Naphthyl and Coumarinyl Biarylpiperazine Derivatives as Highly Potent Human β-Secretase Inhibitors. Design, Synthesis, and Enzymatic BACE-1 and Cell Assays

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Twenty novel β-secretase inhibitors containing biarylpiperazine moieties belonging to naphthyl and coumarinyl series were designed for their potential use in Alzheimer's disease therapy. Enzymatic and cell-based assays have been carried out. The biological results clearly demonstrate that specific substituents located at the N_4-position of the piperazine ring result in excellent in vitro inhibitory potency (IC_{50} values ranging between 40 and 70 nM). Variable temperature NMR and modeling studies are consistent with the obtained biological data, since these studies confirmed that introduction at the N_4-position of the piperazine ring allows productive interactions within the BACE-1 active site, which appear to be determinative for high BACE-1 inhibitory activity. These results are of particular interest since some of the new analogues belonging to the naphthyl series are almost one log more active than the best inhibitor of the similar family recently reported.

Introduction

As the number of cases of Alzheimer's disease (AD) rises in developed countries, the unmet medical need for disease-modifying pharmacotherapy continues to grow. Much of AD research has been focused on the amyloid cascade hypothesis, which states that amyloid-β-42 ($A\beta_{42}$), a proteolytic derivative of the large transmembrane protein amyloid precursor protein (APP), plays an early role in any case of AD. Consequently, blocking the production of $A\beta_{42}$ by specific inhibition of key proteases required for $A\beta_{42}$ 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.[1] Taking into account those observations developing β-secretase inhibitors appears to be more encouraging.

β-Secretase is an aspartic protease that generates the N-terminus of $A\beta_{42}$[2] whose therapeutic promise is based on the absence of major problems as a result of β-secretase ablation. Indeed, the phenotype of young BACE knock-out mice was analyzed, and they were found to be healthy and fertile.[3] Taken together, the absence of Aβ production and distinct pathology in the BACE knock-out mice led us to search for β-secretase drug inhibitors, particularly in view of the situation regarding the γ-secretase target. An overview on the design and development of β-amyloid cleaving enzyme-1 (BACE-1) has been recently published by Cumming et al.[4]

Several types of BACE-1 inhibitors are reported:

(i) Substrate-Based BACE-1 Inhibitors: The first generation of these inhibitors was predominantly of peptidic nature which precludes their full development as AD drugs. Less peptidic second generation inhibitors are under investigation.[5]

(ii) Statin and Homostatine BACE-1 Inhibitors. These types of inhibitors have been mostly developed by Elan and Pharmacia & Upjohn. (Note: 18 patents have been published in the (homo)-statine field but are not listed in this paper).

(iii) Hydroxyethylamine (HEA) BACE-1 Inhibitors. The HEA dipeptide isostere is a known motif of the aspartyl protease inhibitors, notably from early research on HIV protease and renin inhibitors. Elan, Pharmacia, Bristol Myers Squibb, and Takeda chemical industries have made important contributions with closely related core structures. Several patent applications have been disclosed.

(iv) Other Inhibitors. These include various piperidine derivatives developed by different pharmaceutical companies such as Vertex, Actelion Pharmaceuticals, De Novo Pharmaceuticals, Elan, and Pharmacia. These BACE-1 inhibitors have been recently reviewed.[4]

From this survey, it appears that the ideal properties of a BACE-1 inhibitor for clinical use are (i) ability to potently and effectively lower Aβ in transgenic mice, (ii) CNS penetration, (iii) oral bioavailability, (iv) good pharmacokinetic profile, and finally (v) selectivity against other aspartyl proteases.

To overcome the historical problems associated with peptide like structures such as low oral bioavailability, poor blood−barrier permeability, and susceptibility to P-glycoprotein transport,[6] we chose to focus on the identification of nonpeptide or pseudopeptide inhibitors. In this perspective, starting from already published data on arylpiperazine amide scaffolds as a possible framework for the design of β-secretase inhibitors, we investigated original and judicious modifications at different positions on the aryl piperazine scaffold. Molecular modeling and the Protein Data Bank revealed that the arylpiperazine scaffold allows the design of a large diversity of BACE-1

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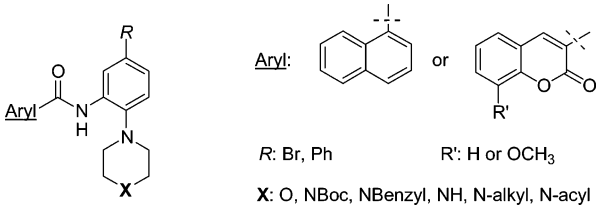


Figure 1. Arylpiperazine amide scaffolds.

inhibitors through the substitution possibilities offered at various positions as shown on Figure 1.

Among the numerous substitution possibilities we have deliberately limited our project to two series of analogues: on one hand, the naphthyl series which has been already studied, and, on the other hand, the coumarinyl series (Figure 1). In the original coumarin series, the replacement of the naphthyl moiety by a coumarin nucleus could rigidify the resulting structure through a possible hydrogen bonding between the carbonyl of the lactone and the NH group of the amide functional group. Such hydrogen bonding possibility could favor specific conformations, which could improve or impair the inhibitory activity of the resulting derivatives. Indeed, individual rotamers of certain drugs involving amide bonds could be required for high-affinity receptor binding[7] or for high-enzymatic active affinity within the β-secretase active site. The rotational process is largely governed by steric and electronic effects provided by substituents around the C−N bond. In the course of our synthetic chemistry program to design these β-secretase inhibitors, variable temperature NMR studies revealed that some interactions between the amide group and the amino group at the 4-position of the piperazine ring most likely occurred in both naphthyl and coumarin series rather than hydrogen bonding with the carbonyl of the lactone ring. 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 substituents.

Chemistry

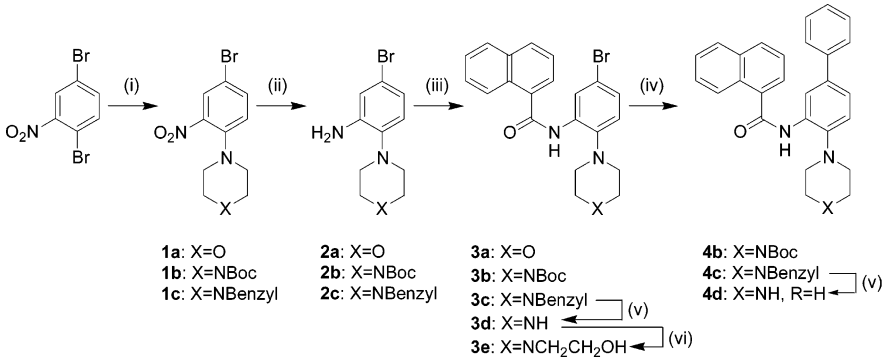
Both series of compounds required common intermediates **2a**−**c**, which were obtained through sequences described in Scheme 1.

Compounds **1a**−**c** were obtained in quite good yields by condensation of commercially available 2,5-dibromonitrobenzene with morpholine, *N*-Boc piperazine, or *N*-benzyl piperazine in refluxed 2-propanol.[8] Reduction of the nitro group was assayed under different conditions in order to optimize the yields. Indeed, classical catalytic hydrogenation with palladium on charcoal led to the loss of the bromine atom, while other conditions, such as $NiCl_2/NaBH_4$ in CH_3OH/CH_2Cl_2[9] or Fe/NH_4Cl in refluxed ethanol,[10,11] led to low yields of desired compounds. In contrast, the use of Zn powder in KH_2PO_4 in refluxed THF[12,13] led to high yields of the expected aniline derivatives **2a**−**c**. These anilines were subsequently acylated with 1-naphthoyl chloride affording intermediates **3a**−**c**. The *N*-benzyl protecting group of compound **3c** was removed by using ammonium formate as hydric source and Pd/C as catalyst.[14] The resulting amino derivative **3d** was alkylated[15] with bromoethanol to give **3e**. Phenyl substituents were then introduced on derivatives **3b** and **3c** through a Suzuki cross-coupling reaction[16,17] with the commercially available phenyl boronic acid, leading to the corresponding biphenyl piperazine derivatives **4b** and **4c**. This reaction represents the limiting step of this synthetic scheme. We observed that the yield of this reaction depends on the nature of the substituent located at the N_4-position of the piperazine ring. Compound **4c** bearing an *N*-benzyl substituent was obtained in high yields while compound **4b** with a *N*-Boc protecting group was isolated in low yield. Compound **4c** was then deprotected with ammonium formate[14] to afford the corresponding free amino derivative **4d**.

This key intermediate **4d** was subsequently functionalized with various halide derivatives or carboxylic acids (Scheme 2). Compound **4d** was N-alkylated[15] with different bromide or chloride derivatives such as bromoethanol, ethyl bromoacetate, *N*-(5-bromopentyl)phthalimide, (*S*)-(−)-3-chloro-1-phenyl-1-propanol, or (*R*)-(+)-3-chloro-1-phenyl-1-propanol, in the presence of potassium carbonate in acetonitrile to give respectively compounds **5a**, **5b**, **5c**, **5e**, and **5f**. Compound **5d** was obtained after removal of the phthalimide protecting group of **5c** by hydrazine monohydrate in ethanol.[18] N-Acylation of **4d** by benzoyl chloride led to compound **6a**, while **6b** was isolated in good yield by acylation of (±)-α-lipoic acid[19] using 1,3-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) as coupling system.

