabolic precursors thereof was established in a cellular amyloid secretion assay. The modulatory activity was observed for acidic moieties and metabolically labile esters only, which supports our hypothesis of an acid-lysine interaction to be relevant for this type of γ-secretase modulators.

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disease, it is a devastating illness effecting more than 20 million patients globally. The brain atrophy associated with AD is accompanied by the hallmark amyloid plaques and fibrillary tangles. The plaques consist of aggregated oligomeric amyloid-β-peptides (Aβ) of various lengths wherein $Aβ_{42}$ is more prone to aggregate than $Aβ_{40}$ or $Aβ_{38}$.[1] These Aβ-peptides are generated by sequential processing of the amyloid precursor protein (APP) by β- and γ-secretase. The γ-secretase complex catalyzes the critical step in the liberation of these Aβ isoforms, and is thus a promising target in the prevention of AD. The membrane located γ-secretase-complex consists of four proteins: nicastrin (Nct), anterior-pharynx defective-1 (Aph-1), presenilin enhancer (Pen-2) and presenilin (PS1 or PS2).[2] PS1 is a nine transmembrane domain (TMD) protein which bears two catalytic aspartates Asp^{257} and Asp^{385} in the transmembrane domains 6 and 7.[3–5] These catalytic domains cleave APP within the membrane, implicating an unusual regulated intramembrane proteolysis (RIP) in the lipophilic membrane.[6–8]

Several inhibitors for this process have been reported recently.[7] However, most of these inhibit the cleavage of other γ-secretase substrates such as Notch, which is responsible for cell proliferation. Some NSAIDs (non-steroidal anti-inflammatory drugs) show a partial inhibition, the so called modulation of the γ-secretase cleavage, which is characterized by increased $Aβ_{38}$ secretion and decreased $Aβ_{42}$ secretion.[9] Photoaffinity labeling experiments with an *R*-flurbiprofen derivative suggest a binding site for NSAID-derived γ-secretase modulators directly on the substrate APP close to the GxxxG region,[10,11] which is responsible for substrate dimerization and may control the processing by γ-secretase.[18] We suggested an interaction of the NSAID's carboxylic acid with a basic amino acid, for example, lysine[624] on APP, which is located in the vicinity of the GxxxG motif, at the membrane interface.[12] We reported on lipophilic *N*-alkylated carprofen-derivatives and *N*-sulfonylated or *N*-alkylated carbazolyloxyacetic acids which are structurally related to the substrate targeting γ-secretase modulator flurbiprofen (sGSMs).[13,14] The *N*-alkyl chain strongly enhances the modulator activity; this may be partially due to modulator orientation via a membrane anchoring effect (Scheme 1). The structural differences between acidic NSAID derived GSMs and the basic GSMs[17] stimulated us to explore the structure activity relationship of the carboxylic acid. This acid may interact with the substrate and thereby influence the positioning of the substrate in relation to PS1, which has been shown to be crucial for the cleavage event.[19]

2. Methods and results

Herein we report the replacement of the carboxylic acid on carprofen-derived GSMs **2** (IC_{50} ($Aβ_{38}$) = not determined at max concn 100 μM, IC_{50} ($Aβ_{40}$) >40 μM, IC_{50} ($Aβ_{42}$) = 6.9 μM) and **3** (EC_{50} ($Aβ_{38}$) = 24 μM, IC_{50} ($Aβ_{40}$) >40 μM, IC_{50} ($Aβ_{42}$) = 19 μM) by carboxylic acid isosteres and carboxylic acid derivatives. This

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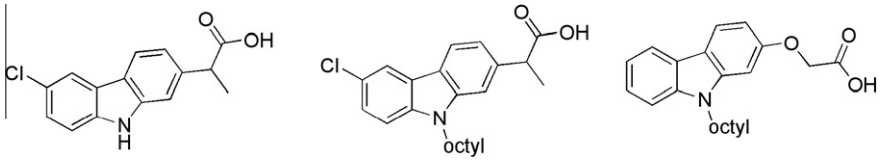


1 Carprofen **2 BSc3039** **3 BSc3030**

Scheme 1. Carprofen (1)[9] is the GSM-lead structure of **2** and **3**.[14]

