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BACE-1 inhibitory activities of new substituted phenyl-piperazine coupled to various heterocycles: Chromene, coumarin and quinoline

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Abstract—The protease β-secretase plays a central role in the synthesis of pathogenic amyloid-β in Alzheimer's disease. Here, we report a new series of analogues based on the phenyl-piperazine scaffold coupled to various heterocyclic moieties, which demonstrate improved inhibitory activities on BACE-1 (FRET assay) compared to already known naphthyl counterparts. The obtained results suggest further structural modifications to access to more potent BACE-1 inhibitors.

Alzheimer's disease (AD) is the biggest unmet medical need in neurology, with >12 million AD sufferers worldwide. Actually the 'amyloid cascade hypothesis' claimed that amyloid β-42 (Aβ42), a proteolytic derivative of the large transmembrane protein (APP), plays an early and crucial role in all cases of AD. Aβ42 forms aggregates that are thought to initiate a pathogenic cascade that ultimately leads to neuronal loss and dementia.[1] Two key enzymes β-secretase (BACE-1 and 2)[2] and γ-secretase have been identified as ideal targets for therapeutic intervention.[3] Since cell-based screens in which cells expressing the endogenous β- and γ-secretases overexpressing human APP were exposed to different drugs, it was possible to observe a reduction in Aβ42 production. Numerous compounds reducing amyloid peptide production have been reported so far in the scientific and patent literature.[4–6] Unfortunately inhibition of γ-secretase is associated with toxicity issues in both animal models and in clinic,[7,8] mainly because γ-secretase is an unusual transmembrane protease complex, which involved four components including presinilin. As presinilins are also involved in Notch cleavage, specific inhibitors, which do not affect Notch pathway, are required, since this later pathway plays an important role in development stages.[9] For this reason, β-secretase appears to be a therapeutic promise. As far as a only few mammalian aspartic proteases exist, it could be emphasized that the number of enzymes that could be cross-inhibited and lead to toxic side effects is restricted. Moreover after analyzing the phenotype of young BACE knockout mice, it was found that these latter were healthy and fertile.[10]

In this paper, we wish to report the synthesis and the β-secretase inhibitory activity of new nonpeptidomimetic derivatives which incorporate in their structure various heterocyclic moieties: coumarin, quinoline or chromene. These moieties were linked through an amide bond to substituted phenyl-piperazine scaffold (Fig. 1) Through the present study our objective was to define some fundamental structural requirements which will allow us to design in second round of synthesis, new analogues with optimized BACE-1 inhibitory properties.

These new analogues were designed following the disclosure by Vertex via a published patent application,[11,12] of several BACE-1 inhibitors based on substituted phenyl-piperazine scaffold but linked to a naphthyl moiety (Fig. 1). Taking into account that our new analogues have relatively the same topological shape as Vertex compounds, we believe that the substitution of the

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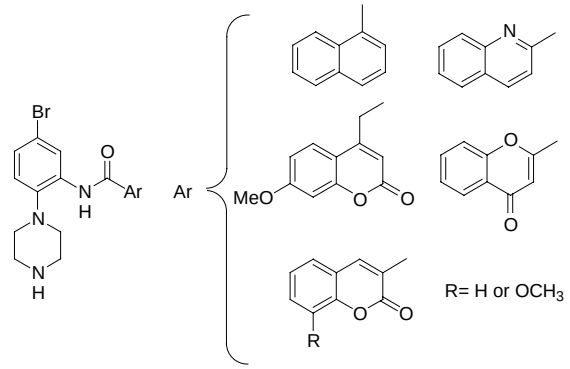