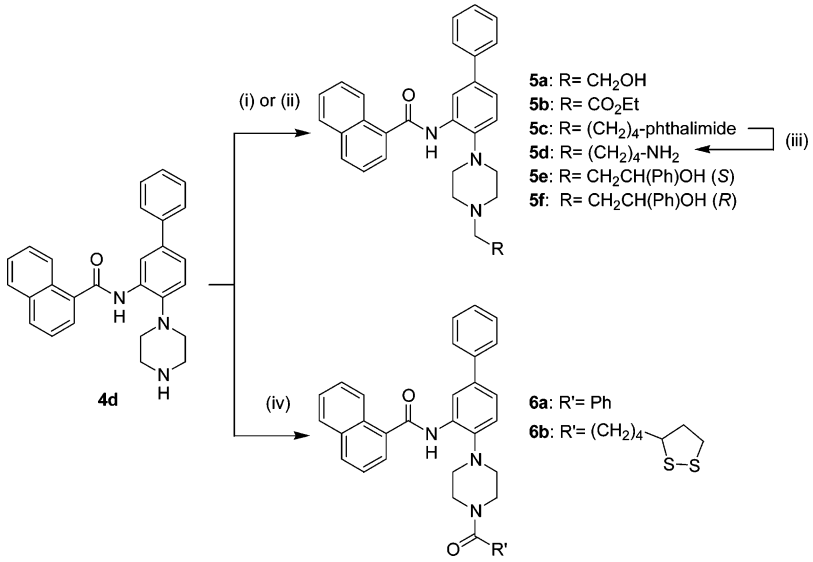
Coumarin-3-carboxylic acids **7** and **8** were obtained in high yields from the corresponding *o*-hydroxybenzaldehydes and Meldrum's acid in refluxed ethanol (Scheme 3).[20] N-Acylation of anilines **2a**−**c** by the carboxylic acids **7** or **8** was achieved by using phosphorus oxychloride ($POCl_3$) in pyridine as a coupling system at a temperature ranging from −20 °C to room temperature.[21] The corresponding adducts **9a**−**c** and **10a**−**c** were obtained in satisfactory yields. Standard removal of the *N*-Boc protecting group of **9b** and **10b** allowed the isolation of the corresponding TFA salts **9d** and **10d** which were subsequently N-alkylated with bromoethanol[15] to give respectively **9e** and

Scheme 1[a]



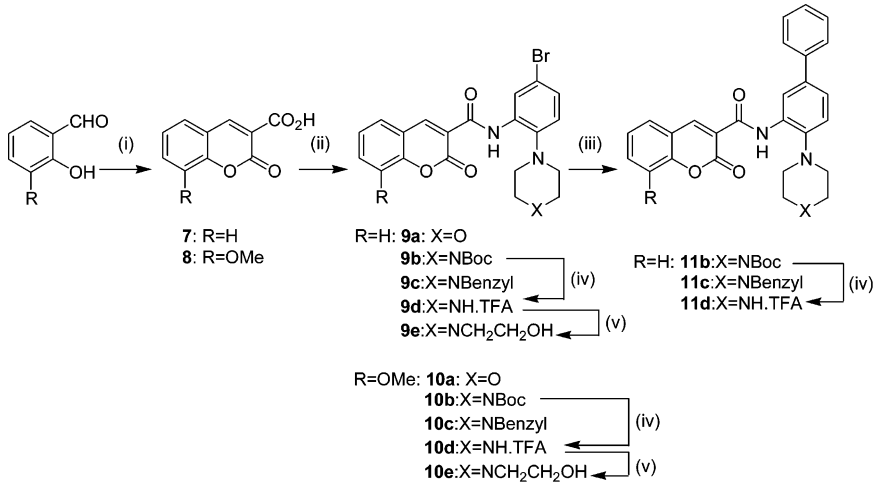
[a] Reagents and conditions: (i) morpholine (**a**) or *N*-Boc-piperazine (**b**) or *N*-benzylpiperazine (**c**), ^{i}PrOH, TEA, reflux, 12 h; (ii) Zn powder, 1 M KH_2PO_4/THF (v:v 1:6), reflux, 8 h; (iii) 1-naphthoyl chloride, CH_2Cl_2, TEA, rt, 2 h; (iv) phenyl boronic acid, 1,4-dioxane, dppf, dppfPd^{II}, KOAc, 80 °C, 24 h; (v) HCO_2NH_4, 10 wt % Pd/C, CH_3OH, reflux, 6 h; (vi) $BrCH_2CH_2OH$, CH_3CN, K_2CO_3, 60 °C, 12 h.

Scheme 2[a]



[a] Reagents and conditions: (i) $BrCH_2R$, CH_3CN, K_2CO_3, reflux, 12 h; (ii) $ClCH_2CH_2CH(Ph)OH$ (*S*) or (*R*), CH_3CN, K_2CO_3, 35 °C, 24 h; (iii) $H_2N-NH_2 \cdot H_2O$, EtOH, reflux, 6 h; (iv) $R'CO_2H$, DCC, HOBt, DMAP, rt, 2 h.

Scheme 3[a]



[a] Reagents and conditions: (i) Meldrum's acid, piperidinium acetate, EtOH, reflux, 2 h; (ii) **2a**−**c**, $POCl_3$, pyridine, −2 0°C to room temperature; (iii) phenyl boronic acid, 1,4-dioxane, dppf, $dppfPd^{II}$, KOAc, 80 °C, 24 h; (iv) TFA, CH_2Cl_2, rt, 12 h; (v) $BrCH_2CH_2OH$, CH_3CN, K_2CO_3, 60 °C, 12 h.

10e. Bromophenyl piperazine **9b** and **9c** were involved in a Suzuki cross-coupling reaction[16,17] in order to synthesize the corresponding biphenyl piperazine derivatives **11b** and **11c**. The *N*-Boc protected biphenylpiperazine analogue was treated by TFA to ensure the removal of the protecting group and to isolate the corresponding TFA salt **11d**.

NMR Studies

The magnetic anisotropy induced by the amide function has been the subject of numerous NMR studies.[22] This anisotropic effect might be used to predict the preferential conformation around N−Cα bond. The interconversion of diastereotropic ^1H NMR signals can be followed by variable temperature nuclear magnetic resonance (VT NMR) techniques. In some cases, coalescence of peaks arising from Ar−CO and/or C−N rotations can be observed. Two pairs of analogues belonging to both the naphthyl (**3b** and **3d**) and coumarin (**10b** and **10d**) series were selected for this study.

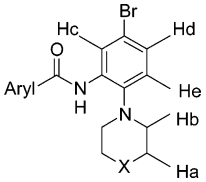
The NMR spectra of compounds dissolved in DMSO-d_6 were recorded at various temperatures ranging from 30 to 130 °C. Variations in chemical shifts and/or coalescence of signals for representative piperazine ring protons Ha, Hb or for aromatic protons Hc, Hd, and He of the halogenated phenyl ring were observed (Table 1).

For compounds unsubstituted at the N_4-position of the piperazine ring (**3d** and **10d**), no significant temperature effects on the chemical shifts as well as on the patterns of signal coalescence for the protons Ha, Hb, Hc, and Hd were observed. We concluded that the hypothesis that replacing a naphthyl moiety by a coumarin, to favor the formation of a hydrogen bond between the NH of the exocyclic amide and the carbonyl group of the coumarin moiety to hinder the rotation around N−Cα or Ar−CO bonds, was not fulfilled.

In contrast, VT NMR carried out for compounds bearing a Boc substituent at the N_4-position of the piperazine ring (**3b** and **10b**) induced chemical shift variations, broadened line shapes, and coalesced signals of some of the specific protons Ha, Hb, Hc, Hd, and He. In Table 1 we report chemical shifts and patterns of signal coalescence of identified protons.

As it can be observed, for compounds (**3d** and **10d**) with a free amino group at the N_4-position of the piperazinyl ring, no pattern of coalescence occurred at 130 °C. In contrast, for the

Table 1. VT NMR of Analogues **3b**, **3d**, **10b**, and **10d** in DMSO-d_6[a]



	aryl/X	*T*, °C	δ Ha	δ Hb	δ Hc	δ Hd	δ He
3b	1-naphthyl/NBoc	100	n. d.	n. d.	8.43 (d)	7.37 (q)	7.23
		110	n. d.	n. d.	8.42 (bs)	7.37 (bd)	7.22
3d	1-naphthyl/NH	110	3.40	2.87	8.47	7.32	7.22
		130	no change				
10b	**8**/NBoc	100	3.31 (bs)	3.30 (bs)	8.70	7.32	7.24
		110	3.64 (t)	2.86 (t)	8.69	7.28	7.22
10d	**8**/NH	100	3.64 (t)	2.86 (t)	8.69	7.28	7.21
		130	no change				

[a] t = triplet, bs = broad singlet, q = quintet, bd = broad doublet; d =doublet, n.d. = not determined; δ = chemical shift (ppm).

corresponding *N*-Boc protected analogues (**3b** and **10b**), between 100 °C to 130 °C, a pattern of coalescence is observed for some signals of the piperazinyl ring (**10b**) as well as for the signals of the aromatic ring (**3b**).

These results suggest that some substituents at the N_4-position of the piperazine ring stabilize the interconversion of the different possible conformations of the piperazine ring, favoring the most energetically stable conformation. It cannot be excluded that the presence of a bulky group at the N_4-position of the piperazine ring could also affect the Ar−CO and C−N bond rotations. The same effects are not observed in the case of free N_4-amino analogues.

Molecular Modeling 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 OM-2,[23] since the structure of this complex has allowed the identification of critical ligand−active site interactions. The 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 (nonpeptidic) have been performed. The most detailed account of nonpeptidic BACE inhibitors comes from Vertex Pharmaceuticals Inc.,[24a] 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 **4d**, in which the N_4-position of the piperazine ring is free. Vertex proposed the first 3-D pharmacophore map of BACE.[24a] Hydrogen interactions within the active site and with other key residues, as well as other hydrophobic interactions with BACE subsites, were determined. Unfortunately, crystallographic data to substantiate this 3-D model was not provided. Simulated molecular dynamics analysis of superimposition of analogue **4d** and its coumarin analogue **9c** in their low energy structures (not shown) reveals only small distortions between both structures, indicating that both structures can make direct contact through amide hydrogen bonding interactions with the catalytic aspartic acids Asp 32 and Asp 228.[23]

Compounds **4d** and **9c** were placed in the most favorable position in the BACE-1 active site, allowing both hydrogen bonding within the active site as well as hydrophobic interactions with enzyme subsites (Figure 2). To make Figure 2 more clear,

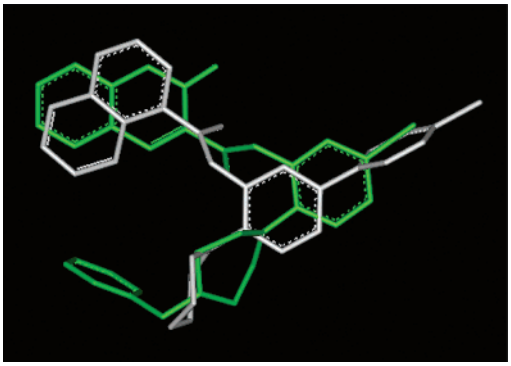


Figure 2. Superposition of **4d** and **9c** structures placed in their favored position within the BACE-1 active sites.

enzyme residues were not represented; only the superimposition of the inhibitors **4d** (white) and **9c** (green) placed in their best fitting position is shown.

From this representation, it appears that the N_4-position of the piperazine ring could be suitable to accommodate various substituents capable of additional interactions with enzyme subsites. Taking into account that such N_4-substituted analogues have never been described in both series, aryl naphthyl or coumarinyl, N_4-piperazine substitution represented an opportunity to design new optimized β-secretase inhibitors.

