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# New $N_4$-substituted piperazine naphthamide derivatives as BACE-1 inhibitors

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**Abstract**
Synthesis and enzymatic evaluation of new series of $N_4$-substituted piperazine naphthamide derivatives as BACE-1 inhibitors for the treatment of Alzheimer's disease are reported.

**Keywords:** *Alzheimer's disease, BACE-1,* $N_4$-*substituted piperazine naphthamide*

## Introduction

The most prominent neuropathological finding in Alzheimer's disease (AD) brains is the occurrence of cortical plaques containing beta-amyloid (Aβ) peptide. Genetic evidence points to a causal role of Aβ during development of AD as it is postulated by the amyloid cascade hypothesis [1]. Consequently, blocking the production of Aβ peptides by specific inhibition of key proteases required for Aβ peptide generation is a major focus of research into AD therapy. The identification of secretase inhibitors has triggered a race to develop drug-like inhibitors of these enzymes, which have become major AD targets. Since it has been established that γ-secretase cleaves various substrates, some of which might have important physiological roles (Notch cleavage), γ-secretase inhibitors might have insurmountable mechanism-based toxicity [2]. Taking into account those observations developing β-secretase inhibitors appear to be more encouraging. Several independent approaches led to the identification of β-site amyloid precursor protein cleavage enzyme (BACE-1) as the first enzyme of the amyloid cascade. BACE-1 cleaves the 695 or 751 amino acid amyloid precursor protein to a 99 amino acid long precursor peptide which is later trimmed by γ-secretase to form the pathological 40 or 42 amino acid long β-amyloid peptide. BACE-1 is an integral membrane protein with high homology to other aspartic proteases. Given the lack of adverse effects in gene knock out mice, BACE-1 is considered a prime target for the development of AD therapeutics. BACE-1 possesses a bilobal structure [3]. Typical for eukaryotic aspartic proteases [4], with the catalytical aspartate residues Asp32 and Asp228 located in the substrate-binding cleft between the N- and C-terminal lobes [3]. To exert their pharmacological activity *in vivo*, BACE-1 inhibitors have to traverse the blood–brain barrier. Most peptidomimetic BACE-1 inhibitors reported so far are large [5],

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moderately permeable compounds not ideally suited for a central nervous system indication. An overview on the design and development of BACE-1 inhibitors has been published by Cummings et al. [6].

## Materials and methods

Starting from already published data on arylpiperazine amide scaffolds as a possible framework for the design of β-secretase inhibitors [7–9], we investigated original and subtitle modifications at different positions on the aryl piperazine scaffold. Molecular modelisation and Protein Data Bank revealed that the aryl piperazine scaffold allows the design of a large diversity of BACE-1 inhibitors through the substitution possibilities offered at various positions as shown in Figure 1.

Through variation temperature NMR and modelisation studies, we have recently confirmed that introduction at the $N_4$-position of the piperazine ring allows productive interactions within the BACE-1 active site, interactions which appear to be determinative for high BACE-1 inhibitory activity [9]. This observation led us to focus our synthetic efforts on the synthesis of a library of derivatives in which the $N_4$-position of the piperazine ring was substituted by a wide diversity of $R_2$ substituents. We reported here the
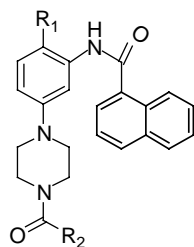
synthesis and the inhibitory potency of a new series of $N_4$-substituted piperazine naphthamide.
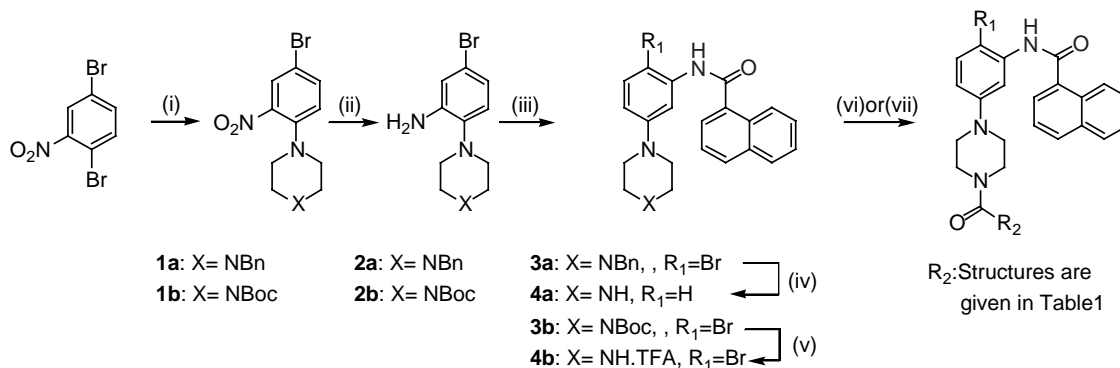
The required intermediates **4a** ($R_1$ = H) and **4b** ($R_1$ = Br; Scheme 1), have been synthesised according to reported procedure [9].