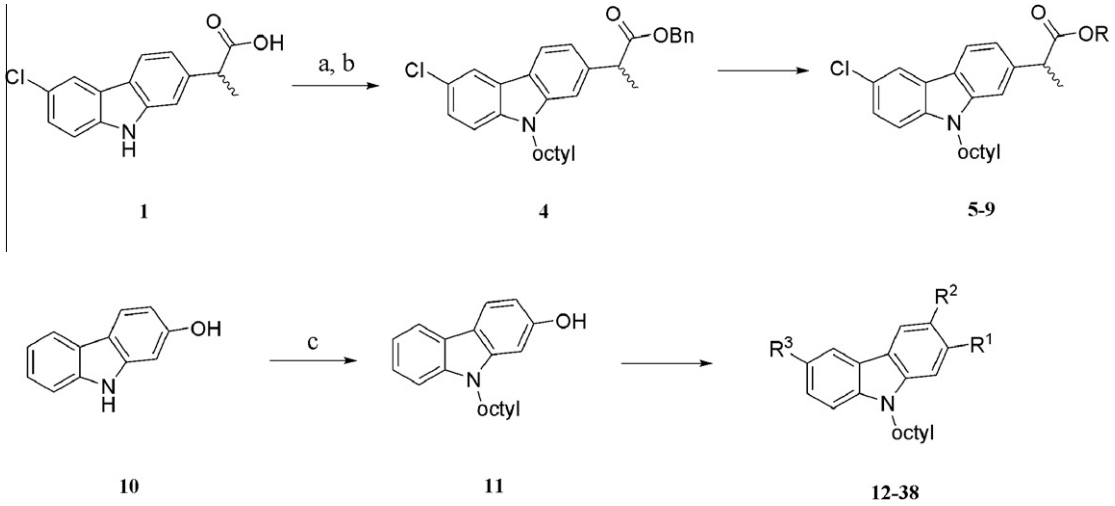
replacement aimed to reduce the topological polar surface area and the amphiphilicity of the lipophilic acids. This was expected to effect the blood–brain barrier (BBB) permeation.[13,14] Carboxylic acid isosteres require related environments as the carboxylic acid for target binding and thus some of them were expected to display γ-secretase modulatory effects. We chose several established carboxylic acid isosteres such as tetrazole, sulfonic acid, amides, sulfone amides and tetronates. We included alcohols, nitriles and amines as controls to obtain either straight or inverse γ-secretase modulators. In addition, we explored the modulator binding site by the introduction of sterically demanding groups. The synthesis was carried out according to Scheme 2 (explicit synthesis: see Section 3 and Supplementary data). The amphiphilicity of these alkylated acids may result in micelle formation at higher concentration, therefore the dose response evaluation was limited to a max concn of 100 μM. This max concn excluded the IC_{50}/EC_{50} determination of less active compounds. However, the shape of the dose response (see Supplementary data for examples) allowed us to classify the compounds as reported in 2006.[14] We observed four different modes of actions in our cellular assay: γ-secretase modulation (increase of $A\beta_{38}$, decrease of $A\beta_{42}$), inverse γ-secretase modulation (decrease of $A\beta_{38}$, increase of $A\beta_{42}$), γ-secretase inhibition (decrease of $A\beta_{38}$, decrease of $A\beta_{42}$) and lack of activity.

We kept the length of the supposed lipid anchor at eight carbon atoms, making a compromise between lipophilicity and activity, although this anchor does not provide the most active sGSM. The side chain of **3** (BSc3030) bears a methylene spacer, which is an important structural feature as an additional methylene diminished activity. This effect was observed for the carboxylic acid **17** and to a lesser extent for the sulfonic acid **18**. Surprisingly, the dimethylation (**29**) diminished activity, although moderately potent γ-secretase activity modulating fenofibrate-derivatives have been reported.[12,17] However, the replacement by a salicylic acid (**19**, Table 1) displayed an $A\beta_{42}$level reducing effect (IC_{50} ($A\beta_{42}$) = 38 μM). The salicylic acid locks the extended carbon chain in a *Z*-configuration resulting in close positioning of the carboxylic acid. Equipotent $A\beta_{42}$level reducing activity compared to **19** was achieved by a tetronic acid (**20**) and a tetramic acid (**21**), which are rarely employed as carboxylic acid isosteres. Only a sterically more demanding tetramate (**22**) displayed a full inhibitory mode of action (Scheme 3).