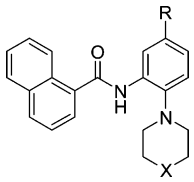
Biological Results and Discussion

Enzymatic Assay Evaluation. 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-EVNLDAEFK-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 six times. The IC_{50} values obtained for both naphthyl and coumarinyl series of compounds are summarized respectively in Tables 2 and 3.

1-Aryl Naphthyl Series. Identification of nonpeptidomimetic BACE inhibitors, has led to the discovery of biaryl N-substituted piperazine naphthalenes. Among the compounds described by Vertex Pharmaceuticals Inc.,[24] compound **4d** was reported as one of the most potent. We used this compound as the standard inhibitor in this study. From Table 2, it can be seen that monoaryl analogues **3a**−**d** are less active inhibitors than their biaryl counterparts, with the exception of compound **3e**. These results lead to the finding that the lack of a biphenyl moiety is detrimental for β-secretase inhibitory activity. In contrast in this series, substitution at the N_4-position of the piperazinyl moiety was determinative for inhibitory activity. Specific substituents induced inhibitory activities with IC_{50} values ranging from 0.07 to 0.1 μM, as illustrated by the inhibitory potencies found for compounds **5a**−**f** and **6a** which are more active than the reference compound **4d** (IC_{50} = 0.3 μM). The most active compound **5f** shows an IC_{50} value of 0.046 μM. These results are of particular interest since to our knowledge; analogues incorporating a wide diversity of substituents at the N_4-position of the piperazinyl have not been described.[24]

The observed enhancement of the inhibitory potency of these N_4-substituted analogues is supported by modeling conformational analysis as well as by NMR studies. Modeling studies have shown that BACE-1 active site allows quite a large diversity of substituents at the N_4-position of the piperazinyl ring. These substituents induce conformational changes at the piperazinyl ring level which could influence the planar position

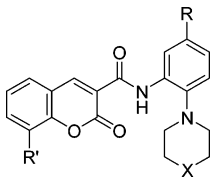
Table 2. BACE-1 Inhibition Activity of Naphthyl Series



	R	X	BACE-1[a] IC_{50} (μM)	cLogP
3a	Br	O	>20	4.55
3b	Br	*N*-Boc	>1	5.44
3c	Br	*N*-benzyl	>5	6.44
3d	Br	NH	1*	4.33
3e	Br	NCH_2CH_2OH	0.079**	4.19
4b	Ph	*N*-Boc	nd	6.84
4c	Ph	*N*-benzyl	1.25*	7.84
4d	Ph	NH	0.30**	5.73
5a	Ph	NCH_2CH_2OH	0.11**	5.59
5b	Ph	NCH_2CO_2Et	0.09**	5.98
5c	Ph	$N(CH_2)_5$-phthalimide	0.10**	7.83
5d	Ph	$N(CH_2)_5NH_2$	0.068**	6.19
5e	Ph	$NCH_2CH_2CH(Ph)OH$ (*S*)	0.069**	7.41
5f	Ph	$NCH_2CH_2CH(Ph)OH$ (*R*)	0.046**	7.41
6a	Ph	NC(O)Ph	0.079**	7.28
6b	Ph	$NC(O)(CH_2)_4$-dithiolan	3.8*	7.77

[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 $p < 0.01$ (**).

Table 3. BACE-1 Inhibition Activity of Coumarin Series



	R′	R	X	Bace-1[a] IC_{50} (μM)	cLogP
9a	H	Br	O	>10	3.21
9b	H	Br	N-Boc	1*	4.09
9c	H	Br	N-benzyl	0.76**	5.10
9d	H	Br	NH·TFA	0.67**	2.99
9e	H	Br	NCH_2CH_2OH	0.63**	2.85
10a	OMe	Br	O	>10	3.08
10b	OMe	Br	N-Boc	>1	3.97
10c	OMe	Br	N-benzyl	>5	4.97
10d	OMe	Br	NH·TFA	5*	2.86
10e	OMe	Br	NCH_2CH_2OH	1*	2.72
11b	H	Ph	N-Boc	10	5.50
11c	H	Ph	N-benzyl	0.151**	6.50
11d	H	Ph	NH·TFA	0.58**	4.39

[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 (s.e.m). $p < 0.05$ (*) and $p < 0.01$ (**).

of the piperazinyl ring relative to the naphthyl planar ring, generating specific favored conformations to the resulting molecule. These later conformations may allow better productive interactions through hydrogen bonding within the active site. The presence of hydrogen bonding donnors (hydroxyl or amino groups introduced at the N_4-piperazinyl position (compounds **5d** and **5e**) may have favored those conformations.

It should also be noted, that introduction of a chiral center in the side chain at the N_4-piperazinyl position does not significantly influence the observed inhibitory activity of the resulting derivatives. As illustrated on Table 2, enantiomers **5e** and **5f** have very similar inhibitory activities, with IC_{50} values of 0.069 μM for the (*S*) isomer **5e** and 0.046 μM for its corresponding (*R*) isomer **5f**. It was also observed that compound **6b** bearing a N_4-acylalkyldithiolane moiety is two log less active than the most active inhibitor **5f**, strengthening the prime importance of the nature of the substitution at the N_4-piperazine position.

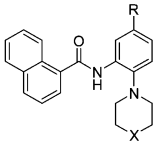
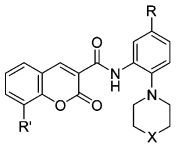
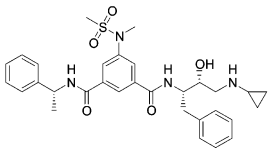
2-Coumarinyl Series. Replacement of the naphthyl moiety by a coumarin nucleus was envisaged in order to possibly restrict the rotation along the amide bond. Indeed, the possibility for the carbonyl group of the lactone function to accept a hydrogen bond with the hydrogen atom of the NH of the exocyclic amide through a possible six-membered ring formation appears to be energetically favorable. Increased hindrance could favor specific constrained conformations which could influence the inhibitory activities (increase or decrease). NMR analysis carried out within the coumarin series of analogues suggests that such a hypothesis is rather unlikely, since VT NMR did not show any effect on the chemical shifts of representative protons mentioned in the NMR studies

As shown on Table 3, generally speaking, the average BACE-1 inhibitory activities for the coumarin analogues (**9a**, **9b**, and **10a**−**e**) were lower than that of the corresponding naphthyl analogues. However some compounds, bearing various groups at the N_4-position piperazinyl were found almost equipotent (**9c**−**e** and **11d**) or more active (**11c**), than the reference compound **4d**. These results are of interest since it confirms NMR observations obtained in both naphthyl and coumarin series, showing that substitution at the N_4-piperazinyl position by various groups could stabilize specific favored conformations, which influence BACE-1 inhibitory activity. Introduction of substituents at the N_4-position such as hydroxy ethyl (**9e**) and benzyl (**9c** and **11c**) groups increases the inhibitory activity, while in contrast introduction at this position of other substituents such as Boc (**9b** and **10b**) or oxygen (**9a** and **10a**) results in a decrease of the inhibitory activity. Besides it could also be observed that in this series, the presence of a biphenyl moiety does not influence noticeably the inhibitory activity of the resulting analogues, as exemplified by the IC_{50} values found for compound **9c** and its biphenyl analogue **11c** which are very similar.

From a pharmacokinetic perspective, most of the reported analogues are sufficiently lipophilic to allow high brain penetration. Indeed, log *P* values reported in Tables 2 and 3 were calculated using ACD (Advanced Chemistry Development, Inc; log *P* 1.0 base calculations) ranged from 2.72 to 7.84. On average compounds belonging to the coumarin series have log *P* values lower than their corresponding naphthyl analogues.

Cell-Based Assay Evaluation. Considering the BACE-1 enzymatic assay as a primary screening of two series of analogues, we have selected almost the most active analogues and analyzed the effect on the production of Aβ peptides from mouse Neuro2a (N2a) cells. IC_{50} values are reported in Table 4. Unexpectedly, analogue **4d** was unable to diminish Aβ generation, whereas it showed the tendency for inhibition with significant toxicity over 10 μM concentration. Compound **3e** decreased both $A\beta_{40}$ and $A\beta_{42}$ secretion (IC_{50} = 2.37 and 0.9 μM, respectively) without obvious toxicity. In contrast, many compounds with strong inhibitory potencies (i.e., **5a**, **5d**, **5e**, **5f**) were unable to inhibit Aβ secretion. Compound **9e** showed moderate inhibitory potency against $A\beta_{40}$ and $A\beta_{42}$ generation (IC_{50} = 3.9 and 2.49 μM, respectively), while it was toxic for cells over 10 μM. Finally, no compounds were affected the processing of C100, the direct γ-secretase substrate (data not

Table 4. Cell-Based Assay Inhibition Activity[a]

	General structure	R	X	Cell-based assay IC_{50} (μM)	
				Aβ40	Aβ42
3e		Br	NCH_2CH_2OH	2.37	0.9
4d		Ph	NH	>10	>10
5a		Ph	NCH_2CH_2OH	>10	>10
5d		Ph	$N\text{-}(CH_2)_5\text{-}NH_2$	>10	>10
5e		Ph	$N(CH_2)_2CH(Ph)OH$ (*S*)	>10	>10
5f		Ph	$N(CH_2)_2CH(Ph)OH$ (*R*)	>10	>10
9e		Br	NCH_2CH_2OH	3.86	2.49
Calbiochem IV*				0.36	nd

[a] *Compound used as control (β-secretase inhibitor IV from Calbiochem cat. no. 565788).[24]

shown), suggesting that these compounds (i.e., compounds **3e** and **9e**) are genuine β-secretase inhibitors.

Discussion

From these results, several comments can be made: Compound **3e** appears to be the most active compound (IC_{50} = 2.37 μM) in the cell assay. This analogue is more active than the analogue **4d** which was used as reference compound (IC_{50} > 10 μM). It should be also noted that these results confirm the enzymatic assay, since the IC_{50} values for these two analogues were respectively: 0.079 μM and 0.3 μM. It should be noted that to our knowledge, no data have been reported to the inhibitory activity of Vertex compound on the Aβ-peptides production.

One explanation for these observed differences in inhibitory potency could be support by the differences in lipophilicity of the analogue. It seems that the presence of a supplementary phenyl ring in compound **4d** increased their corresponding log *P* values (5.73) compared to compound **3e**, which lop *P* value is 4.19.

Compound **3e** appears to be a genuine BACE-1 inhibitor, since it has no effect on SC100 processing. We verified that the inhibitory activity of **3e** was similar to that of β-secretase inhibitor IV (Calbiochem cat. no. 565788),[24] analogue known to be a genuine BACE-1 inhibitor.