The new analogues have been synthesised, using standard methodologies, which involved the direct condensation of the corresponding acid chlorides (compounds **6a, 7a, 9a, 10a, 6b, 7b, 9b** and **10b**), or chloroformiate (compounds **11a** and **11b**), in the presence of triethylamine in methylene chloride at room temperature, and in the case of the carboxylic acids, the new derivatives (compounds **5a, 8a, 5b** and **8b**) were obtained in good yields using standard coupling reactions using dicyclohexylcarbodiimide (DCC), hydroxybenzotriazole (HOBt) in the presence of dimethylaminopyridine (DMAP) in methylene chloride at room temperature. It should be underlined that the corresponding $4',4$-diallyloxybenzoic acid was obtained through a methodology reported by Eicher et al. [10]

## Synthesis

### General procedures

Unless otherwise noted, starting materials and reagents were obtained from commercial suppliers and were used without purification. Methylene dichloride ($CH_2Cl_2$) was distilled over $P_2O_5$ just prior to use. NMR spectra were recorded at 250 MHz for $^1$H and 62.9 MHz for $^{13}$C on a Brüker AC-250 spectrometer. Chemical shifts are expressed as *d* units (parts per million) downfield from tetramethylsilane. Electrospray mass spectra were obtained on a Waters Micromass ZMD spectrometer by direct injection of the sample solubilised in acetonitrile. Elemental analyses were within ± 0.4% of theorical values for all compounds. Analytical thin layer chromatographies (TLC) and preparative thin layers chromatographies were performed using silica gel plates 0.2



Figure 1. General structure of new $N_4$-substituted piperazine naphthamide derivatives as BACE-1 inhibitors.



Scheme 1. Reagents and conditions: (i) (a) *N*-Boc-piperazine (b) or *N*-benzylpiperazine, *i*-PrOH, reflux, 24 h; (ii) Zn powder, 1 M KH$_2$PO$_4$/THF, reflux, 24 h; (iii) 1-naphthoyl chloride, CH$_2$Cl$_2$, TEA, rt, 4 h; (iv) HCO$_2$NH$_4$, 10 wt% Pd/C, CH$_3$OH, reflux, 6 h; (v) TFA, CH$_2$Cl$_2$, rt, 12 h; (vi) R$_2$COCl, CH$_2$Cl$_2$, Et$_3$N; (vii) R$_2$CO$_2$H, DCC, HOBt, DMAP, rt, 2 h.

and 1 mm thick, respectively (60F254, Merck, Darmstadt, Germany). Preparative flash column chromatographies were carried out on silica gel (230–240 mesh, G60, Merck).

### General procedure for compounds 3a and 3b

To a mixture of 2,5-dibromonitrobenzene (2.23 g, 7.94 mmol) in *i*-PrOH (40 ml) was added *N*-benzyl piperazine (2.1 g, 11.9 mmol). The resulting mixture was stirred at 70°C for 24 h. The reaction mixture was cooled to room temperature and then concentrated under reduced pressure. The brown residue was purified by chromatography on silica gel. Elution with increasing proportions of EtOAc–cHex (1:9–1:1) gave compound **1a** (2 g, 69%) as an orange oil.

Zinc dust (0.55 g, 8.45 mmol) was added to a stirred solution of **1a** (0.64 g, 1.69 mmol) in tetrahydrofuran (THF; 45 ml) followed by $KH_2PO_4$ (30 ml, 1 M). The resulting mixture was stirred at 80°C for 24 h. The reaction mixture was cooled to room temperature, filtered and evaporated to a small volume. The mixture was extracted with $CH_2Cl_2$, and the organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by chromatography on silica gel, with EtOAc–cHex as eluant (1:9), giving aniline **2a** (0.40 g, 68%) as a pale yellow foam.

To a solution of **2a** (0.2 g, 1.15 mmol) in methylene chloride (10 ml) with diisopropylethylamine (DIEA) (300 µl, 1.73 mmol) was added 1-naphthoyl chloride (1.5 mmol). The reaction mixture was stirred for 4 h at room temperature. The mixture was extracted with water (15 ml), and the organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by chromatography on silica gel, with EtOAc–cHex eluant (1:3) giving **3a** (0.50 mg, 90%) as a yellow solid. Compound **3b** was prepared by a method similar to that described for **3a** using **1b**.