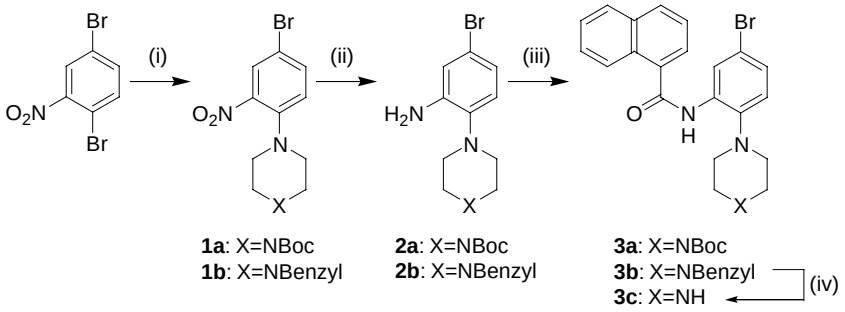
Figure 1. Phenyl-piperazine scaffold general structure.

naphthyl moiety by heterocyclic ones could induce changes in polarity, conformation, and hydrogen-bonding potential, which can infer more specific interactions within the active site of the β-secretase and subsequently improved the inhibitory properties.

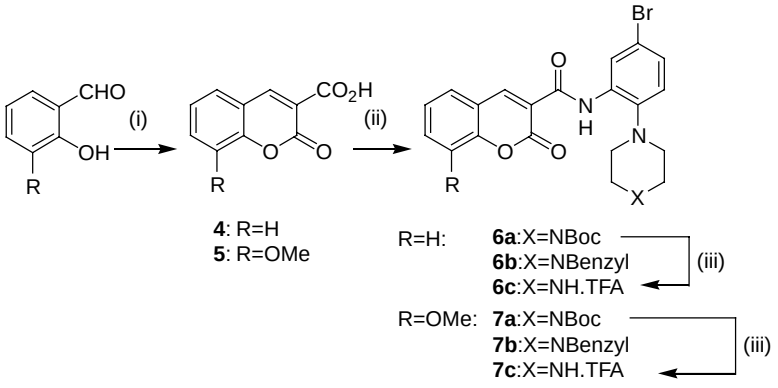
Both series of compounds required common intermediates **2a**,**b**, which were obtained through sequences described in Scheme 1. Compounds **1a**,**b** were obtained in quite good yields by condensation of commercially available 2,5-dibromo-nitrobenzene with *N*-Boc-piperazine or *N*-benzyl-piperazine in refluxed isopropanol.[13] Reduction of the nitro group achieved using Zn powder in KH_2PO_4 in refluxed THF[14,15] led to good yields of the expected aniline derivatives, **2a**,**b**. These anilines were subsequently acylated with 1-naphthoyl chloride affording intermediates **3a**,**b**. The *N*-benzyl protecting group of compound **3b** was removed by using ammonium formate as hydric source and Pd/C as catalyst.[16,17]

Coumarin-3-carboxylic acids **4** and **5** were obtained in high yields from the corresponding *ortho*-hydroxybenzaldehydes and Meldrum's acid in refluxed ethanol (Scheme 2).[18] N-Acylation of anilines **2a**,**b** by the carboxylic acids **4** or **5** was achieved by using phosphorus oxychloride ($POCl_3$) in pyridine as coupling system at temperature ranging from −20 to 20 °C, as we recently reported[19] leading to the corresponding adducts **6a,b** and **7a**,**b** in satisfactory yields. Standard removal of the *N*-Boc protecting groups of **6a** and **7a** allowed the isolation of the corresponding TFA salts **6c** and **7c**.