It is also interesting to note that compounds **3e** and **5a**, which structurally only differ by the presence of a biphenyl piperazine moiety in analogue **5a** instead of a phenyl piperazine in analogue **3e**, are almost equipotent in the enzyme assay but differ with respect to IC_{50} values, 0.11 μM and 0.079 μM. Their inhibitory activities in cell assay are different, respectively 2.37 μM for **3e** and up to 10 μM for **5a**. Again it can be observed that log *P* for the less potent analogue **5a** (log *P* = 5.59) is higher than analogue **3e** (log *P* = 4.19).

As mentioned before, taking BACE-1 enzymatic inhibitory activity as a primary screening, we selected one of the most potent coumarinyl analogues **9e** (IC_{50} = 0.63 μM) as the representative analogue of the coumarin series, to be screened in the cell-based assay.

Moreover, this analogue has a calculated log *P* value around 2.85. We found that this new analogue, which differs from the most potent analogue **3e** by the replacement of the naphthyl ring by a coumarinyl heterocycle, shows a moderate inhibitory activity (IC_{50} = 3.86 μM). This result shows that in this case the log *P* value is not the only parameter which influences the inhibitory potency, since the log *P* value for **9e** is much lower than the corresponding log *P* value for compound **3e**.

From the reported results it can seen that compounds which are the most potent in the cell-based assay are also the most active inhibitors of BACE-1 enzyme. This observation could be of interest since BACE-1 assay could be viewed as a primary screening before performing the cell assay. Moreover, these results confirmed the NMR and modeling studies which suggest that substitution at the *N*-piperazinyl position was determinative for β-secretase activity. This was confirmed by compound **3e** which is substituted by an hydroxyethyl moiety. If lipophilicity of the analogues influences the inhibitory potency in cell assay, it is not the only parameter.

Conclusions

We have synthesized two series of new aryl-piperazine derivatives, possessing either naphthyl or coumarinyl moieties, and determined their inhibitory activities on BACE-1 enzyme. The structure−activity data for the two series of derivatives bring to light the importance of substituents located at the N_4-position of the piperazinyl ring. Some new analogues bearing at this position a side chain with a hydroxy group were highly potent BACE-1 inhibitors in both enzymatic and cell assays.

In contrast, substitution of the naphthyl moiety by a coumarin ring led to a significant decrease of their inhibitory properties. Compound **3e**, belonging to the naphthyl series, was found more active than the reported analogues by Vertex Pharmaceuticals, Inc.[25,26] Inhibitory activities of compound **4d** from cell-based assays have not been previously reported, at least to our knowledge. By its inhibitory activity on Aβ-peptide production in a cell-based assay, compound **3e**, bearing an *N*-hydroxyethyl substituent, could be considered as a new representative hit in these series. Unfortunately, cytotoxicity of new active analogues could represent a serious obstacle for their future development. It should be also noted that these new nonpeptidic analogues should have good catabolic stabilities compared to recently described inhibitors which are of peptidomimetic nature, such as compound JMV2764.[27]

Experimental Section

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. Acetonitrile was of anhydrous quality from commercial suppliers (Aldrich, Carlo Erba Reagents). Melting points were determinated on an Electrothermal IA-9100 digital, and all temperatures are given in degrees Celsius and are uncorrected. ^{1}H NMR spectra were recorded at 250 MHz on a Brüker AC-250 spectrometer. Chemical shifts are expressed as δ units (part per million) downfield from TMS (tetramethylsilane). Electro-spray mass spectra were obtained on a Waters Micromass ZMD spectrometer by direct injection of the sample solubilized in acetonitrile. Elemental analyses were within ±0.4% of theorical values for all compounds. All reactions were monitored by thin-layer chromatography. Analytical thin layer chromatographies (TLC) were performed using silica gel plates 0.2 mm thick ($60F_{254}$ Merck). Preparative flash column chromatographies were carried out on silica gel (230−400 mesh, G60 Merck).

4-(4-Bromo-2-nitro-phenyl)-morpholine (1a). To a mixture of 2,5-dibromonitrobenzene (4.0 g, 14.2 mmol), triethylamine (1 mL, 7.2 mmol) in *i*-PrOH (15 mL) was added morpholine (2.5 mL, 28.4 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 (1:3 to 1:1) gave the desired compound **1a** (3.0 g, 73%) as an orange oil. R_f 0.18 (cHex:EtOAc, 3:1). ^{1}H NMR ($CDCl_3$) δ_H 3.02 (t, 4H, J = 4.5 Hz, NCH_2CH$_2$O), 3.82 (t, 4H, J = 4.5 Hz, NCH$_2$CH_2O), 7.02 (d, 1H, J = 8.8 Hz, Ar*H*), 7.58 (dd, 1H, J = 2.3 and 8.8 Hz, Ar*H*), 7.91 (d, 1H, J = 2.3 Hz, Ar*H*). ESI-MS m/z [M + H]$^+$ = 287. Anal. ($C_{10}H_{11}BrN_2O_3$) C, H, N.

4-(4-Bromo-2-nitro-phenyl)-1-*tert*-butoxycarbonyl-piperazine (1b). Compound **1b** was prepared by a method similar to that described for **1a**. Yield 72%, R_f 0.79 (cHex:EtOAc, 1:1). ^{1}H NMR ($CDCl_3$) δ_H 1.46 (s, 9H, CH_3, *t*Bu), 2.98 (t, 4H, J = 4.7 Hz, NCH_2CH$_2$NBoc), 3.57 (t, 4H, J = 4.7 Hz, NCH$_2$CH_2NBoc), 7.01 (d, 1H, J = 8.9 Hz, Ar*H*), 7.58 (dd, 1H, J = 2.4 and 8.9 Hz, Ar*H*), 7.91 (d, 1H, J = 2.4 Hz, Ar*H*). ESI-MS m/z [M + H]$^+$ = 386. Anal. ($C_{15}H_{20}BrN_3O_4$) C, H, N.

1-Benzyl-4-(4-bromo-2-nitro-phenyl)-piperazine (1c). Compound **1c** was prepared by a method similar to that described for **1a**. Yield 85%, R_f 0.51 (cHexane:EtOAc, 1:1). ^{1}H NMR ($CDCl_3$) δ_H 2.59 (t, J = 4.8 Hz, 4H, NCH$_2$CH_2NCH$_2$Ph), 3.06 (t, J = 4.8 Hz, 4H, NCH_2CH$_2$NCH$_2$Ph), 3.56 (s, 2H, NCH_2Ph), 7.01 (d, J = 8.7 Hz, Ar*H*), 7.28−7.34 (m, 5H, Ar*H*), 7.54 (dd, J = 8.7 and 2.4 Hz, 1H, Ar*H*), 7.89 (d, J = 2.4 Hz, 1H, Ar*H*). ESI-MS m/z [M + H]$^+$ = 376. Anal. ($C_{17}H_{18}BrN_3O_2$) C, H, N.

4-(2-Amino-4-bromo-phenyl)-morpholine (2a). Zinc dust (3.4 g, 52.3 mmol) was added to a stirred solution of **1a** (3.0 g, 10.4 mmol) in THF (60 mL) followed by KH_2PO_4 (20 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. 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 (1:6), giving aniline **2a** (1.7 g, 63%) as a pale yellow foam. M.p. 123°. R_f 0.53 (EtOAc:cHex, 1:1). ^{1}H NMR ($CDCl_3$) δ_H 2.59 (bs, 4H, NCH$_2$CH$_2$O), 2.89 (t, J = 4.8 Hz, 4H, NCH$_2$CH$_2$O), 4.01 (brs, 2H, NH_2), 6.78−6.87 (m, 3H). ESI-MS m/z [M + H]$^+$ = 257. Anal. ($C_{10}H_{13}BrN_2O$) C, H, N.

4-(2-Amino-4-bromo-phenyl)-1-*tert*-butoxycarbonyl-piperazine (2b). Compound **2b** was prepared by a method similar to that described for **2a**. Yield 63%, R_f 0.42 (EtOAc:toluene, 1:6). ^{1}H NMR ($CDCl_3$) δ_H 1.48 (s, 9H, CH_3, *t*Bu), 2.81 (t, 4H, J = 4.7 Hz, CH_2CH$_2$NBoc), 3.56 (br s, 4H, CH$_2$CH_2NBoc), 4.08 (br s, 2H, NH_2), 6.81−6.87 (m, 3H, Ar*H*). ESI-MS m/z [M + H]$^+$ = 356. Anal. ($C_{15}H_{22}BrN_3O_2$) C, H, N.

1-Benzyl-4-(2-amino-4-bromo-phenyl)-piperazine (2c). Compound **2c** was prepared by a method similar to that described for **2a**. Yield 86%, mp 85° C. R_f 0.66 (EtOAc:cHex, 1:1). ^{1}H NMR ($CDCl_3$) δ_H 2.62 (brs, 4H, NCH$_2$CH_2NCH$_2$Ph), 2.91 (t, 4H, J = 4.7 Hz, NCH_2CH$_2$NCH$_2$Ph), 3.59 (s, 2H, NCH_2Ph), 4.00 (brs, 1H, NH_2), 6.82−6.85 (m, 3H, Ar*H*), 7.29−7.36 (m, 5H, Ar*H*). ESI-MS m/z [M + H]$^+$ = 346. Anal. ($C_{17}H_{20}BrN_3$) C, H, N.

{4-Bromo-2-[(naphthalene-1-carbonyl)-amino]-phenyl}-morpholine (3a). To a solution of **2a** (210 mg, 0.81 mmol) in methylene chloride (10 mL) with DIEA (210 μL, 1.21 mmol) was added 1-naphthoyl chloride (200 mg, 1.05 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 (1:9) giving **3c** (300 mg, 90%) as a yellow solid. M.p. 172°. R_f 0.61 (EtOAc:cHex,1:1). ^{1}H NMR ($CDCl_3$) δ_H 2.86 (t, 4H, J = 4.4 Hz, NCH_2CH$_2$O), 3.70 (t, 4H, J = 4.4 Hz, NCH$_2$CH_2O), 7.08 (d, 1H, J = 8.4 Hz, Ar*H*), 7.27 (dd, 1H, J = 2.2 and 8.4 Hz, Ar*H*), 7.51−7.60 (m, 3H, Ar*H*), 7.71−7.75 (m, 1H, Ar*H*), 7.91−7.95 (m, 1H, Ar*H*), 8.01 (d, 1H, J = 8.2 Hz, Ar*H*), 8.44−8.48 (m, 1H, Ar*H*), 8.9 3 (d, 1H, J = 2.2 Hz, Ar*H*), 9.15 (brs, 1H, N*H*C(O)). ESI-MS m/z [M + H]$^+$ = 411. Anal. ($C_{21}H_{19}BrN_2O_2$) C, H, N.