### Procedure for compounds 4a

To a stirred suspension of the appropriate *N*-benzyl compound **3a** (0.36 g, 0.72 mmol) and an equal weight of 10% Pd–C in dry methanol (10 ml) was added anhydrous ammonium formate (0.23 g, 3.6 mmol) in a single portion under nitrogen. The resulting mixture was stirred at reflux temperature, and the reaction was monitored by TLC. After completion, the catalyst was removed by filtration through Celite and washed with methylene chloride (10 ml). The solvent was removed under reduced pressure, and the crude product was purified by chromatography on silica gel with methylene dichloride (DCM), 0.5% MeOH as eluent, to yield the desired compound **4a** (0.23 g, 98%) as a yellow oil. $R_f$ 0.30 ($CH_2Cl_2$/MeOH 10%). $^1$H NMR ($CDCl_3$,

250 MHz) δ: 9.16 (1H, s), 8.69 (1H, d), 8.47 (1H, dd), 7.98 (1H, d), 7.91 (1H, dd), 7.75 (1H, d), 7.60–7.50 (3H, m), 7.28–7.20 (2H, m), 7.14 (1H, dd), 2.91 (8H, s), 2.80 (1H, l). ESI-MS *m/z* [M + H]$^+$ 332. Anal. ($C_{21}H_{21}N_3O$) C, H, N.

### Procedure for compound 4b

To a stirred solution of the appropriate *N*-Boc compound **3b** (0.51 mg, 1 mmol) in dry $CH_2Cl_2$ (10 ml) was added trifluoroacetic acid (TFA; 770 µl, 10 mmol). The reaction mixture was stirred overnight at room temperature. After completion, the solvent and excess TFA were removed under reduced pressure, and the corresponding TFA salt was identified as the desired compound **4b** (0.38 g, 93%) as a TFA salt. $R_f$ 0.11 ($CH_2Cl_2$/MeOH 10%) $^1$H NMR ($CDCl_3$, 250 MHz) δ: 9.20 (1H, s), 8.94 (1H, s), 8.50 (1H, t), 8.03 (1H, d), 7.94 (1H, t), 7.76 (1H, d), 7.59 (3H, m), 7.27 (1H, dd), 7.09 (1H, d), 2.87 (8H, l), 1.97 (1H, l). ESI-MS *m/z* [M + H]$^+$ 411. Anal. ($C_{21}H_{20}BrN_3O$) C, H, N.

### General procedure for compounds 6a, 7a, 9a, 10a, 6b, 7b, 9b and 10b

To a solution of **4a** (60 mg, 0.18 mmol) in methylene chloride (8 ml) with DIEA (0.27 mmol) was added in 4-trifluoromethylbenzoyl chloride (47 mg, 0.23 mmol). The reaction mixture was stirred for 12 h at room temperature. The mixture was extracted with water (15 ml), and the organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by chromatography on silica gel, with EtOAc–cHex eluant (1:2), to give the desired compound **6a** (85 mg, yield 94%) as a white solid. $R_f$ 0.53 (cHex–AcOEt, 1:1). $^1$H NMR ($CDCl_3$, 250 MHz) δ: 8.98 (s, 1H), 8.62 (d, 1H), 8.37 (t, 1H), 7.93 (d, 1H), 7.86 (t, 1H), 7.68–7.41 (m, 7H), 7.23–7.08 (m, 3H), 3.51 (d, 4H), 2.85 (d, 4H). $^{13}$C NMR ($CDCl_3$, 62.9 MHz) δ: 169.01, 167.22, 140.31, 138.87, 134.74, 132.16, 131.27, 133.82, 130.10, 128.61, 127.45, 126.72, 125.75, 124.89, 124.31, 120.87, 119.84, 52.44, 48.52. ESI-MS *m/z* [M + H]$^+$ 504. Anal. ($C_{29}H_{24}F_3N_3O_2$) C, H, N.