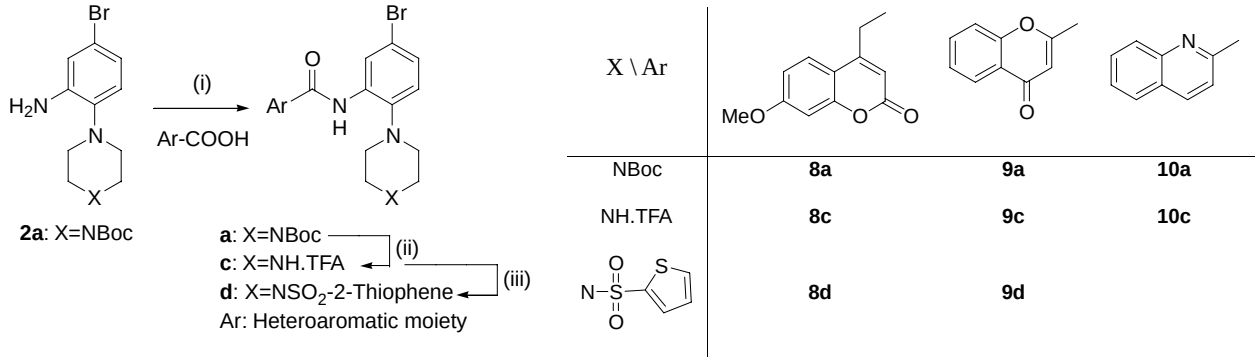
Aniline **2a** was then N-acylated by different commercially available carboxylic acids, 7-methoxycoumarin-4-acetic acid, chromene-2-carboxylic acid and with 2-quinoline carboxylic acid (Scheme 3). N-acylation was achieved using phosphorus oxychloride ($POCl_3$) in pyridine as coupling reagent at temperature ranging from −20 °C to room temperature[19] leading to the corresponding *N*-Boc intermediates **8a**, **9a** and **10a** in satisfactory yields. Standard removal of the *N*-Boc protecting group allowed the isolation of the corresponding TFA



Scheme 1. Reagents and conditions: (i) *N*-Boc-piperazine or *N*-benzyl-piperazine, *i*-PrOH, TEA, reflux, 12 h; (ii) Zn powder, 1 M KH_2PO_4/THF (v/v, 1:6), reflux, 8 h; (iii) 1-naphthoylchloride, CH_2Cl_2, TEA, rt, 2 h; (iv) HCO_2NH_4, 10% wt. Pd/C, CH_3OH, reflux, 6 h.



Scheme 2. Reagents and conditions: (i) Meldrum's acid, piperidinium acetate, EtOH, reflux, 2 h; (ii) **2a**,**b**, $POCl_3$, pyridine, −20 °C to rt; (iii) TFA, CH_2Cl_2, rt, 12 h.



Scheme 3. Reagents and conditions: (i) $POCl_3$, pyridine, −20 °C to rt; (ii) TFA, CH_2Cl_2, rt, 12 h; (iii) Cl-SO_2-2-thiophene, $NaHCO_3$, CH_2Cl_2, rt, 4 h.

salts **8c**, **9c** and **10c** followed by sulfonylation of **8c** and **9c** with 2-thiophene sulfonyl chloride[20] in the presence of $NaHCO_3$ in methylene chloride to give, respectively, compounds **8d** and **9d**.

All intermediates and final compounds of both series were fully characterized by ^1H NMR, ^{13}C NMR and MS (data available on request).

All the compounds were assayed as BACE-1 inhibitors, using a fluorescence resonance energy transfer (FRET) assay, which uses purified baculovirus-expressed (BACE-1) and a specific substrate (Rh-EVNL-DAEFK-quencher) based on the Swedish mutation of the amyloid precursor protein (APP). This peptidic substrate becomes highly fluorescent upon enzymatic cleavage.

IC_{50} inhibition values were determined at least three times. The IC_{50} values obtained for both naphthyl and coumarinyl series of compounds are summarized in Table 1.

In order to compare the BACE-1 inhibitory activities of the new heterocyclic derivatives **6a**–**c**, **7a**–**c**, **8a**,**c**,**d**, **9a**,**c**,**d** and **10a**,**c** with those of described Vertex derivatives, we synthesized as bench-marking some Vertex compounds **3a**–**c** according to the known procedures.[9]

From the obtained results it can be seen that rough replacement of the naphthyl group by chromone, coumarin or quinoline moieties does not significantly impair the inhibitory activity of the resulting derivatives since the IC_{50} values for the whole series of compounds ranged from 0.09 to 5 μM.

Furthermore, some of the new synthesized analogues belonging to the coumarinyl series (**6b**,**c**) were found to be more active than the corresponding Vertex analogues. The most potent inhibitor **8a** with an IC_{50} of 0.093 μM is one log more active than its corresponding naphthyl analogue **3a** (IC_{50} value of 1.7 μM).