On the contrary, the established isosteres: tetrazole (**24**) or the 2,2,2-trifluoroethanol (**12**) did not affect γ-secretase activity up to a concentration of 100 μM or even caused increased $A\beta_{42}$ secretion. The linear alcohol **23** and bromide **13** were inactive. However, the α-branched alcohol **8** increases $A\beta_{42}$ secretion. The amines (**25, 30–32)** did not show modulatory activity. The nitrile (**14)** caused increased $A\beta_{42}$ secretion, whereas the amide (**36**) and the acidic sulfonimide (**9)** showed reduction of the $A\beta_{42}$ level. These results indicate the necessity of an acidic moiety on this scaffold to achieve modulatory activity. This supports our hypothesis that modulators interact with a basic amine on the substrate, because all GSMs of this series exhibit a pKa value in the range of 2–5.

Additional substituents in the C-3 position of **3** revealed a tolerance for small functional groups like an aldehyde (**33**), but an additional propionic acid side chain (**37**) diminished activity (Scheme 4). These findings suggest a defined binding pocket for the modulators.

The methyl ester **15** displayed unexpected $A\beta_{42}$level reducing activity (Table 2, entry 4, IC_{50} ($A\beta_{42}$) = 27 μM), a marginal loss in activity compared to the carboxylic acid **3** with an IC_{50} for $A\beta_{42}$ of 19 μM. Further investigation confirmed the tolerance of short linear esters. However, the activity decreased with increasing length of the ester. The ethyl ester **34** showed $A\beta_{42}$ level reducing activity (IC_{50} ($A\beta_{42}$) = 31 μM), but *iso*-propyl (**35**) or *iso*-butyl ester (**26**) did not reduce $A\beta_{42}$ secretion. On the contrary, the tertiary

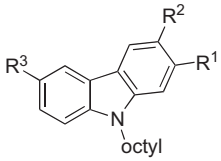


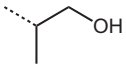
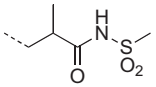
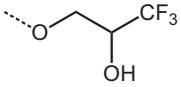
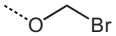
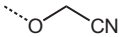
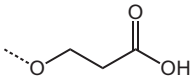
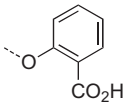
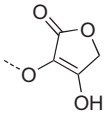
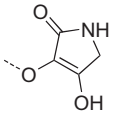
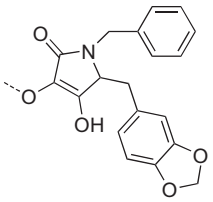
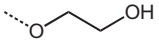
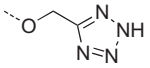
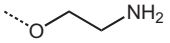
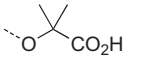
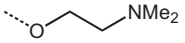
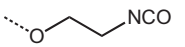
1 **4** **5-9**

10 **11** **12-38**

Scheme 2. Reagents: General synthesis of the carbazole derivatives. (a) Benzylbromide, K_2CO_3, acetone, rt; (b) octylbromide, NaH, THF, 0 °C-rt; (c) octylbromide, NaH, THF, and 0 °C-rt.

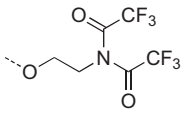
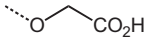
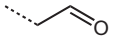
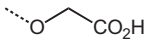
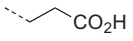
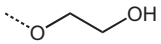
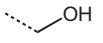
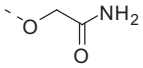
Table 1
Activity of *N*-octylcarprofen and *N*-octylcarbazole derivatives



Entry	Compd	Mode of action	R^1	R^2	R^3	IC_{50}/EC_{50} μM		
						$A\beta_{38}$	$A\beta_{40}$	$A\beta_{42}$
1	5	iGSM		H	Cl	n.d.[c]	n.d.[c]	50[a]
2	8	iGSM		H	Cl	n.d.	n.d.[c]	21[a]
3	9	GSM		H	Cl	n.d.	n.d.[c]	32[a]
4	11	GSI	–OH	H	H	n.d.[c]	n.d.[c]	>80
5	12	iGSM		H	H	n.d.[c]	n.d.[c]	12[a]
6	13	iGSM		H	H	>40	>40	>40
7	14	GSI		H	H	74	105	252[b]
8	17	GSM		H	H	>80[a]	>80	>80[b]
9	18	GSM		H	H	n.d.[c]	n.d.[c]	>80[b]
10	19	GSM		H	H	23[a]	>80	38[b]
11	20	GSM		H	H	21[a]	>80	37[b]
12	21	GSM		H	H	8[a]	72	28[b]
13	22	GSI		H	H	31	32	24
14	23	iGSM		H	H	n.d.[c]	n.d.[c]	>40
15	24	GSI		H	H	165[b]	102[b]	113[b]
16	25	iGSM		H	H	>80	>80	>80[a]
17	29	GSM		H	H	>40[a]	>40	>40
18	30	iGSM		H	H	n.d.[c]	n.d.[c]	>10[a]
19	31	GSM		H	H	n.d.[c]	n.d.[c]	>80