*Compound 7a*. Yield: 74%. $R_f$ 0.11 (cHex–AcOEt, 2:1). $^1$H NMR ($CDCl_3$, 250 MHz) δ: 9.11 (s, 1H), 8.70 (d, 1H), 8.45 (t, 1H), 8.00 (d, 1H), 7.92 (t, 1H), 7.73 (d, 1H), 7.57 (m, 3H), 7.37 (d, 2H), 7.30–7.10 (m, 3H), 6.90 (d, 2H), 3.82 (s, 3H), 3.62 (m, 4H), 2.91 (m, 4H). $^{13}$C NMR ($CDCl_3$, 62.9 MHz) δ: 169.69, 166.39, 160.11, 139.75, 133.91, 133.11, 130.41, 129.28, 128.38, 127.74, 126.55, 125.85, 125.51, 124.39, 124.06, 123.41, 120.080, 118.88, 112.95, 54.53, 51.69, 26.075. ESI-MS *m/z* [M + H]$^+$ 466. Anal. ($C_{29}H_{27}N_3O_3$) C, H, N.

*Compound 9a*. Yield: 98%. $R_f$ 0.27 (cHex−AcOEt, 1:1). ^{1}H NMR (CDCl$_3$, 250 MHz) δ: 8.62 (d, 1H), 8.38 (t, 1H), 7.92 (d, 1H), 7.84 (t, 1H), 7.67 (d, 1H), 7.49 (m, 3H), 7.23–7.08 (m, 3H), 6.45 (q, 1H), 6.22 (d, 1H), 5.63 (d, 1H), 3.54 (d, 4H), 2.82 (s, 4H). ^{13}C NMR (CDCl$_3$, 62.9 MHz) δ: 167.22, 165.51, 140.49, 134.72, 133.96, 131.27, 130.10, 128.61, 127.38, 127.14, 126.70, 125.21, 124.92, 124.27, 120.91, 119.72, 52.53, 52.51, 46.29, 42.00. ESI-MS *m/z* [M + H]$^+$ 386. Anal. (C$_{24}$H$_{23}$N$_3$O$_2$) C, H, N.

*Compound 10a*. Yield: 94%. $R_f$ 0.89 (CH$_2$Cl$_2$−MeOH, 9:1). ^{1}H NMR (CDCl$_3$, 250 MHz) δ: 9.06 (s, 1H), 8.62 (d, 1H), 8.39 (t, 1H), 7.92 (d, 1H), 7.85 (t, 1H), 7.67 (d, 1H), 7.50 (m, 3H), 7.20–7.02 (m, 3H), 3.36 (m, 4H), 2.77 (t, 4H), 2.12–1.97 (m, 10H), 1.60 (m, 5H). ^{13}C NMR (CDCl$_3$, 62.9 MHz) δ: 167.23, 154.21, 133.9, 131.20, 130.16, 128.53, 127.35, 126.65, 125.32, 124.87, 124.18, 120.83, 79.95, 52.27, 41.68. ESI-MS *m/z* [M + H]$^+$ 510. Anal. (C$_{32}$H$_{35}$N$_3$O$_3$) C, H, N.

*Compound 6b*. Yield: 87%. $R_f$ 0.13 (cHex−AcOEt, 3:1). ^{1}H NMR (CDCl$_3$, 250 MHz) δ: 8.94 (s, 1H), 8.83 (d, 1H), 8.32 (t, 1H), 7.91 (d, 1H), 7.83 (t, 1H), 7.59 (m, 3H), 7.45 (m, 5H), 7.15 (dd, 1H), 6.95 (d, 1H), 3.69 (m, 2H), 3.29 (m, 2H), 2.78 (m, 4H). ^{13}C NMR (CDCl$_3$, 62.9 MHz) δ: 168.99, 167.15, 139.25, 138.75, 135.01, 134.16, 133.95, 131.58, 130.04, 128.66, 127.52, 127.47, 126.81, 124.85, 122.43, 119.81, 52.29, 48.03, 42.60. ESI-MS *m/z* [M + H]$^+$ 583. Anal. (C$_{29}$H$_{23}$BrF$_3$N$_3$O$_2$) C, H, N.

*Compound 7b*. Yield: 94%. $R_f$ 0.22 (cHex−AcOEt, 2:1). ^{1}H NMR (CDCl$_3$, 250 MHz) δ: 9.00 (s, 1H), 8.84 (s, 1H), 8.35 (t, 1H), 7.92 (d, 1H), 7.84 (t, 1H), 7.63 (d, 1H), 7.48 (m, 3H), 7.28 (d, 2H), 7.17 (dd, 1H), 6.97 (d, 1H), 6.71 (d, 2H), 3.73 (s, 3H), 3.53 (m, 4Hl), 2.78 (s, 4H). ^{13}C NMR (CDCl$_3$, 62.9 MHz) δ: 170.53, 167.16, 160.99, 139.49, 135.06, 134.20, 133.95, 131.55, 130.07, 128.63, 127.52, 127.04, 126.79, 125.13, 124.86, 122.45, 119.47, 113.81, 55.39, 52.43, 29.72, 26.92. ESI-MS *m/z* [M + H]$^+$ 545. Anal. (C$_{29}$H$_{26}$BrN$_3$O$_3$) C, H, N.