Moreover, the coumarinyl inhibitor **8a** is linked to the amide function through a methylene spacer. This observation could be of interest to design new series of analogues in which various lengths of linkers could be introduced between the heterocycles and the carbonyl of the amide group.

Another observation stands on the influence of the position by which the coumarine moiety is linked to the amide group. Position 4 in the case of analogue **8a** appears to be the most favourable, while position 3 for compounds bearing a *N*-Boc group on the piperazine ring (**6a** and **7a**) is a slightly less active inhibitor.

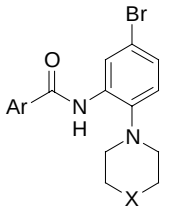
It could be also observed that at least in this in vitro enzymatic assay, the hydrophobicity of the derivatives exemplified by C log *P* does not appear to be a determinative parameter in the BACE-1 inhibitory activities. Indeed, no correlation between C log *P* values and inhibitory activities can be deduced from the obtained results. Nevertheless, for a hypothetical therapeutical use, taking into account the well-known rule of five of Lipinsky et al.,[21] compounds with C log *P* values lower than 5 should have improved drug ability properties compared to more hydrophobic analogues (C log *P* greater than 5).

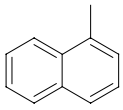
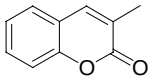
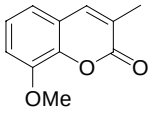
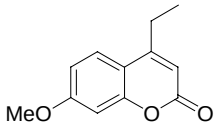
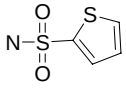
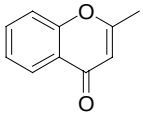
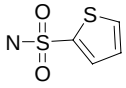
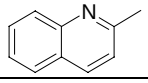
From these results it could be now possible to envisage the design of new BACE-1 inhibitors:

Coumarinyl analogues in which the coumarinyl moiety should be linked through different lengths of linkers to the amide function.
The linkers between the coumarin nucleus and the amide function should be preferably branched at the 4-position of the coumarinyl moiety.
Substituents on the nitrogen atom of the piperazine ring (H; Boc; benzyl; and SO_2-2-thiophene) could be also important features to consider in the design of optimized BACE-1 inhibitor candidates.

In conclusion, the obtained data suggest that starting from an initial structure (Vertex like analogues), there is some latitude with regard to functional group identity (coumarin, chromene and quinoline) that allows further refinement of inhibitory potency relative to β-secretase (BACE-1). BACE inhibitor **8a** is at this moment a typical example of refined structure.

Table 1. BACE-1 inhibition activity of phenyl-piperazine derivatives



Compound	Ar	X	IC_{50}[a] (μM)	Clog *P*
3a		*N*-Boc	1.7*	5.44
3b		*N*-Benzyl	5.2*	6.44
3c		NH·TFA	1.0*	4.33
6a		*N*-Boc	1*	4.09
6b		*N*-Benzyl	0.76**	5.10
6c		NH·TFA	0.67**	2.99
7a		*N*-Boc	2.4*	3.97
7b		*N*-Benzyl	5.5*	4.97
7c		NH·TFA	5.0*	2.86
8a		*N*-Boc	0.093**	3.74
8c		NH·TFA	1.7*	2.63
8d			1.13**	4.19
9a		*N*-Boc	1.9*	3.12
9c		NH·TFA	1.6*	2.02
9d			2.6*	3.58
10a		*N*-Boc	1.7*	5.95
10c		NH.TFA	3.5*	3.84

[a] The BACE-1 fluorescence resonance energy transfer assay kit was purchased from PanVera (Madison, WI; 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 (SEM). $p < 0.05$ (*) and $p < 0.01$ (**). Compound **3c** was used as a standard reference in BACE-1 FRET assay.