(*continued on next page*)

Table 1 (*continued*)

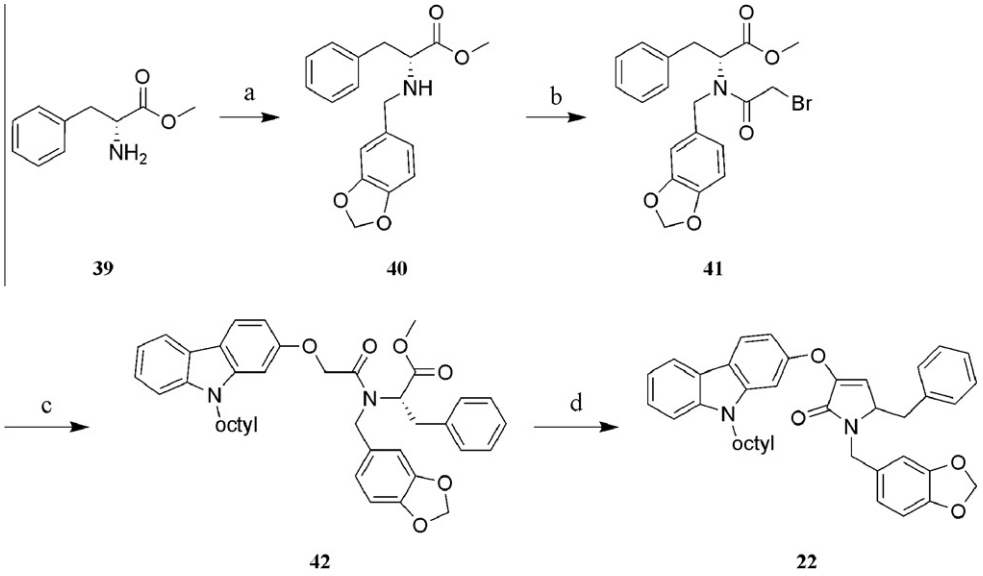
Entry	Compd	Mode of action	R^1	R^2	R^3	IC_{50}/EC_{50} μM		
						$Aβ_{38}$	$Aβ_{40}$	$Aβ_{42}$
20	32	GSI		H	H	n.d.[c]	n.d.[c]	>80
21	33	GSM			H	n.d.[c]	n.d.[c]	32
22	37	GSM			H	n.d.[c]	n.d.[c]	>80
23	38	GSI			H	n.d.[c]	n.d.[c]	80
24	36	GSI		H	H	n.d.[c]	n.d.[c]	104

Modulators (*GSM*) display increased production of $Aβ_{38}$ (EC_{50}) and reduction of $Aβ_{42}$ (IC_{50}). Inverse modulators (iGSM) display increased production of $Aβ_{42}$ (EC_{50}) and reduction of $Aβ_{38}$ (IC_{50}). γ-secretase inhibitors (GSI) reduce $Aβ_{38}$ and $Aβ_{42}$.

[a] EC_{50} values are displayed.

[b] IC_{50} values are displayed.