*Compound 9b*. Yield: 57%. $R_f$ 0.19 (cHex−AcOEt, 1:1). ^{1}H NMR (CDCl$_3$, 250 MHz) δ: 8.99 (s, 1H), 8.85 (s, 1H), 8.37 (t, 1H), 7.92 (d, 1H), 7.84 (t, 1H), 7.64 (d, 1H), 7.48 (m, 2H), 7.16 (dd, 1H), 6.94 (d, 1H), 6.44 (q, 1H), 6.22 (d, 1H), 5.62 (d, 1H), 3.48 (d, 4H), 2.77 (s, 4H). ^{13}C NMR (CDCl$_3$, 62.9 MHz) δ: 167.15, 165.50, 139.38, 135.05, 134.17, 133.95, 131.57, 130.04, 128.62, 127.51, 127.05, 124.97, 122.44, 119.73, 52.44, 52.16, 46.18,

43.45. ESI-MS *m/z* [M + H]$^+$ 465. Anal. (C$_{24}$H$_{22}$BrN$_3$O$_2$) C, H, N.

*Compound 10b*. Yield: 97%. $R_f$ 0.38 (cHex−AcOEt, 5:1). ^{1}H NMR (CDCl$_3$, 250 MHz) δ: 9.03 (s, 1H), 8.86 (s, 1H), 8.38 (t, 1H), 7.94 (d, 1H), 7.87 (t, 1H), 7.65 (d, 1H), 7.53 (m, 3H), 7.19 (s, 1H), 6.97 (d, 1H), 3.36 (d, 4H), 2.73 (s, 4H), 2.10–2.02 (m, 10H), 1.57 (m, 5H). ^{13}C NMR (CDCl$_3$, 62.9 MHz) δ: 167.19, 154.15, 139.90, 135.07, 134.22, 133.96, 131.51, 130.12, 128.58, 127.50, 126.76, 125.21, 124.83, 122.35, 119.48, 80.07, 52.21, 41.67, 40.30, 36.17, 35.70, 30.85. ESI-MS *m/z* [M + H]$^+$ 589. Anal. (C$_{32}$H$_{34}$BrN$_3$O$_3$) C, H, N.

### General procedure for compounds *5a, 8a, 5b and 8b*

Compound **4a** (60 mg, 0.18 mmol), 2-chlorobenzoic acid (45 mg, 0.29 mmol), DCC (59 mg, 0.29 mmol), HOBt (39 mg, 0.29 mmol) and DMAP (35 mg, 0.29 mmo) were stirred in dry methylene chloride (15 ml) at room temperature for 10 h. After this time, the insoluble residue was filtered off and washed with 5 ml of methylene chloride. The filtrate was concentrated, and the resulting crude residue was purified by column chromatography with toluene−EtOAc (5:1) as eluent, to yield compound **5a** as a white solid (92 mg, 98%; as a white powder). $R_f$ 0.29 (toluene−AcOEt, 4:1). ^{1}H NMR (CDCl$_3$, 250 MHz) δ: 8.98 (s, 1H), 8.63 (d, 1H), 8.36 (t, 1H), 7.92 (d, 1H), 7.84 (t, 1H), 7.66 (d, 1H), 7.47 (m, 3H), 7.33–7.03 (m, 7H), 3.75 (d, 2H), 3.17 (m, 2H), 2.90 (d, 2H), 2.75 (dd, 2H). ^{13}C NMR (CDCl$_3$, 62.9 MHz) δ: 167.24, 167.00, 140.56, 135.38, 134.73, 133.81, 131.28, 130.44, 129.75, 128.61, 127.81, 127.41, 126.715, 126.38, 125.21, 124.93, 124.29, 120.90, 119.78, 52.43, 47.24, 42.18. ESI-MS *m/z* [M + H]$^+$ 470. Anal. (C$_{28}$H$_{24}$ClN$_3$O$_2$) C, H, N.