1-{4-Bromo-2-[(naphthalene-1-carbonyl)-amino]-phenyl}-4-*tert*-butoxycarbonyl piperazine (3b). Compound **3b** was prepared by a method similar to that described for **3a**. Yield 84%, R_f 0.59 (EtOAc:cHex, 1:2). ^{1}H NMR ($CDCl_3$) δ_H 1.45 (s, 9H, CH_3, *t*Bu), 2.81 (t, 4H, J = 4.7 Hz, NCH_2CH$_2$NBoc), 3.43 (brs, 4H, NCH$_2$CH_2-NBoc), 7.04 (d, 1H, J = 8.4 Hz, Ar*H*), 7.24 (dd, 1H, J = 2.3 and 8.4 Hz, Ar*H*), 7.51−7.60 (m, 3H, Ar*H*), 7.70−7.84 (m, 1H, Ar*H*), 7.91−7.95 (m, 1H, Ar*H*), 7.99 (d, 1H, J = 8.2 Hz, Ar*H*), 8.43−8.47 (m, 1H, Ar*H*), 8.92 (d, 1H, J = 2.3 Hz, Ar*H*), 9.10 (brs, 1H, N*H*C(O)). ESI-MS m/z [M + H]$^+$ = 510. Anal. ($C_{26}H_{28}BrN_3O_3$) C, H, N.

4-Benzyl-1-{4-bromo-2-[(naphthalene-1-carbonyl)-amino]-phenyl}-piperazine (3c). Compound **3c** was prepared by a method similar to that described for **3a**. Yield 75%, mp 147°C. R_f 0.31 (EtOAc:cHex,1:9). ^{1}H NMR ($CDCl_3$) δ_H 2.49 (brs, 4H, NCH$_2$CH_2-NCH$_2$Ph), 2.89 (t, 4H, J = 4.7 Hz, NCH_2CH$_2$NCH$_2$Ph), 3.48 (s, 2H, NCH_2Ph), 7.08 (d, 1H, J = 8.5 Hz, Ar*H*), 7.24 (dd, 1H, J = 2.3 and 8.5 Hz, Ar*H*), 7.29−7.33 (m, 5H, Ar*H*), 7.52−7.61 (m, 3H, Ar*H*), 7.71−7.75 (m, 1H, Ar*H*), 7.93−7.95 (m, 1H, Ar*H*), 8.02 (d, 1H, J = 8.2 Hz, Ar*H*), 8.47−8.51 (m, 1H, Ar*H*), 8.91 (d, 1H, J = 2.3 Hz, Ar*H*), 9.17 (brs, 1H, N*H*C(O)). ESI-MS m/z [M + H]$^+$ = 500. Anal. ($C_{28}H_{26}BrN_3O$) C, H, N.

1-{4-Bromo-2-[(naphthalene-1-carbonyl)-amino]-phenyl}-piperazine (3d). To a stirred suspension of the appropriate *N*-benzyl compound **3c** (1.0 g, 2 mmol) and an equal weight of 10% Pd−C in dry methanol (10 mL) was added anhydrous ammonium formate (630 mg, 10 mmol) in a single portion under nitrogen. The resulting mixture was stirred at relux temperature, and the reaction was monitored by TLC. After completion, the catalyst was removed by filtration through Celite and washed with methyle chloride (10 mL). The solvent was removed under reduced pressure, and the crude product was purified by chromatography on silica gel with DCM, 0.5% MeOH as eluent, to yield the desired compound **3d**

(0.8 g, 97%) as a yellow oil. R_f 0.09 (EtOAc:cHex,2:1). ^{1}H NMR ($CDCl_3$) δ_H 3.08 (brs, 4H, NCH_2CH_2NH), 3.19 (brs, 4H, NCH_2-CH_2NH), 7.19 (d, 1H, J = 8.4 Hz, Ar*H*), 7.40 (dd, 1H, J = 2.3 and 8.4 Hz, Ar*H*), 7.58−7.64 (m, 3H, Ar*H*), 7.77−7.80 (m, 1H, Ar*H*), 8.02−8.05 (m, 1H, Ar*H*), 8.09 (d, 1H, J = 8.2 Hz, Ar*H*), 8.22−8.26 (m, 1H, Ar*H*), 8.43 (d, 1H, J = 2.3 Hz, Ar*H*), 8.77 (brs, 2H, N*H*), 9.78 (brs, 1H, N*H*C(O)). ESI-MS m/z $[M + H]^+$ = 410. Anal. ($C_{21}H_{20}BrN_3O$) C, H, N.

1-{4-Bromo-2-[(naphthalene-1-carbonyl)-amino]-phenyl}-4-(2-hydroxyethyl)-piperazine (3e). 2-Bromoethanol (5.0 μL, 0.070 mmol) was added to a vigorous stirred mixture of **3d** (34 mg, 0.065) and anhydrous K_2CO_3 (18 mg, 0.13 mmol) in dry acetonitrile (5 mL). The suspension was refluxed for 10 h under nitrogen. The solvent was then removed under reduced pressure. The crude product was dissolved in CH_2Cl_2 (5 mL) and extracted with water (2 × 5 mL), and the organic layer was dried over $MgSO_4$ and then purified by chromatography on silica gel, using methylene chloride−methanol (9:1) as eluent, to yield the compound **3e** (27 mg, 92%) as a white powder. M.p. 88°. R_f 0.55 (CH_2Cl_2:MeOH, 5:1). ^{1}H NMR ($CDCl_3$) δ_H 2.53 (brs, 6H, NCH_2CH$_2$OH and NCH$_2$CH_2NCH$_2$-CH$_2$OH), 2.91 (brs, 4H, NCH_2CH$_2$NCH$_2$CH$_2$OH), 3.62 (t, 2H, J = 4.3 Hz, NCH$_2$CH_2OH), 7.08 (d, 1H, J = 8.4 Hz, Ar*H*), 7.25 (dd, 1H, J = 2.2 and 8.4 Hz, Ar*H*), 7.51−7.60 (m, 3H, Ar*H*), 7.71 (d, 1H, J = 7.0 Hz, Ar*H*), 7.91−7.95 (m, 1H, Ar*H*), 8.00 (d, 1H, J = 8.3 Hz, Ar*H*), 8.45−8.48 (m, 1H, Ar*H*), 8.92 (d, 1H, J = 2.2 Hz, Ar*H*), 9.10 (brs, 1H, N*H*C(O)). ESI-MS m/z $[M + H]^+$ = 454. Anal. ($C_{23}H_{24}BrN_3O_2$) C, H, N.

1-{4-(Phenyl)-2-[(naphthalene-1-carbonyl)-amino]-phenyl}-4-*tert*-butoxy Carbonyl Piperazine (4b). Phenyl boronic acid (132 mg, 1.1 mmol) was dissolved in anhydrous 1,3-dioxane (30 mL). The appropriate bromo derivative **3b** (500 mg, 1 mmol) was dissolved in anhydrous dioxane (30 mL), and the two solutions were mixed with dppf (17 mg, 0.03 mmol), dppf-Pd^{II} (25 mg, 0.03 mmol), and KOAc (300 mg, 3 mmol). The resulting suspension was refluxed for 72 h. The dioxane was removed under reduced pressure, and the catalysts were removed by filtration through silica gel. The crude product was purified by flash chromatography with cHex−EtOAc (9:1) as eluent, to yield the desired compound **4b** as a white oil (180 mg, 36%). R_f 0.26 (EtOAc:cHex,1:9). ^{1}H NMR ($CDCl_3$) δ_H 1.45 (s, 9H, CH_3, *t*Bu), 2.81 (t, 4H, J = 4.7 Hz, NCH_2CH$_2$NBoc), 3.43 (brs, 4H, NCH$_2$CH_2NBoc), 7.27−7.35 (m, 8H, Ar*H*), 7.42 (d, 2H, J = 8.5 Hz, Ar*H*), 7.75−7.78 (m, 1H, Ar*H*), 7.91−7.93 (m, 1H, Ar*H*), 8.02 (d, 1H, J = 8.3 Hz, Ar*H*), 8.50−8.54 (m, 1H, Ar*H*), 8.98 (brs, 1H, Ar*H*), 9.20 (brs, 1H, N*H*C(O)) ESI-MS m/z $[M + H]^+$ = 508. Anal. ($C_{32}H_{33}N_3O_3$) C, H, N.

4-Benzyl-1-{4-(phenyl)-2-[(naphthalene-1-carbonyl)-amino]-phenyl}-piperazine (4c). Compound **4c** was prepared by a method similar to that described for **4b**. Yield 80%, mp 156 °C. R_f 0.23 (EtOAc:cHex,1:9). ^{1}H NMR ($CDCl_3$) δ_H 2.51 (brs, 4H, NCH$_2$CH_2-NCH$_2$Ph), 2.96 (t, 4H, J = 4.7 Hz, NCH_2CH$_2$NCH$_2$Ph), 3.49 (s, 2H, NCH_2Ph), 7.27−7.35 (m, 8H, Ar*H*), 7.42 (d, 2H, J = 8.5 Hz, Ar*H*), 7.53−7.63 (m, 5H, Ar*H*), 7.75−7.78 (m, 1H, Ar*H*), 7.91−7.93 (m, 1H, Ar*H*), 8.02 (d, 1H, J = 8.3 Hz, Ar*H*), 8.50−8.54 (m, 1H, Ar*H*), 8.98 (brs, 1H, Ar*H*), 9.20 (brs, 1H, N*H*C(O)). ESI-MS m/z $[M + H]^+$ = 498. Anal. ($C_{34}H_{31}N_3O$) C, H, N.

1-{4-(Phenyl)-2-[(naphthalene-1-carbonyl)-amino]-phenyl}-piperazine (4d). Compound **4d** was prepared by a method similar to that described for **3d**. Yield 88%, mp 118°C. R_f 0.45 (MeOH:CH_2Cl_2, 1:9). ^{1}H NMR ($CDCl_3$) δ_H 3.03 (brs, 8H, NCH_2CH_2NH), 4.32 (brs, 1H, NCH$_2$CH$_2$N*H*), 7.31−7.41 (m, 2H, Ar*H*), 7.43−7.49 (m, 2H, Ar*H*), 7.55−7.59 (m, 3H, Ar*H*), 7.67−7.71 (brd, 2H, Ar*H*), 7.75 (dd, 1H, J = 1.3 and 7.0 Hz, Ar*H*), 7.91−7.95 (m, 2H, Ar*H*), 8.00 (brd, 1H, Ar*H*), 8.46−8.50 (m, 1H, Ar*H*), 9.00 (brs, 1H, Ar*H*), 9.06 (brs, 1H, N*H*C(O)). ESI-MS m/z $[M + H]^+$ = 408. Anal. ($C_{27}H_{25}N_3O$) C, H, N.