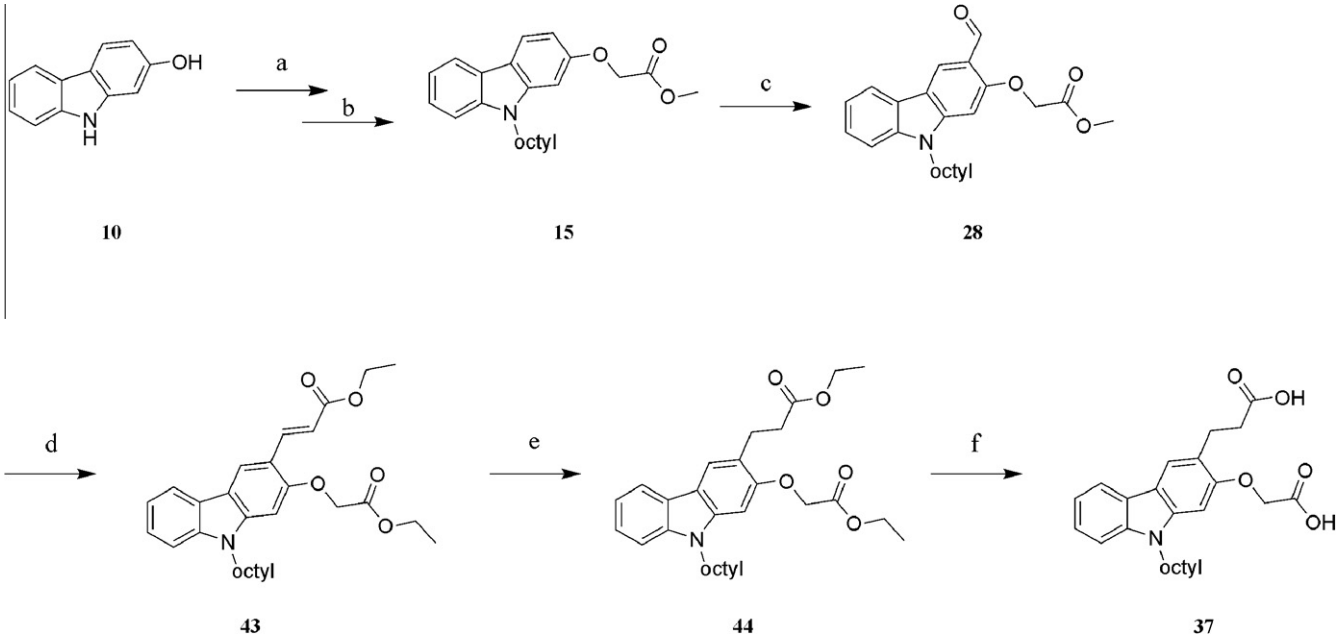
[c] Not determinable at max concn: 100 μM.



Scheme 3. Reagents: Synthesis of the higher substituted tetramate (**22**). (a) Piperonal, $Na(AcO)_3BH$, DCE, rt, 5 h; (b) bromoacetyl bromide, TEA, DCM, rt, 2,5 h; (c) 11, K_2CO_3, acetone, rt; (d) KO*t*Bu, THF, rt.

butyl ester (**16)** increased $Aβ_{42}$ levels at EC_{50} = 19 μM. Equally the phosphonic diethyl ester (**27**) caused an increased $Aβ_{42}$ secretion with an EC_{50} ($Aβ_{42}$) = 13 μM. These findings suggest a metabolic activation related to fenofibrate hydrolysis, for example, an esterase releasing the active carboxylic acid. This hydrolysis seems to tolerate short linear esters as substrates. This presumption was confirmed by investigation of the α-branched side chain of carprofen esters (**4, 6, and 7**), which inhibit or inversely modulate γ-secretase activity. Consequently, short α,α′-linear esters fulfill criteria for a fenofibrate analog prodrug system in this cellular assay. This potential prodrug system is still under investigation. Finally, an additional aldehyde in the C-3 position (**28**) of the methyl ester was not tolerated and resulted in straight γ-secretase inhibition. All attempts to hydrolyze the phosphonic ester (**27**) resulted in decomposition, excluding the resulting acid from the investigation.