*Compound 8a*. Yield: 90%. $R_f$ 0.16 (cHex−AcOEt, 2:1). ^{1}H NMR (CDCl$_3$, 250 MHz) δ: 9.02 (s, 1H), 8.62 (d, 1H), 8.37 (t, 1H), 7.93 (d, 1H), 7.86 (t, 1H), 7.67 (d, 1H), 7.50 (m, 3H), 7.18 (m, 3H), 6.87 (m, 3H), 5.97 (m, 2H), 5.29 (m, 4H), 4.54 (t, 4H), 3.52 (d, 4H), 2.83 (s, 4H). ^{13}C NMR (CDCl$_3$, 62.9 MHz) δ: 170.20, 167.06, 156.88, 149.85, 148.08, 140.46, 134.58, 133.69, 132.73, 131.08, 129.96, 128.43, 127.24, 126.55, 126.17, 124.75, 124.13, 120.74, 119.60, 120.511, 113.32, 112.95, 69.63, 52.36, 48.63. ESI-MS *m/z* [M + H]$^+$ 548. Anal. (C$_{34}$H$_{33}$N$_3$O$_4$) C, H, N.

*Compound 5b*. Yield: 98%. $R_f$ 0.25 (toluene−AcOEt, 4:1). ^{1}H NMR (CDCl$_3$, 250 MHz) δ: 8.96 (s, 1H), 8.83 (s, 1H), 8.33 (t, 1H), 7.92 (d, 1H), 7.84 (t, 1H), 7.62 (d, 1H), 7.48 (m, 3H), 7.31–6.98 (m, 5H), 6.96

(d, 1H), 3.50 (d, 4H), 2.77 (d, 4H). $^{13}$C NMR (CDCl$_3$, 62.9 MHz) $\delta$: 169.47, 167.15, 139.30, 136.15, 135.03, 134.18, 133.96, 133.48, 131.57, 130.04, 128.90, 128.66, 127.53, 127.08, 126.81, 124.86, 122.54, 119.80, 52.35, 47.92, 42.80, 29.72, 26.92. ESI-MS $m/z$ [M + H]$^+$ 549. Anal. (C$_{28}$H$_{23}$BrClN$_3$O$_2$) C, H, N.

*Compound 8b*. Yield: 95%. $R_f$ 0.27 (cHex−AcOEt, 2:1). $^1$H NMR (CDCl$_3$, 250 MHz) $\delta$: 8.99 (s, 1H), 8.85 (d, 1H), 8.34 (t, 1H), 7.93 (d, 1H), 7.85 (t, 1H), 7.64 (d, 1H), 7.49 (m, 3H), 7.16 (dd, 1H), 6.97 (d, 1H), 6.86 (d, 2H), 6.77 (d, 1H), 5.96 (d, 2H), 5.36−5.16 (m, 4H), 4.53 (t, 4H), 3.52 (d, 4H), 2.78 (s, 4H). $^{13}$C NMR (CDCl$_3$, 62.9 MHz) $\delta$: 170.39, 167.08, 150.05, 148.23, 139.43, 135.03, 133.95, 133.00, 132.84, 131.55, 130.05, 128.63, 127.52, 127.06, 126.80, 124.96, 122.43, 120.65, 119.70, 118.03, 113.44, 113.04, 69.77, 52.43, 33.88, 30.16, 29.91, 24.96. ESI-MS $m/z$ [M + H]$^+$ 627. Anal. (C$_{34}$H$_{32}$BrN$_3$O$_4$) C, H, N.

*General procedure for compounds 11a and 11b*

To a solution of **4a** (94 mg, 0.28 mmol) in methylene chloride (8 ml) with triethylenamine (TEA) (0.28 mmol) was added succinic anhydride (28 mg, 0.28 mmol). The reaction mixture was stirred for 24 h at room temperature. The reaction mixture was adjusted to pH 2 (with 1 N HCl), and the organic layer was dried over MgSO$_4$ and concentrated under reduced pressure. The residue was purified by chromatography on silica gel, with CH$_2$Cl$_2$−MeOH eluant (9:1), to give the desired compound **11a** (75 mg, yield 62%) as a white solid. $R_f$ 0.41 (CH$_2$Cl$_2$−MeOH, 9:1). $^1$H NMR (DMSO-$d_6$, 250 MHz) $\delta$: 9.73 (s, 1H), 8.43 (d, 1H), 8.26 (d, 1H), 8.19 (m, 2H), 7.91 (d, 1H), 7.72 (t, 3H), 7.29 (m, 3H), 3.63 (s, 4H), 2.98 (m, 4H), 2.59 (m, 4H). $^{13}$C NMR (DMSO-$d_6$, 62.9 MHz) $\delta$: 175.28, 170.69, 167.92, 144.74, 135.46, 134.24, 133.83, 131.35, 130.63, 129.33, 127.99, 127.39, 126.093, 125.44, 123.45, 121.81, 55.86, 52.44, 46.02, 42.48, 30.15, 28.48. ESI-MS $m/z$ [M + H]$^+$ 432. Anal. (C$_{25}$H$_{25}$N$_3$O$_4$) C, H, N.