Acknowledgments

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References and notes

1. Hardy, J.; Selkoe, D. J. *Science* **2002**, *297*, 353.
2. Vassar, R.; Bennett, B. D.; Babu-Khan, S.; Khan, S.; Mendiaz, E. A.; Denis, P.; Teplow, D. B.; Ross, S.; Amarante, P.; Loeloff, R.; Luo, Y.; Fisher, S.; Fuller, J.; Edenson, S.; Lile, J.; Jarosinski, M. A.; Leona Biere, A.; Curran, E.; Burgess, T.; Louis, J.-C.; Collins, F.; Treanor, J.; Rogers, G.; Citron, M. *Science* **1999**, *286*, 735.
3. De Strooper, B. *Neuron* **2003**, *38*(1), 9.
4. Schimdt, B. *ChemBioChem* **2003**, *4*, 366.
5. Thompson, L. A.; Bronson, J. J.; Zusi, F. C. *Curr. Pharm. Des.* **2005**, *11*, 3383.
6. Cumming, J. N.; Iserloh, U.; Kennedy, M. E. *Curr. Opin. Drug Discovery Dev.* **2004**, *7*, 536.
7. Wong, G. T.; Manfra, D.; Poulet, F. M.; Zhang, Q.; Josien, H.; Bara, T.; Engstrom, L.; Pinzon-Ortiz, M.; Fine, J. S.; Lee, H. J.; Zhang, L.; Higgins, G. A.; Parker, E. M. *J. Biol. Chem.* **2004**, *279*, 12876.
8. Orgogozo, J. M.; Gilman, S.; Dartigues, J. F.; Laurent, B.; Puel, M.; Kirby, L. C.; Jouanny, P.; Dubois, B.; Eisner, L.; Flitman, S.; Michel, B. F.; Boada, M.; Frank, A.; Hock, C. *Neurology* **2003**, *61*, 46.
9. Sisodia, S. S.; St. George-Hyslop, P. H. *Nat. Rev. Neurosci.* **2002**, *3*, 281.
10. Roberds, S. L.; Anderson, J.; Basi, G.; Bienkowski, M. J.; Branstetter, D. G.; Chen, K. S.; Freedman, S. B.; Frigon, N. L.; Games, D.; Hu, K.; Johnson-Wood, K.; Kappen-

man, K. E.; Kawabe, T. T.; Kola, I.; Kuehn, R.; Lee, M.; Liu, W.; Motter, R.; Nichols, N. F.; Power, M.; Robertson, D. W.; Schenk, D.; Schoor, M.; Shopp, G. M.; Shuck, M. E.; Sinha, S.; Svensson, K. A.; Tatsuno, G.; Tintrup, H.; Wijsman, J.; Wright, S.; McConlogue, L. *Hum. Mol. Genet.* **2001**, *10*, 1317.
11. Bhisetti, G. R.; Saunders, J. O.; Murcko, M. A.; Lepre, C. A.; Britt, S. D.; Come, J. H.; Deninger, D. D.; Wang, T. PCT Int. Appl. WO 02/088101, 2002.
12. Park, H.; Lee, S. *J. Am. Chem. Soc.* **2003**, *125*, 16416.
13. Tobe, M.; Isobe, Y.; Tomizawa, H.; Nagasaki, T.; Obara, F.; Matsumoto, M.; Hayashi, H. *Chem. Pharm. Bull.* **2002**, *50*, 1073.
14. Babler, J. H.; Sarussi, S. J. *Synth. Commun.* **1981**, *11*, 925.
15. Ono, A.; Terasaki, S.; Tsuruoka, Y. *Chem. Ind. (London)* **1983**, 477.
16. Ram, S.; Spicer, L. D. *Synth. Commun.* **1987**, *17*, 415.
17. Ram, S.; Spicer, L. D. *Tetrahedron Lett.* **1987**, *28*, 515.
18. Song, A.; Wang, X.; Lam, K. S. *Tetrahedron Lett.* **2003**, *44*, 1755.
19. Quéléver, G.; Burlet, S.; Garino, C.; Pietrancosta, N.; Laras, Y.; Kraus, J.-L. *J. Comb. Chem.* **2004**, *6*, 695.
20. Clare, B. W.; Scozzafava, A.; Supuran, C. T. *J. Med. Chem.* **2001**, *44*, 2253.
21. Linpinsky, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev.* **1997**, *23*, 3.