

Supporting information

Fatty acid amide hydrolase (FAAH), acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE): networked targets for the development of carbamates as potential anti Alzheimer's Disease agents

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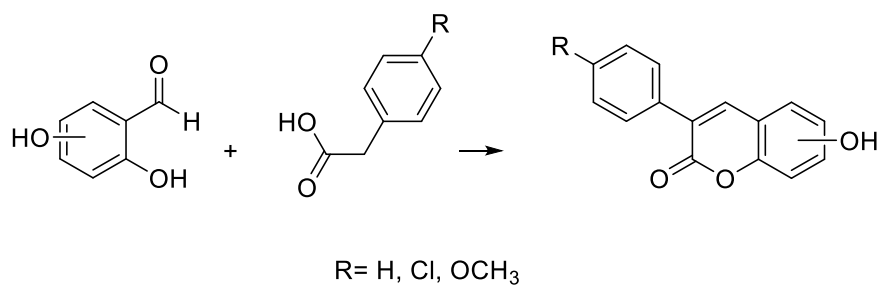
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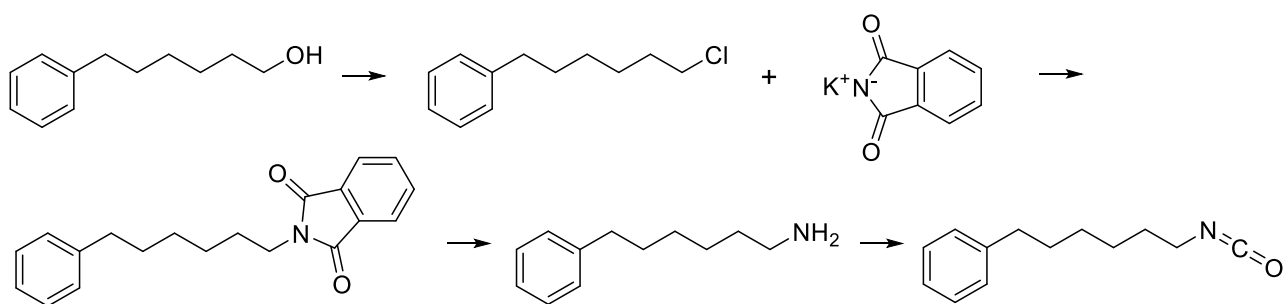
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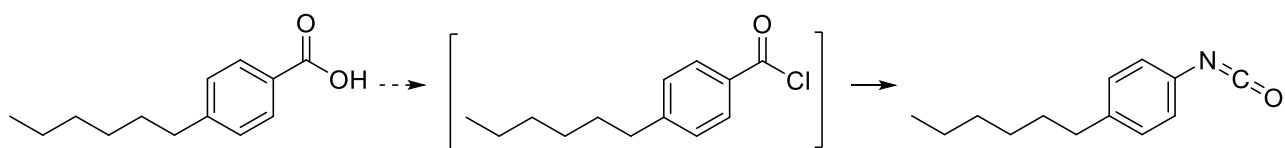
Scheme 1S. Synthesis of phenylcoumarins.



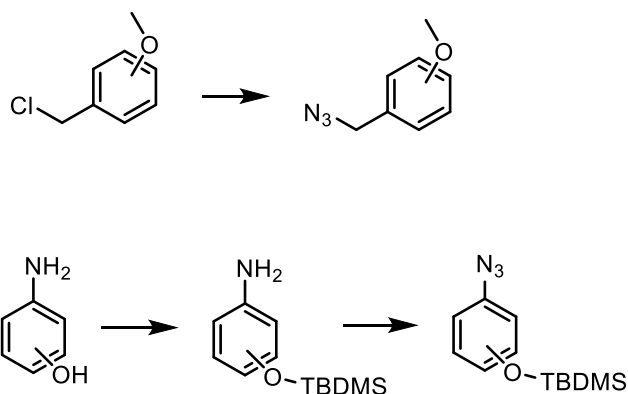
Scheme 2S. Synthesis of (6-isocyanatohexyl)benzene.



Scheme 3S. Synthesis of 1-hexyl-4-isocyanatobenzene.



Scheme 4S. Synthesis of azides.