The brain permeability of carboxylic acids is regarded with suspicion despite the identification of the MCT-1 transporter in human brain.[23] Therefore we decided to challenge the paradigm of poor brain permeability of such acids and selected the lead carprofen derivative **45** (Fig. 1, IC_{50} $Aβ_{42}$ = 2.9 μM, IC_{50} $Aβ_{40}$ >40 μM, IC_{50} $Aβ_{38}$ = 5.8 μM).[14] It does not interfere with γ-secretase cleavage activity at the ε-site as determined by de novo production of AICD.[14] This indicated that **45** may not exert Notch inhibition at the required physiological concentration. The ratio of the carprofen concentrations in plasma (AUC_F) versus cerebrospinal fluid (AUC_{CSF}) over time was reported for rats ($AUC_R = AUC_{CSF}$: $AUC_F = 0.23$), this relates to an $AUC_R = 5.31$ for flurbiprofen.[20] Moreover, Péhourcq et al. observed a parabolic relationship for brain permeation and the lipophilicity of deprotonated NSAIDs. This relationship stimulated us to select C-10-derivative **45** for in vivo evaluation while the SAR study of the acid derivates was ongoing. Indeed, **45** showed a slow, yet significant BBB permeation in APP_{swe} Tg mice at a single 10 mg/kg oral dosage suspended in NaCl and gelatine. The plasma and brain concentration of the modulator were monitored over a period of 5 h. The brain concentration was found to continuously increase to a plasma/brain ratio of approximately 2:1 (Fig. 1). The AUC_R <0.1 indicates reduced brain permeation versus carprofen. Moreover, the significant brain levels of the acid **45** do not imply localization of the compound at



Scheme 4. Reagents: Synthesis of C3-substituted carbazole **37**. (a) Octyl iodide, NaH, THF, DMF, −70 °C-rt, 12 h; (b) bromoacetic acid methylester, K_2CO_3, acetone, rt, 12 h; (c) *N*,*N*-diisopropylformamide, $POCl_3$, H_2O, DCE, 80 °C, 2–5 d; (d) phosphonium salt, NaOEt, EtOH, 80 °C, 20 h; (e) Pd/C, H_2, EtOH, HCl, rt, 24 h; (f) KOH, MeOH, rt/64 °C, 2 d.

the site required for modulatory activity. Furthermore, no significant $A\beta_{42}$ changes were observed at this timepoint. These unchanged Aβ levels were linked to the double transgenic mice exhibiting NSAID-resistant PS1 mutations recently.[15,16] This lack of activity in murine cell lines was reported for several first generation γ-secretase modulators and PS1 mutations both by Weggen et al.[21] and Steiner et al.[22] Thus the less potent and even more lipophilic carbazole derivatives were excluded from evaluation in this mouse model.

Our findings indicate the necessity of an acidic moiety in a pKa-range of 2–5 on this class of γ-secretase modulators. This small pKa-range supports our hypothetical interaction with lysine[624]. The location of this lysine, directly at the membrane interface close to the GxxxG dimerization motif, suggests a modulation mechanism as an APP dimerization inhibitor or impacts substrate orientation in the membrane[19], shifting the cleavage site to the less toxic $A\beta_{38}$. The optimum spacer length between the arylic backbone and carboxylic acid was determined to be 2–3 carbon or oxygen atoms. However, introduction of a rigid aryl spacer allowed an elongation of up to four atoms. We identified a potential prodrug system based on short linear esters, which complements the BBB permeation of the free acid **45**.

3. Experimental

3.1. General experimental informations

The ^1H and ^{13}C spectra were recorded on a Bruker AC 300 (300 MHz) and AC 500 (500 MHz) spectrometer. Chemical shifts are reported in δ (ppm) adjusted to the central line of the deuterated solvent (MeOD, $CDCl_3$, d_6-DMSO, Acetone-d_6). Mass spectrometry was performed on a Bruker-Franzen Esquire LC mass spectrometer (ESI) and a double focused MAT 95 (EI). HPLC analysis was performed on an Agilent 1100 system.

3.2. Purity determination

Analytical reversed phase high-performance liquid chromatography was performed on an Agilent 1100 HPLC system, equipped with an auto sampler. The purity of the final compounds was determined using UV detection (λ = 254 nm). The chromatographic method employed the following: column Zorbax Eclipse XDB-C18; 4.6 × 150 mm; mobile phase A H_2O (0.1% TFA), mobile phase B acetonitrile, flow rate 1 mL/min, gradient elution 30–100% B over 15 min. According to these methods the purities for all compounds were ⩾95% if not indicated otherwise in the experimental details.

The in vitro measurements were performed on a cellular Aβ lowering assay, in H4 APP Δ9 cells to evaluate the compounds for their potencyto modulate Aβ secretion. We used the Aβ liquid phase electrochemiluminescence (LPECL) assay to measure Aβ isoforms.[12,13] Cell viability was measured by a colorimetric cell proliferation assay (CellTiter 96TM AQ assay, Promega) utilizing the bioreduction of MTS (Owen's reagent) to formazan.