1-{4-(Phenyl)-2-[(naphthalene-1-carbonyl)-amino]-phenyl}-4-(2-hydroxyethyl)-piperazine (5a). Compound **5a** was prepared by a method similar to that described for **3e**. Yield 77%, mp 183 °C. R_f 0.65 (MeOH:CH_2Cl_2, 1:9). ^{1}H NMR ($CDCl_3$) δ_H 2.51−2.57 (m, 6H, NCH$_2$CH_2NCH_2CH$_2$OH), 2.74 (brs, 1H, NCH$_2$CH$_2$O*H*), 2.98 (t, 4H, J = 4.7 Hz, NCH_2CH$_2$NCH$_2$CH$_2$OH), 3.62 (t, 2H, J = 5.3 Hz, NCH$_2$CH_2OH), 7.28−7.41 (m, 2H, Ar*H*), 7.43−7.48 (m, 2H, Ar*H*), 7.52−7.60 (m, 3H, Ar*H*), 7.69−7.72 (brd, 2H, Ar*H*), 7.77 (dd, 1H, J = 1.3 and 7.0 Hz, Ar*H*), 7.91−7.95 (m, 2H, Ar*H*), 7.99−8.02 (brd, 1H, Ar*H*), 8.50−8.54 (m, 1H, Ar*H*), 9.02 (brs, 1H, Ar*H*), 9.16 (brs, 1H, N*H*C(O)). ESI-MS m/z $[M + H]^+$ = 452. Anal. ($C_{29}H_{29}N_3O_2$) C, H, N.

1-{4-(Phenyl)-2-[(naphthalene-1-carbonyl)-amino]-phenyl}-4-(ethyl-aceto)-piperazine (5b). Compound **5b** was prepared by a method similar to that described for **3e** using ethyl bromoacetate. Yield 47%, R_f 0.42 (EtOAc:cHex,1:1). ^{1}H NMR ($CDCl_3$) δ_H 1.25 (t, 3H, NCH$_2$CO$_2$CH$_2$CH_3), 2.65 (brs, 4H, NCH$_2$CH_2NCH$_2$-CO$_2$Et), 3.03 (t, 4H, J = 4.7 Hz, NCH_2CH$_2$NCH$_2$CO$_2$Et), 3.20 (s, 2H, NCH_2CO$_2$Et), 4.17 (q, 2H, NCH$_2$CO$_2$CH_2CH$_3$), 7.29−7.40 (m, 2H, Ar*H*), 7.42−7.48 (m, 2H, Ar*H*), 7.53−7.60 (m, 3H, Ar*H*), 7.68−7.72 (brd, 2H, Ar*H*), 7.77 (dd, 1H, J = 1.3 and 7.0 Hz, Ar*H*), 7.91−7.95 (m, 2H, Ar*H*), 7.99−8.02 (brd, 1H, Ar*H*), 8.51−8.55 (m, 1H, Ar*H*), 9.01 (brs, 1H, Ar*H*), 9.20 (brs, 1H, N*H*C(O)). ESI-MS m/z $[M + H]^+$ = 494. Anal. ($C_{31}H_{31}N_3O_3$) C, H, N.

1-{4-(Phenyl)-2-[(naphthalene-1-carbonyl)-amino]-phenyl}-4-[1-(5-phthalimido)-pentyl]-piperazine (5c). Compound **5c** was prepared by a method similar to that described for **3e** using *N*-(5-bromopentyl) phthalimide. Yield 99%, mp 72 °C. R_f 0.60 (MeOH:CH_2Cl_2, 1:9). ^{1}H NMR ($CDCl_3$) δ_H 1.32−1.38 (m, 2H), 1.47−1.58 (m, 2H), 1.64−1.75 (m, 2H), 2.28−2.34 (m, 2H), 2.49 (brs, 4H, NCH$_2$CH_2N(CH$_2$)$_5$-phthalimide), 2.96 (t, 4H, J = 4.7 Hz, NCH_2CH$_2$N(CH$_2$)$_5$-phthalimide), 3.68 (t, 2H, J = 7.1 Hz), 7.27−7.40 (m, 2H, Ar*H*), 7.42−7.48 (m, 2H, Ar*H*), 7.53−7.60 (m, 3H, Ar*H*), 7.68−7.72 (m, 4H, Ar*H*), 7.73−7.80 (m, 1H, Ar*H*), 7.82−7.85 (m, 2H, Ar*H*), 7.91−7.94 (m, 2H, Ar*H*), 7.99−8.02 (brd, 1H, Ar*H*), 8.52−8.57 (m, 1H, Ar*H*), 9.02 (brs, 1H, Ar*H*), 9.20 (brs, 1H, N*H*C(O)). ESI-MS m/z $[M + H]^+$ = 623. Anal. ($C_{40}H_{38}N_4O_3$) C, H, N.

4-[1-(5-Amino)-pentyl]-1-{4-(phenyl)-2-[(naphthalene-1-carbonyl)-amino]-phenyl}-piperazine (5d). Compound **5c** (75 mg, 0.12 mmol) was dissolved in warm ethanol (5 mL), hydrazine monohydrate (10 mg, 0.18 mmol) was added, and the solution was refluxed for 3 h, cooled to room temperature, and filtered. The filtrate was concentrated under reduced pressure, and the residue was diluted with $NaHCO_3$ and extracted with methylene chloride. The organic layer was dried with $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash chromatography with methylene chloride−methanol (9/1) as eluent to give the compound **5d** (50 mg, 84%) as brown oil. R_f 0.09 (MeOH:CH_2Cl_2, 1:9). ^{1}H NMR ($CDCl_3$) δ_H 1.26−1.36 (m, 2H), 1.42−1.53 (m, 4H), 2.30 (m, 2H), 2.49 (brs, 4H, NCH$_2$CH_2N(CH$_2$)$_5$NH$_2$), 2.71 (t, 2H, J = 6.9 Hz), 2.98 (t, 4H, J = 4.7 Hz, NCH_2CH$_2$N(CH$_2$)$_5$NH$_2$), 3.49 (brs, 2H, NCH$_2$CH$_2$N(CH$_2$)$_5$NH_2), 7.29 (d, 1H, J = 8.3 Hz, Ar*H*), 7.36−7.40 (m, 1H, Ar*H*), 7.46 (t, 2H, J = 7.3 Hz, Ar*H*), 7.53−7.61 (m, 3H, Ar*H*), 7.69−7.72 (m, 2H, Ar*H*), 7.77−7.79 (m, 1H, Ar*H*), 7.92−7.95 (m, 2H, Ar*H*), 8.01 (d, 1H, J = 8.2 Hz, Ar*H*), 8.52−8.56 (m, 1H, Ar*H*), 9.02 (brs, 1H, Ar*H*), 9.22 (brs, 1H, N*H*C(O)). ESI-MS m/z $[M + H]^+$ = 493. Anal. ($C_{32}H_{36}N_4O$) C, H, N.

(*S*)-(−)-1-{4-(Phenyl)-2-[(naphthalene-1-carbonyl)-amino]-phenyl}-4-[1-(3-phenyl)-propan-3-ol]-piperazine (5e). (*S*)-(−)-3-Chloro-1-phenyl-1-propanol (17 mg, 0.09 mmol) was added to a vigorous stirred mixture of **4d** (40 mg, 0.09 mmol) and anhydrous potassium carbonate (31 mg, 0.22 mmol) in dry acetonitrile (5 mL), and then the suspension was stirred at room temperature for 10 h under nitrogen. After that time, the suspension was concentrated. The crude product was chromatographied with methylene chloride−methanol (9/1) as eluent to give **5e** (42 mg, 86%) as a yellow oil. R_f 0.64 (MeOH:CH_2Cl_2, 1:9). ^{1}H NMR ($CDCl_3$) δ_H 1.86−1.90 (m, 2H), 2.51−2.74 (m, 5H), 3.03 (t, 4H, J = 4.7 Hz, NCH_2CH$_2$NCH$_2$-CH$_2$CH(Ph)OH), 3.36−3.49 (m, 1H), 4.92−4.95 (m, 1H), 7.27−7.39 (m, 8H, Ar*H*), 7.43−7.49 (m, 2H, Ar*H*), 7.52−7.62 (m, 3H, Ar*H*), 7.70 (brd, 2H, Ar*H*), 7.77 (dd, 1H, J = 1.3 and 7.0 Hz, Ar*H*), 7.91−7.95 (m, 2H, Ar*H*), 7.99 (brd, 1H, Ar*H*), 8.50−8.54 (m, 1H, Ar*H*), 9.02 (brs, 1H, Ar*H*), 9.14 (brs, 1H, N*H*C(O)). ESI-MS m/z $[M + H]^+$ = 542. $[\alpha]^{20}_D$ = −24° (c = 1.10, $CHCl_3$). Anal. ($C_{36}H_{35}N_3O_2$) C, H, N.

(*R*)-(+)-1-{4-(Phenyl)-2-[(naphthalene-1-carbonyl)-amino]-phenyl}-4-[1-(3-phenyl)-propan-3-ol]-piperazine (5f). Compound **5f** was prepared by a method similar to that described for **5e** using (*R*)-(+)-3-chloro-1-phenyl-1-propanol. Yield 51%, R_f 0.64 (MeOH: CH_2Cl_2, 1:9). ^{1}H NMR ($CDCl_3$) δ_H 1.83−1.90 (m, 2H), 2.52−2.74 (m, 5H), 3.03 (t, 4H, J = 4.7 Hz, NC*H*$_2$CH$_2$NCH$_2$CH$_2$-CH(Ph)OH), 3.36−3.49 (m, 1H), 4.93−4.98 (m, 1H), 7.25−7.39 (m, 8H, Ar*H*), 7.43−7.49 (m, 2H, Ar*H*), 7.52−7.62 (m, 3H, Ar*H*), 7.70 (brd, 2H, Ar*H*), 7.77 (dd, 1H, J = 1.3 and 7.0 Hz, Ar*H*), 7.91−7.95 (m, 2H, Ar*H*), 8.09 (brd, 1H, Ar*H*), 8.49−8.53 (m, 1H, Ar*H*), 9.02 (brs, 1H, Ar*H*), 9.13 (brs, 1H, N*H*C(O)). ESI-MS m/z [M + H]$^+$ = 542. $[\alpha]^{20}_D$ = + 19.5° (c = 1.07, $CHCl_3$). Anal. ($C_{36}H_{35}N_3O_2$) C, H, N.