Experimental section

Chemistry. General Methods. Melting points were measured in glass capillary tubes on a Büchi SMP-20 apparatus and are uncorrected. Direct infusion ES-MS spectra were recorded on a Waters Micromass ZQ 4000 apparatus. ^1H NMR spectra were recorded in CDCl_3 solution on a Varian Gemini 200/400 MHz spectrometer. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS), and spin multiplicities are given as s (singlet), d (doublet), t (triplet), m (multiplet) or br (broad). Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040-0.063 mm; Merck) by flash chromatography. Compounds were named following IUPAC rules as applied by Beilstein-Institut AutoNom (version 2.1), a PC integrated software package for systematic names in organic chemistry.

7-hydroxy-3-phenyl-2H-chromen-2-one. A mixture of 2,4-dihydroxybenzaldehyde (1.01 g, 7.35 mmol), 2-phenylacetic acid sodium salt (1.16 g, 7.35 mmol) and an excess of acetic anhydride was heated under stirring at 180-190 °C for 5h. After cooling the mixture was poured in 10 % solution of NaHCO_3 . The separated solid was collected by filtration; then 20 mL of EtOH and 3 mL of HCl conc were added and the mixture was refluxed for 8 h. The solid was collected by filtration to give 0.65 g (37 %) of neat compound, mp 175.5-177 °C. ^1H NMR δ 9.49 (s, 1H), 8.05 (s, 1H), 7.79 (d, J = 8.8 Hz, 2H), 7.63 (d, J = 8.4 Hz, 1H), 7.48-7.54 (m, 3H), 6.91 (d, J = 10.8 Hz, 1H), 6.81 (s, 1H).

7-hydroxy-3-(4-methoxyphenyl)-2H-chromen-2-one. Using the previous procedure and starting from p-methoxyphenylacetic acid sodium salt and 2,4-dihydroxybenzaldehyde. Yield: 10 %, mp 158.7-160.8 °C. ^1H NMR δ 9.49 (s, 1H), 8.05 (s, 1H), 7.79 (d, J = 8.8 Hz, 2H), 7.63 (d, J = 8.4 Hz, 1H), 7.08 (dd, J = 8.4 Hz, 2H), 6.91 (d, J = 10.8 Hz, 1H), 6.81 (s, 1H), 3.92 (s, 3H).

6-hydroxy-3-(4-methoxyphenyl)-2H-chromen-2-one. Using the previous procedure and starting from p-methoxyphenylacetic acid sodium salt and 2,5-dihydroxybenzaldehyde. Yield: 49.7 %, mp 186.6-187.3 °C. ^1H NMR δ 9.49 (s, 1H), 8.11 (s, 1H), 7.63 (d, J = 8.8 Hz, 2H), 7.48-7.32 (m, 3H), 7.08-6.91 (m, 2H), 3.68 (s, 3H).

3-(4-chlorophenyl)-7-hydroxy-2H-chromen-2-one. Using the previous procedure and starting from p-chlorophenylacetic acid sodium salt and 2,4-dihydroxybenzaldehyde. Yield: 48.27 %, mp >250 °C. ^1H NMR δ 9.49 (s, 1H), 8.11 (s, 1H), 7.79 (d, J = 8.8 Hz, 2H), 7.62 (d, J = 8.4 Hz, 1H), 7.48 (dd, J = 8.4 Hz, 2H), 6.91 (d, J = 10.8 Hz, 1H), 6.81 (s, 1H).

(6-chlorohexyl)benzene. To a cold solution of 6-phenyl-1-hexanol (1.00g, 5.62 mmol) and TEA (0.57g, 5.62 mmol) in DCM (30 mL), SOCl_2 (0.67 g, 5.62 mmol) were added. The solution was stirred at 0° C for 1h, then at rt for 48h. The solution was alkalized with Na_2CO_3 and extracted with DCM. The solvent was removed under reduced pressure, 0.90g of neat compound were obtained. Yield: 81.81 %, oil. ^1H NMR δ 7.25-7.21 (m, 2H), 7.14-7.12 (m, 3H), 3.44 (t, J = 13.2 Hz, 2H), 2.59 (t, J = 15.2 Hz, 2H), 1.72-1.68 (m, 2H), 1.61-1.57 (m, 2H), 1.43-1.39 (m, 2H), 1.34-1.28 (m, 2H).

2-(6-phenylhexyl)isoindoline-1,3-dione. A mixture of (6-chlorohexyl)benzene (0.91 g, 4.65 mmol) and K-phthalimide (0.78 g, 4.23 mmol) in DMF (2.5 mL) was refluxed for 2h. After cooling the mixture was poured in H_2O and the separated solid was collected by filtration. 1.15 g of neat compound were obtained. Yield: 88.60 %, mp 40-41 °C. ^1H NMR δ 7.85-7.83 (m, 1H), 7.71-7.69 (m, 1H), 7.27-7.26 (m, 4H), 7.17-7.14 (m, 3H), 3.67 (t, J = 14.8 Hz, 2H), 2.59 (t, J = 15.6 Hz, 2H), 1.67-1.59 (m, 4H), 1.38-1.36 (m, 4H).