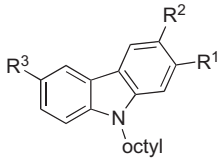
See Supplementary data for details of the in vivo mouse experiments.

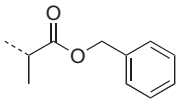
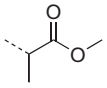
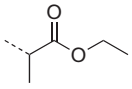
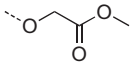
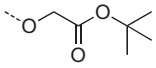
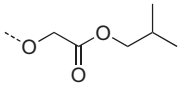
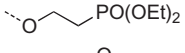
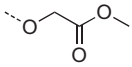
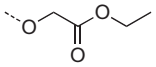
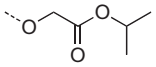
3.3. 2-Hydroxy-*N*-octylcarbazole 11

To a suspension of NaH (0.82 g, 20.48 mmol) in THF (20 mL) under argon atmosphere was added 2-hydroxycarbazole (1.50 g, 8.19 mmol) at a temperature of 0 °C. After 30 min stirring octylbromide (1.50 g, 7.78 mmol) was added and the reaction mixture was allowed to warm to rt. After 12 h the reaction mixture was quenched by drop wise addition of H_2O until gas formation ceased. The obtained mixture was extracted three times with CH_2Cl_2 (30 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, concentrated in vacuo and purified by silica gel column chromatography (gradient starting with cyclohexane to cyclohexane: CH_2Cl_2 1:2) to give 1.58 g (74%) of **11** (BSc4029) as a colorless solid.

HPLC: 9.2 min (96%), ^1H NMR (500 MHz, $CDCl_3$): δ = 7.96 (d, *J* = 8.3 Hz, 1H), 7.90 (d, *J* = 8.3 Hz, 1H), 7.36 (m, 1H), 7.34 (m, 1H), 7.19 (td, *J* = 7.0, 1.2 Hz, 1H), 6.82 (d, *J* = 2.2 Hz, 1H), 6.72 (dd, *J* = 8.3, 2.2 Hz, 1H), 4.81–5.43 (b.s., 1H), 4.18 (t, *J* = 7.5 Hz, 2H), 1.82 (q, *J* = 7.5 Hz, 2H), 1.20–1.41 (m, 10H), 0.86 (t, *J* = 6.8 Hz, 3H) ppm, ^{13}C NMR (125 MHz, $CDCl_3$): δ = 154.7, 141.9, 140.6, 124.4, 123.0, 121.2, 119.4, 118.9, 116.9, 108.4, 108.0, 95.0, 43.1, 31.8, 29.4, 29.2, 28.8, 27.3, 22.6, and 14.1 ppm, MS (*m*/*z*, 70 eV, ESI) = 318.4 [M+Na].

Table 2
Activity of *N*-octylcarbazole and *N*-octylcarprofen esters



Entry	Compd	Mode of action	R^1	R^2	R^3	IC_{50}/EC_{50} μM		
						$A\beta_{38}$	$A\beta_{40}$	$A\beta_{42}$
1	4	GSI		H	Cl	n.d.[b]	n.d.[b]	>80
2	6	iGSM		H	Cl	n.d.[b]	n.d.[b]	15[a]
3	7	iGSM		H	Cl	n.d.[b]	n.d.[b]	21[a]
4	15	GSM		H	H	n.d.[b]	n.d.[b]	27
5	16	iGSM		H	H	n.d.[b]	n.d.[b]	19[a]
6	26	GSM		H	H	n.d.[b]	n.d.[b]	>80
7	27	iGSM		H	H	n.d.[b]	n.d.[b]	13[a]
8	28	GSI			H	n.d.[b]	n.d.[b]	89
9	34	GSM		H	H	n.d.[b]	n.d.[b]	31
10	35	iGSM		H	H	n.d.[b]	n.d.[b]	49[a]

Modulators (*GSM*) display increased β production of A_{38} (EC_{50}) β and reduction of A_{42} (IC_{50}). Inverse modulators (iGSM) display increased production of ×(EC_{50} β) and reduction of A_{38} (IC_{50}). β-Secretase inhibitors (GSI) reduce $A\gamma_{38}$ β and A_{42}.

[a] EC_{50} values are displayed.

[b] Not determinable at max concn: 100 μM.