*Compound 11b*. Yield: 49%. $R_f$ 0.47 (CH$_2$Cl$_2$−MeOH, 9:1). $^1$H NMR (DMSO-$d_6$, 250 MHz) $\delta$: 8.97 (s, 1H), 8.84 (s, 1H), 8.35 (t, 1H), 7.92 (d, 1H), 7.84 (t, 1H), 7.64 (d, 1H), 7.48 (m, 3H), 7.17 (d, 1H), 6.94 (d, 1H), 3.53 (m, 2H), 3.39 (m, 2H), 2.76 (m, 4H), 2.54 (d, 4H). $^{13}$C NMR (DMSO-$d_6$, 62.9 MHz) $\delta$: 177.2, 170.34, 167.20, 139.37, 135.02, 133.95, 131.59, 130.05, 128.66, 127.53, 127.10, 126.81, 125.09, 122.56, 119.77, 52.12, 45.80, 42.35, 29.42, 29.38, 27.89. ESI-MS $m/z$ [M + H]$^+$ 511. Anal. (C$_{25}$H$_{24}$BrN$_3$O$_4$) C, H, N.

## Computational studies

The development of substrate-based inhibitors has been significantly aided by X-ray crystallographic analysis of BACE-1 complexes with a standard peptide-based inhibitor known as OM-2 [3], since the structure of this complex has allowed the identification of critical ligand−active site interactions. BACE-1 active site was found more open than that of other aspartic proteases and exhibited conformational flexibility. Since peptide-based or hydroxyethylene inhibitors were relatively large and "undrug-like", other X-ray crystallographic studies of BACE-1 complexes with other inhibitors (non-peptidic) have been performed. The most detailed account of non-peptidic BACE inhibitors comes from Vertex Pharmaceuticals Inc. (Cambridge, MA, USA) [11], which disclosed several hundreds of compounds along with their associated BACE inhibition $K_i$ values. Among the more potent classes reported, were the biaryl naphthalenes represented by compound **4a**, in which the $N_4$-position of the piperazine ring is free. Vertex proposed the first 3D pharmacophore map of BACE [11]. Hydrogen interactions within the active site, with other key residues as well as other hydrophobic interactions with BACE sub-sites were determined. Unfortunately crystallographic data to substantiate this 3D model was not provided. Simulated molecular dynamics analysis reveals that a direct contact through amide hydrogen bonding interactions with the catalytic aspartic acids Asp32 and Asp228 [3], could favoured BACE-1 inhibition. The compound **4a** was placed in the most favourable position in the BACE-1 active site, allowing both hydrogen bonding within the active site as well as hydrophobic interactions with enzyme sub-sites. From this study, it appears that $N_4$-position of the piperazine ring could be suitable to accommodate various substituents capable of additional interactions with enzyme sub-sites

In this perspective, we have synthesised a new series of compounds (16 analogues) which included at the $N_4$-position different substituents, which differ from each other by different structural parameters: steric hindrance, polarity in terms of hydrophobic or hydrogen bondingand also by the organic function (amides, amines or carbamates) which links the substituents to the $N_4$ nitrogen atom.

## Computational studies (experimental procedure)

Molecular dynamics and molecular mechanics (InsightII, Discover module, Gromacs) is used in order to obtain the most realistic structures from known X-ray structures deposited at the Protein Data Bank. Starting from a model generated from the 1FKN structure [3], a virtual docking of known inhibitors into the active site of the BACE-1 allows the selection of the best descriptor among those previously generated. To reach this goal,