6-phenylhexan-1-amine. To a solution of 2-(6-phenylhexyl)isoindoline-1,3-dione (1.15 g, 3.75 mmol) in ethanol, hydrazine monohydrate (0.37 g, 11.61 mmol) was added. The reaction was refluxed for 5h. After cooling HCl 37 % (1.3 mL) was added and the solution was refluxed for 30 minutes, then it was concentrated under reduced pressure. The mixture was washed with water and extracted with DCM to obtain 0.69 g as an oil. Yield: 96.53%. ¹H NMR δ 7.27-7.25 (m, 2H), 7.17-7.14 (m, 4H), 2.71 (t, J = 14 Hz, 2H), 2.58 (t, J = 15.2 Hz, 2H), 1.62-1.58 (m, 2H), 1.48-1.43 (m, 2H), 1.37-1.33 (m, 4H).

(6-isocyanatohexyl)benzene. Phosgene solution 20% in toluene (0.57 g, 5.84 mmol) was added dropwise to a solution of 6-phenylhexan-1-amine (0.69 g, 3.89 mmol) and TEA (2.83 mL) in toluene at 0° C. The solution was stirred for 2h at rt; phosgene solution 20% in toluene (0.57 g, 5.84 mmol) was added again and the solution was stirred for 90 minutes. The reaction was bubbled with N₂, then it was washed with water. The solvent was removed. The compound was used crude for next step of reaction.

1-hexyl-4-isocyanatobenzene. A mixture of 1g (5.21 mmol) of 4-hexylbenzoic acid and 10 mL of SOCl₂ and was refluxed for 5h, then SOCl₂ was removed. To an ice-cold mixture of 0.42g (7.28 mmol, 1.4eq) of NaN₃ previously dissolved in 2 mL of water, a solution of 4-hexylbenzoyl chloride dissolved in 2 mL of acetone was added, keeping the temperature below 10° C. The mixture was stirred for 1h, then the layers were separated and the lower aqueous layer was discarded; the upper layer was slowly and dropwise added to 30 mL of benzene heated to 60° C and the mixture was kept at 60-70° C until gas production ceased; then it was filtered and the solvent was removed. Compound was used crude, without any further purification, for next step of reaction; oil

1-(azidomethyl)-4-methoxybenzene. To a solution of 4-methoxybenzylchloride (0.55 g, 3.50 mmol) in anhydrous DMF (15 mL), NaN₃ (0.46 g, 7.00 mmol) were added and the mixture was stirred at 80 °C for 6h. The reaction was quenched with H₂O and the crude was extracted with diethyl ether. The compound was used without any further purification for the next step (0.53 g, 93

%, colorless oil). ^1H NMR δ 7.23 (d, $J = 7.2$ Hz, 2H), 6.88 (d, $J = 7.2$ Hz, 2H), 4.24 (s, 2H), 3.79 (s, 3H).

1-(azidomethyl)-3-methoxybenzene. Using the previous procedure and starting from 3-methoxybenzylchloride. Yield: 92.73 %, oil. ^1H NMR δ 6.99 (t, $J = 8$ Hz, 1H), 6.60-6.56 (m, 3H), 4.01 (s, 2H), 3.50 (s, 3H).

4-((tert-butyldimethylsilyl)oxy)aniline. To a solution of p-aminophenol (0.50 g, 4.582 mmol) in DMF (10 mL), imidazole (0.94 g, 13.75 mmol) and TBDMS-Cl (0.83 g, 5.49 mmol) were added. The mixture was stirred at rt for 12h, then the solution was poured in water and it was extracted with ether. The compound was purified via flash chromatography: petroleum ether/ethyl acetate 4:1; 0.82g of neat compound were obtained. Yield: 58.42%, dark red oil. ^1H NMR δ 6.64 (d, $J = 8.5$ Hz, 2H), 6.56 (d, $J = 8.5$ Hz, 2H), 0.93 (s, 9H), 0.13 (s, 6H).

3-((tert-butyldimethylsilyl)oxy)aniline. Using the previous procedure and starting from 3-aminophenol. Yield: 79.92 %, brow oil. ^1H NMR δ 7.01 (t, $J = 16$ Hz, 1H), 6.32-6.26 (m, 2H), 6.21 (s, 1H), 3.61 (s, 1H), 0.99 (s, 9H), 0.21 (s, 6H).