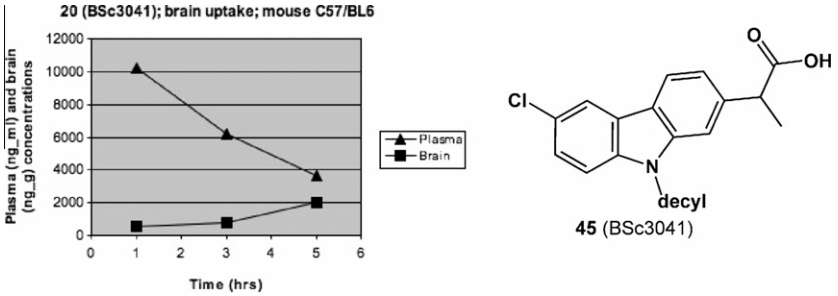


Figure 1. 45 (BSc3041) Penetrate the BBB in C57/BLg mice at an oral dosage of 10 mg/kg.

3.4. 2-(2-Bromoethoxy)-9-octyl-9H-carbazole 13

According to general procedure for ether formation (see Supplementary data): **11** (BSc4029) (0.100 g, 0.34 mmol), 1,2-dibromoethane (0.180 g, 0.97 mmol) and K_2CO_3 (0.141 g, 1.02 mmol), acetone (30 mL) yield 80 mg (51%) **13** as colorless solid.

HPLC: 10.8 min (98%), ^1H NMR (300 MHz, $CDCl_3$): δ = 8.01 (d, J = 7.7 Hz, 1H), 7.97 (d, J = 8.5 Hz, 1H), 7.44 7.35 (m, 2H),

7.24–7.19 (m, 1H), 6.90 (d, J = 2.2 Hz, 1H), 6.85 (dd, J = 8.5, 2.2 Hz, 1H), 4.43 (t, J = 6.3 Hz, 2H), 4.23 (t, J = 7.3 Hz, 2H), 3.74–3.69 (m, 2H), 1.90–1.82 (m, 2H), 1.44–1.23 (m, 10H), 0.89 (t, J = 7.0 Hz, 3H) ppm, ^{13}C NMR (75 MHz, $CDCl_3$): δ = 157.3, 141.6, 140.7, 124.6, 122.9, 121.1, 119.6, 118.9, 117.5, 109.5, 107.3, 94.8, 68.6, 43.1, 31.8, 29.4, 29.3, 29.2, 28.8, 27.3, 22.6, and 14.0 ppm.

3.5. 2-(9-Octyl-9H-carbazol-2-yloxy)-ethanesulfonic acid 18

To a solution of **13** (BSc4004) (0.020 g, 0.05 mmol) in a mixture of H_2O (0.050 mL) and dioxane (0.050 mL) was added Na_2SO_3 (0.032 g, 0.25 mmol) and the resulting mixture was heated at 160 °C under microwave irradiation until the reaction was completed (TLC control). The reaction mixture was concentrated in vacuo, the resulting solid dissolved in $EtOH_{abs}$ and concentrated in vacuo to give 0.007 g (35%) of **18** (BSc4079) as a colorless solid. HPLC: 7.0 min (93%), ^{1}H NMR (500 MHz, MeOD): δ = 7.97–7.94 (m, 1H), 7.93 (d, J = 8.5 Hz, 1H), 7.41 (d, J = 8.2 Hz, 1H), 7.37–7.30 (m, 1H), 7.18–7.10 (m, 1H), 6.84 (dd, J = 8.5, 2.2 Hz, 1H), 7.04 (d, J = 2.1 Hz, 1H), 4.32 (t, J = 7.1 Hz, 2H), 4.53–4.47 (m, 2H), 3.38–3.33 (m, 2H), 1.89–1.81 (m, 2H), 0.86 (m, 10H), 1.32–1.24 (t, J = 7.0 Hz, 3H) ppm, ^{13}C NMR (125 MHz, MeOD): δ = 143.1, 142.0, 125.3, 124.3, 121.8, 120.2, 119.8, 118.2, 109.7, 109.1, 100.5, 95.2, 65.3, 51.9, 43.7, 32.9, 30.7, 30.3, 29.9, 28.2, 23.6, and 14.4 ppm, MS (m/z, 70 eV, ESI) = 402 [M−].

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.06.062.

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