Table I.   BACE-1 inhibition activity of $N_4$-substituted piperazine naphthamide derivatives.
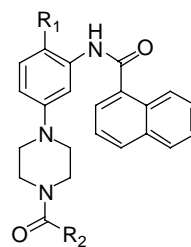


| No. | $R_1$ | $R_2$ | BACE-1[a] IC$_{50}$ (μM) | C log $P$[b] | No. | $R_1$ | $R_2$ | BACE-1[a] IC$_{50}$ (μM) | ClogP[b] |
|---|---|---|---|---|---|---|---|---|---|
| **4a** | H | H | Inactive | 2.72 | **4b** | | Br | Inactive | 4.17 |
| **5a** | H |  | 10★★ | 4.41 | **6a** | Br |  | 1★ | 5.58 |
| **6a** | H |  | 1★ | 4.64 | **6b** | Br |  | 1★ | 5.81 |
| **7a** | H |  | 10★★ | 3.84 | **7b** | Br |  | 10★★ | 5.01 |
| **8a** | H |  | Inactive | 5.07 | **8b** | Br |  | Inactive | 6.24 |
| **9a** | H |  | Inactive | 2.85 | **9b** | Br |  | Inactive | 4.02 |
| **10a** | H |  | Inactive | 6.11 | **10b** | Br |  | Inactive | 7.28 |
| **11a** | H |  | Inactive | 2.13 | **11a** | Br |  | Inactive | 3.30 |

[a] The BACE-1 fluorescence resonance energy transfer assay kit was purchased from PanVera (No. P2985). BACE-1 activity assays were carried out according to the manufacturer's instructions. Average value is from three independent experiments. Values show means of at least three independent experiments each performed in triplicates, with standard errors of mean. ★$P < 0.5$ and ★★$P < 2$; [b] Calculated log $P$ values from ACD (Advanced Chemistry Development, Inc., Toronto, Ont., Canada) log $P$ 1.0 base calculations.

a small number of compounds with known $K_d$ were used (training set 15–20 molecules), and screened using different docking software. Using a simulated annealing procedure (InsightII, Affinity module), and with quicker programs DOCK, and AUTODOCK it is possible to simulate a flexible ligand bound to a rigid receptor, but also a certain amount of flexibility of the protein. Following this methodology, we have selected some substituents, synthesise the corresponding compounds and then assayed as possible BACE-1 inhibitors.

The molecular structure of BACE-1 was obtained from Protein Data bank (pdb code: 1 FKM). Docking calculations were performed using as protonated either Asp32 or Asp228 inner oxygens for each compound.

## Results and discussion

The new compounds were evaluated as BACE-1 inhibitors using BACE-1 fluorescence resonance energy transfer (FRET) Assay. These experiments have been preformed using BACE-1 (β-secretase) FRET Kit assay, from PanVera Corporation (Madison, WI, USA), according to the described protocol and using a multiwell spectrofluorometer instrument capable of 530–545 nm excitation and 570–590 nm emission wavelengths (Wallac Victor [2] 1420, Perkin-Elmer, Turku, Finland). The kinetic assays are performed in duplicate for each inhibitor, using BACE-1 inhibitor (H-Lys-Thr-Glu-Glu-Ile-Ser-Glu-Val-Asn-(3S,4S)-Stat-Val-Ala-Glu-Phe-OH.

$IC_{50} = 30$ nM; Calbiochem, Beeston, UK) as reference. The biological results are summarised in Table I.

The data reported in Table I show that some compounds are potent BACE-1 inhibitors or their inhibitory activity is noticeably influenced by the substituents on the $N_4$ nitrogen atom of the piperidine ring, confirming that this region is crucial for efficient BACE-1 inhibition. As it could be seen, polar ω-carboxylic acid substituents (compounds **11a** and **11b**) or bulky substituents like adamantyl carbamate (compounds **10a** and **10b**) are detrimental for BACE-1 inhibitory activity. In the opposite substitution at the $N_4$ position by halogenated phenyl groups (compounds **5a**, **6a**, **5b** and **6b**) or by methoxy groups (compounds **7a** and **7b**) are the most potent inhibitors. It could be observed that the position of these halogenated substituents on the phenyl ring does affect drastically the inhibitory activity. The presence of a bromine substituent on the phenyl ring instead of hydrogen does not affect the inhibitory activity in the whole series of analogues. One explanation for these observed differences in inhibitory potency could be supported by the differences in lipophilicity inside this new series of analogues. The presence of a substituted phenyl ring (compounds **5a**, **6a**, **5b** and **6b**) increased their corresponding $\log P$ values ranging between 4 and 5, suggesting that these substituted phenyl moieties seem particularly suitable for simultaneously fit the S1 and S'I

pocket, while favourably positioning the protonated nitrogen atom closed to the catalytic diad Asp32 and Asp228. Compounds having lower $\log P$ values (compounds **9a**, **11a** and **11b**) or having higher $\log P$ values are less potent BACE-1 inhibitors. In conclusion, introduction of suitable substituents at the $N_4$ position of $N$-substituted piperazine naphthamide allowed to modulate their corresponding BACE-1 inhibitory activity, mainly hydrophobic aromatic substituents.

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