(4-azidophenoxy)(tert-butyl)dimethylsilane. 4-((tert-butyldimethylsilyl)oxy)aniline (0.82 g, 3.66 mmol), H_2O (9.5 mL) and HCl 37 % (2.85 mL) were added to a round-bottom flask cooled at 0°C in a ice bath. A solution of NaNO_2 (0.25 g, 3.66 mmol) in H_2O was added dropwise to the reaction flask and the mixture was stirred for 10'. NaN_3 (0.29 g, 4.39 mmol) was added in portions and the reaction was stirred for 1h. The reaction was quenched with water and extracted with ethyl acetate. 0.06 g of neat compound were obtained. Yield: 58.83 %, black oil. ^1H NMR δ 6.89-6.76 (m, 4H), 0.90 (s, 9H), 0.09 (s, 6H).

(3-azidophenoxy)(tert-butyl)dimethylsilane. Using the previous procedure and starting from 3-((tert-butyldimethylsilyl)oxy)aniline. Yield: 65.93 %, oil. ^1H NMR δ 7.18 (t, $J = 6.8$ Hz, 1H), 6.60 (d, $J = 7.8$ Hz, 2H), 6.49 (d, $J = 8.9$ Hz, 1H), 0.90 (s, 9H), 0.09 (s, 6H).

Kinetics of ChE carbamylation.

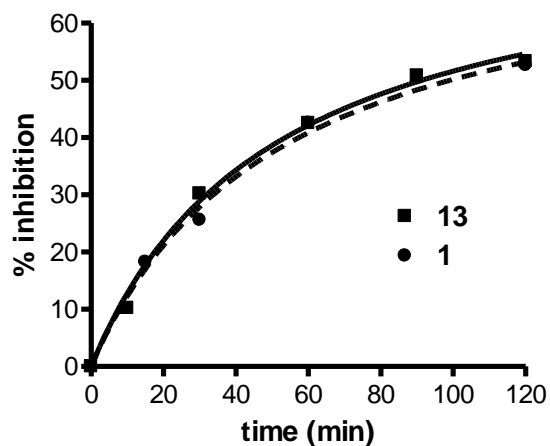


Figure 1S. Time-dependent inhibition of *hAChE* activity by the phenyl-hexyl carbamate **13** and the phenyl-pentyl derivative **1**. Comparison was made using inhibitor concentrations close to the respective IC_{50} values.

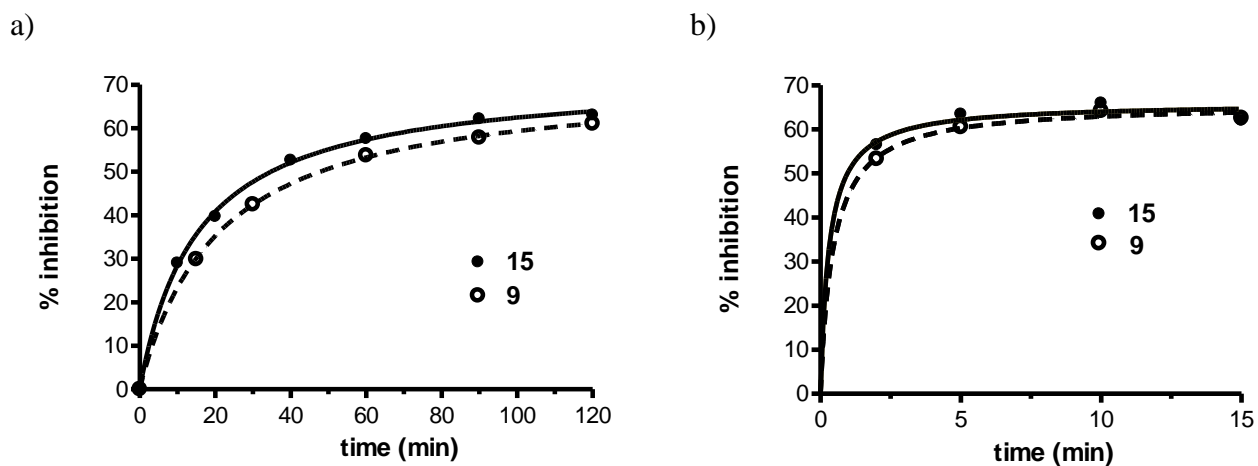


Figure 2S. Influence of the spacer chain length on the kinetic of carbamylation: a) *hAChE* and b) *hBuChE*. Time dependent inhibition at inhibitor concentration close to the IC_{50} values was monitored.