1-{4-(Phenyl)-2-[(naphthalene-1-carbonyl)-amino]-phenyl}-4-benzoyl-piperazine (6a). 4d (50 mg, 0.12 mmol) was dissolved in methylene chloride (5 mL) with DIEA (30 μL, 0.16 mmol). To this solution, benzoyl chloride (17 μL, 0.14 mmol) was added. The reaction mixture was stirred for 2 h at room temperature. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography with cyclohexanes−ethyl acetate (9/1) as eluent to give the desired compound **6a** (56 mg, 91%) as a white powder. M.p. 91°. R_f 0.44 (EtOAc:cHex,1:1). ^{1}H NMR ($CDCl_3$) δ_H 2.98 (brs, 4H, NC*H*$_2$CH$_2$NC(O)Ph), 3.60 (brs, 4H, NCH$_2$C*H*$_2$NC(O)Ph), 7.18 (d, 1H, J = 7.2 Hz, Ar*H*), 7.27−7.40 (m, 8H, Ar*H*), 7.44−7.53 (m, 3H, Ar*H*), 7.60−7.62 (m, 2H, Ar*H*), 7.68 (dd, 1H, J = 1.3 and 7.0 Hz, Ar*H*), 7.84−7.87 (m, 1H, Ar*H*), 7.91−7.95 (brd, 2H, Ar*H*), 8.38−8.41 (m, 1H, Ar*H*), 8.94 (brs, 1H, Ar*H*), 9.03 (brs, 1H, N*H*C(O)). ESI-MS m/z [M + H]$^+$ = 512. Anal. ($C_{34}H_{29}N_3O_2$) C, H, N.

1-{4-(4′-Chlorophenyl)-2-[(naphthalene-1-carbonyl)-amino]-phenyl}-4-{(5-dithiolan)-pentanoyl}-piperazine (6b). 4d (50 mg, 0.12 mmol), racemic (±)-α-lipoic acid (47 mg, 0.22 mmol), DCC (47 mg, 0.22 mmol), HOBt (30 mg, 0.22 mmol), and DMAP (28 mg, 0.22 mmol) were stirred in dry DCM (6 mL) at room temperature for 10 h. After this time, the insoluble residue was filtered off and washed with 2 × 5 mL of DCM. The filtrate was concentrated, and the resulting crude residue was purified by column chromatography with DCM, 0.5% MeOH as eluent, to yield **6b** as a white solid (60 mg, 84%) as a white powder. M.p. 82°. R_f 0.83 (MeOH: CH_2Cl_2, 1:9). ^{1}H NMR ($CDCl_3$) δ_H 1.42−1.54 (m, 2H), 1.60−1.76 (m, 4H), 1.84−1.98 (m, 1H), 2.33 (t, 2H, J = 7.3 Hz), 2.40−2.53 (m, 1H), 2.91 (t, 4H, J = 4.7 Hz, NC*H*$_2$CH$_2$NC(O)−(CH$_2$)$_4$-dithiolan), 3.10−3.20 (m, 2H), 3.47−3.66 (m, 5H), 7.24 (d, 1H, J = 8.3 Hz, Ar*H*), 7.37−7.41 (m, 1H, Ar*H*), 7.46 (t, 2H, J = 7.3 Hz, Ar*H*), 7.55−7.61 (m, 3H, Ar*H*), 7.68−7.71 (d, 2H, Ar*H*), 7.77 (dd, 1H, J = 1.3 and 7.0 Hz, Ar*H*), 7.92−7.96 (m, 2H, Ar*H*), 8.00 (brd, 1H, J = Hz, Ar*H*), 8.47−8.51 (m, 1H, Ar*H*), 9.03 (brs, 1H, Ar*H*), 9.12 (brs, 1H, N*H*C(O)). ESI-MS m/z [M + H]$^+$ = 596. Anal. ($C_{35}H_{37}N_3O_2S_2$) C, H, N.

Coumarin-3-carboxylic Acid or 2-Oxo-2*H*-chromene-3-carboxylic Acid (7). A mixture of salicylaldehyde, 2-hydroxybenzaldehyde, (1.22 g, 10.0 mmol), Meldrum's acid (1.44 g, 10.0 mmol), piperidinium acetate (30 mg, 0.2 mmol), and ethanol (10 mL) was stirred at room temperature for 20 min and then refluxed for 2 h. The reaction mixture was allowed to cool to room temperature. The crystallized product was filtered, washed three times with ethanol, and dried in vacuo to give quantitatively the desired compound **7** as yellow needles (1.9 g, quantitative) as yellow needles. M.p. 188°. R_f 0.12 (cHex−EtOAc, 2:1). ^{1}H NMR (DMSO-d_6) δ_H 7.38 (dd, 1H, J = 1.0 and 7.5 Hz, Ar*H*), 7.41−7.45 (m, 1H, Ar*H*), 7.69−7.76 (m, 1H, Ar*H*), 7.90 (dd, 1H, J = 1.6 and 7.7 Hz, Ar*H*), 8.73 (s, 1H, H_4), 13.12 (brs, 1H, COO*H*). ESI-MS m/z [M + H]$^+$ = 191. Anal. ($C_{10}H_6O_4$) C, H, N.

8-Methoxy-coumarin-3-carboxylic Acid or 8-Methoxy-2-oxo-2*H*-chromene-3-carboxylic Acid (8). Compound **8** was prepared by a method similar to that described for **7** using 2-hydroxy-3-methoxy-benzaldehyde. Yield 99%, mp 182 °C. R_f 0.09 (cHex−EtOAc, 2:1). ^{1}H NMR (DMSO-d_6) δ_H 3.89 (s, 3H, OC*H*$_3$), 7.25−7.41 (m, 3H, Ar*H*), 8.67 (s, 1H, H_4), 13.40 (brs, 1H, COO*H*). ESI-MS m/z [M + H]$^+$ = 221. Anal. ($C_{11}H_8O_5$) C, H, N.

{4-Bromo-2-[(2-Oxo-2*H*-chromene-3-carbonyl)-amino]-phenyl}-morpholine (9a). Coumarin-3-carboxylic acid **7** (350 mg, 1.90 mmol) and the amino moiety **2a** (400 mg, 1.56 mmol) were dissolved in anhydrous pyridine (10 mL). The solution was cooled to −15 °C, and phosphorus oxychloride (160 μL, 1.72 mmol) was added dropwise under vigorous stirring. The reaction mixture was stirred at −15 °C for at least 30 min. The solution was then stirred 12 h at room temperature. The reaction was quenched by addition of crushed ice/water (30 mL). The desired compound was extracted into AcOEt (3 × 30 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The crude material was purified by flash chromatography to lead to the desired compound **9a** (200 mg, 30%) as yellow needles. M.p. 222°. R_f 0.48 (cHex−EtOAc, 1:1). ^{1}H NMR ($CDCl_3$) δ_H 2.90 (t, 4H, J = 4.5 Hz, NC*H*$_2$CH$_2$O), 3.99 (t, 4H, J = 4.5 Hz, NCH$_2$C*H*$_2$O), 7.10 (d, 1H, J = 8.4 Hz, Ar*H*), 7.25 (dd, 1H, J = 2.3 and 8.4 Hz, Ar*H*), 7.42−7.47 (m, 2H, Ar*H*), 7.68−7.78 (m, 2H, Ar*H*), 8.83 (d, 1H, J = 2.3 Hz, Ar*H*), 9.01 (s, 1H, Ar*H*), 11.67 (brs, 1H, N*H*C(O)). ESI-MS m/z [M + H]$^+$ = 429. Anal. ($C_{20}H_{17}$-BrN_2O_4) C, H, N.

1-{4-Bromo-2-[(2-Oxo-2*H*-chromene-3-carbonyl)-amino]-phenyl}-4-*tert*-butoxycarbonyl-piperazine (9b). Compound **9b** was prepared by a method similar to that described for **9a** using **2b**. Yield 71%, mp 230 °C. R_f 0.66 (toluene−EtOAc, 6:1). ^{1}H NMR ($CDCl_3$) δ_H 1.48 (s, 9H, CH_3, *t*Bu), 2.87 (brs, 4H, NC*H*$_2$CH$_2$NBoc), 3.73 (brs, 4H, NCH$_2$C*H*$_2$NBoc), 7.09−7.24 (m, 4H, Ar*H*), 7.37−7.45 (m, 3H, Ar*H*), 7.66−7.76 (m, 3H, Ar*H*), 8.61 (d, 1H, J = 2.3 Hz, Ar*H*), 9.03 (s, 1H, Ar*H*), 11.69 (brs, 1H, N*H*C(O)). ESI-MS m/z [M + H]$^+$ = 528. Anal. ($C_{25}H_{26}BrN_3O_5$) C, H, N.

4-Benzyl-1-{4-bromo-2-[(2-Oxo-2*H*-chromene-3-carbonyl)-amino]-phenyl}-piperazine (9c). Compound **9c** was prepared by a method similar to that described for **9a** using **2c**. Yield 70%, mp 190 °C. R_f 0.27 (cHex−EtOAc, 2:1). ^{1}H NMR ($CDCl_3$) δ_H 2.77 (brs, 4H, NCH$_2$C*H*$_2$NCH$_2$Ph), 2.93 (brs, 4H, NC*H*$_2$CH$_2$NCH$_2$Ph), 3.65 (s, 2H, NC*H*$_2$Ph), 7.09 (d, 1H, J = 8.4 Hz, Ar*H*), 7.23 (dd, 1H, J = 2.3 and 8.4 Hz, Ar*H*), 7.28−7.48 (m, 7H, Ar*H*), 7.67−7.77 (m, 2H, Ar*H*), 8.81 (d, 1H, J = 2.3 Hz, Ar*H*), 9.01 (s, 1H, Ar*H*), 11.57 (brs, 1H, N*H*C(O)). ESI-MS m/z [M + H]$^+$ = 518. Anal. ($C_{27}H_{24}BrN_3O_3$) C, H, N.