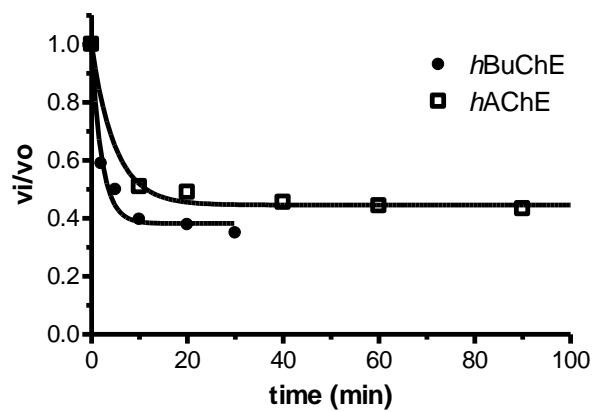


Figure 3S. Carbamoylation of *hAChE* and *hBuChE* by compound **12**.

Computational Studies.

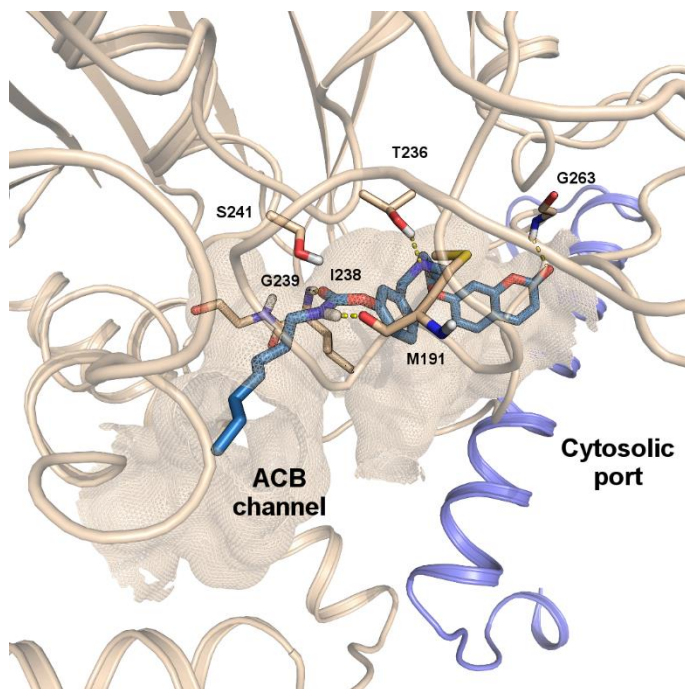


Figure 4S. Binding mode of **9** in its neutral form in *r*FAAH; the *N*-alkyl spacer and the coumarinic head fit the upper portion of the cytosolic port, enabling the neutral amino-group to make a polar interaction with T236 side chain.

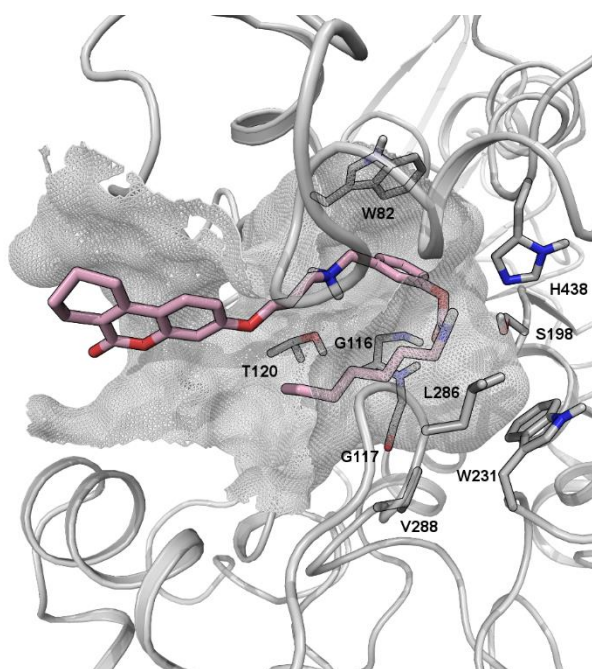


Figure 5S. Docking of compound **10** (pink carbons) within the catalytic gorge of *h*BuChE. The tricyclic nucleus occupies the access channel of the active site, while the heptyl chain is accommodated within the putative acyl chain-binding pocket.