1-{4-Bromo-2-[(2-Oxo-2*H*-chromene-3-carbonyl)-amino]-phenyl}-piperazine as a TFA Salt (9d). To a stirred solution of the appropriate *N*-Boc compound **9c** (60 mg, 0.11 mmol) in dry CH_2Cl_2 (10 mL) was added trifluoroacetic acid (TFA) (90 μL, 1.1 mmol) dropwise under nitrogen at 0 °C. The reaction mixture was stirred overnight at room temperature. After completion, the solvent and excess TFA were removed under reduced pressure, and the corresponding trifluoroacetic acid salt was identified as the desired compound **9d** (60 mg, 97%) as a TFA salt. R_f 0.09 (EtOAc: cyclohexane, 2:1). ^{1}H NMR (DMSO-d_6) δ_H 3.06 (brs, 4H, NC*H*$_2$CH$_2$NH·TFA), 3.32 (brs, 4H, NCH$_2$C*H*$_2$NH·TFA), 7.26−7.38 (m, 2H, Ar*H*), 7.48−7.58 (m, 2H, Ar*H*), 7.83 (t, 1H, J = 8.1 Hz, Ar*H*), 8.10 (d, 1H, J = 6.7 Hz, Ar*H*), 8.72 (d, 1H, J = 2.2 Hz, Ar*H*), 8.85 (brs, 2H, NCH$_2$CH$_2$N*H*·TFA), 9.10 (s, 1H, Ar*H*), 11.53 (brs, 1H, N*H*C(O)). ESI-MS m/z [M + H]$^+$ = 428. Anal. ($C_{22}H_{19}$-$BrF_3N_3O_5$) C, H, N.

1-{4-Bromo-2-[(2-Oxo-2*H*-chromene-3-carbonyl)-amino]-phenyl}-4-(2-hydroxyethyl)-piperazine (9e). Compound **9e** was prepared by a method similar to that described for **3e**. Yield 77%, R_f 0.45 (CH_2Cl_2: MeOH, 5:1). ^{1}H NMR ($CDCl_3$) δ_H 2.76 (brs, 2H, NC*H*$_2$CH$_2$OH), 2.89 (brs, 4H, NCH$_2$C*H*$_2$ NCH$_2$CH$_2$OH), 2.96 (brs, 4H, NC*H*$_2$CH$_2$NCH$_2$CH$_2$OH), 3.67−3.73 (m, 2H, NCH$_2$C*H*$_2$OH), 7.10 (d, 1H, J = 8.3 Hz, Ar*H*), 7.25 (dd, 1H, J = 2.2 and 8.3 Hz, Ar*H*), 7.42−7.46 (m, 2H, Ar*H*), 7.67−7.76 (m, 2H, Ar*H*), 8.82 (d, 1H, J = 2.2 Hz, Ar*H*), 9.01 (s, 1H, Ar*H*), 11.56 (brs, 1H, N*H*C(O)). ESI-MS m/z [M + H]$^+$ = 472. Anal. ($C_{22}H_{22}BrN_3O_4$) C, H, N.

{4-Bromo-2-[(8-methoxy-2-Oxo-2*H*-chromene-3-carbonyl)-amino]-phenyl}-morpholine (10a). Compound **10a** was prepared by a method similar to that described for **9a**. Yield 21%, 229 °C. R_f 0.41 (cHex−EtOAc, 1:1). ^{1}H NMR ($CDCl_3$) δ_H 2.89 (t, 4H, J = 4.5 Hz, NC*H*$_2$CH$_2$O), 3.98 (t, 4H, J = 4.5 Hz, NCH$_2$C*H*$_2$O), 4.01 (s, 3H, OC*H*$_3$), 7.06 (d, 1H, J = 8.4 Hz, Ar*H*), 7.20−7.35 (m, 5H,

Ar*H*), 8.83 (d, 1H, J = 2.3 Hz, Ar*H*), 8.98 (s, 1H, Ar*H*), 11.70 (brs, 1H, N*H*C(O)). ESI-MS m/z $[M + H]^+ = 459$. Anal. ($C_{21}H_{19}BrN_2O_5$) C, H, N.

1-{4-Bromo-2-[(8-methoxy-2-Oxo-2*H*-chromene-3-carbonyl)-amino]-phenyl}-4-*tert*-butoxycarbonyl-piperazine (10b). Compound **10b** was prepared by a method similar to that described for **9b**. Yield 64%, 241 °C. R_f 0.39 (cHex−EtOAc, 2:1). ^{1}H NMR ($CDCl_3$) δ_H 1.49 (s, 9H, CH_3, *t*Bu), 2.83 (brs, 4H, NCH_2CH_2NBoc), 3.71 (brs, 4H, NCH$_2$CH_2NBoc), 4.00 (s, 3H, OCH_3), 7.02 (d, 1H, J = 8.3 Hz, Ar*H*), 7.21−7.24 (m, 1H, Ar*H*), 7.29−7.34 (m, 2H, Ar*H*), 8.83 (d, 1H, J = 2.3 Hz, Ar*H*), 8.98 (s, 1H, Ar*H*), 11.73 (brs, 1H, N*H*C(O)). ESI-MS m/z $[M + H]^+ = 558$. Anal. ($C_{26}H_{28}BrN_3O_6$) C, H, N.

4-Benzyl-1-{4-bromo-2-[(8-methoxy-2-Oxo-2*H*-chromene-3-carbonyl)-amino]-phenyl}-piperazine (10c). Compound **10c** was prepared by a method similar to that described for **9c**. Yield 40%, mp 232 °C. R_f 0.31 (cHex−EtOAc, 2:1). ^{1}H NMR ($CDCl_3$) δ_H 2.79 (brs, 4H, NCH$_2$CH_2NCH$_2$Ph), 2.94 (brs, 4H, NCH_2CH$_2$NCH$_2$Ph), 3.66 (s, 2H, NCH_2Ph), 4.03 (s, 3H, OCH_3), 7.07 (d, 1H, J = 8.4 Hz, Ar*H*), 7.23 (dd, 1H, J = 2.3 and 8.4 Hz, Ar*H*), 7.28−7.34 (m, 5H, Ar*H*), 7.40−7.42 (m, 2H, Ar*H*), 8.81 (d, 1H, J = 2.3 Hz, Ar*H*), 8.98 (s, 1H, Ar*H*), 11.59 (brs, 1H, N*H*C(O)). ESI-MS m/z $[M + H]^+ = 548$. Anal. ($C_{28}H_{26}BrN_3O_4$) C, H, N.

1-{4-Bromo-2-[(8-methoxy-2-Oxo-2*H*-chromene-3-carbonyl)-amino]-phenyl}-piperazine as a TFA Salt (10d). Compound **10d** was prepared by a method similar to that described for **9d**. Yield 99%, R_f 0.08 (EtOAc:cHex,2:1). ^{1}H NMR (DMSO-d_6) δ_H 3.05 (t, 4H, J = 4.4 Hz, NCH_2CH$_2$NH), 3.34 (brs, 4H, NCH$_2$CH_2NH), 3.96 (s, 3H, OCH_3), 7.30 (d, 1H, J = 8.5 Hz, Ar*H*), 7.35 (dd, 1H, J = 2.2 and 8.5 Hz, Ar*H*), 7.43 (d, 1H, J = 7.5 Hz, Ar*H*), 7.48−7.52 (m, 1H, Ar*H*),7.61−7.66 (m, 1H, Ar*H*), 8.71(d, 1H, J = 2.2 Hz, Ar*H*), 8.70 (brs, 2H, NCH$_2$CH$_2$N*H*·TFA), 9.06 (s, 1H, Ar*H*), 11.58 (brs, 1H, N*H*C(O)). ESI-MS m/z $[M + H]^+ = 458$. Anal. ($C_{23}H_{21}BrF_3N_3O_6$) C, H, N.

1-{4-Bromo-2-[(8-methoxy-2-Oxo-2*H*-chromene-3-carbonyl)-amino]-phenyl}-4-(2-hydroxyethyl)-piperazine (10e). Compound **10e** was prepared by a method similar to that described for **3e**. Yield 97%, mp 219 °C. R_f 0.65 (CH_2Cl_2: MeOH, 5:1). ^{1}H NMR (DMSO-d_6) δ_H 2.76 (brs, 2H, NCH_2CH$_2$OH), 3.16 (brs, 4H, NCH$_2$CH_2NCH$_2$CH$_2$OH), 3.36 (brs, 4H, NCH_2CH$_2$NCH$_2$CH$_2$OH), 3.79 (brs, 2H, NCH$_2$CH_2OH), 3.96 (s, 3H, OCH_3), 7.29 (d, 1H, J = 8.3 Hz, Ar*H*), 7.36 (dd, 1H, J = 2.2 and 8.3 Hz, Ar*H*), 7.42−7.46 (m, 2H, Ar*H*), 7.67−7.76 (m, 1H, Ar*H*), 8.74 (d, 1H, J = 2.2 Hz, Ar*H*), 9.07 (s, 1H, Ar*H*), 11.50 (brs, 1H, N*H*C(O)). ESI-MS m/z $[M + H]^+ = 502$. Anal. ($C_{23}H_{24}BrN_3O_5$) C, H, N.

1-{4-(Phenyl)-2-[(2-Oxo-2*H*-chromene-3-carbonyl)-amino]-phenyl}-4-*tert*-butoxycarbonyl-piperazine (11b). Compound **11b** was prepared by a method similar to that described for **4b**. Yield 45%, mp 224 °C. R_f 0.76 (EtOAc:cHex,1:1). ^{1}H NMR ($CDCl_3$) δ_H 1.45 (s, 9H, CH_3, *t*Bu), 2.98 (brs, 4H, NCH_2CH$_2$NBoc), 3.92 (brs, 4H, NCH$_2$CH_2NBoc), 7.23−7.26 (m, 1H, Ar*H*), 7.32−7.47 (m, 7H, Ar*H*), 7.56−7.69 (m, 2H, Ar*H*), 7.71−7.78 (m, 2H, Ar*H*), 8.92 (d, 1H, J = 2.0 Hz, Ar*H*), 9.05 (s, 1H, Ar*H*), 11.75 (brs, 1H, N*H*C(O)). ESI-MS m/z $[M + H]^+ = 526$. Anal. ($C_{31}H_{31}N_3O_5$) C, H, N.

4-Benzyl-1-{4-(phenyl)-2-[(2-Oxo-2*H*-chromene-3-carbonyl)-amino]-phenyl}-piperazine (11c). Compound **11c** was prepared by a method similar to that described for **4c**. Yield 51%, mp 235 °C. R_f 0.61 (EtOAc:cHex,1:1). ^{1}H NMR ($CDCl_3$) δ_H 2.79 (brs, 4H, NCH$_2$CH_2NCH$_2$Ph), 3.00 (t, 4H, J = 4.7 Hz, NCH_2CH$_2$NCH$_2$Ph), 3.66 (s, 2H, NCH_2Ph), 7.29−7.44 (m, 10H, Ar*H*), 7.46−7.49 (m, 2H, Ar*H*), 7.57 (d, 2H, J = 8.7 Hz, Ar*H*), 7.67−7.75 (m, 2H, Ar*H*), 8.89 (d, 1H, J = 1.7 Hz, Ar*H*), 9.03 (s, 1H, Ar*H*), 11.60 (brs, 1H, N*H*C(O)). ESI-MS m/z $[M + H]^+ = 516$. Anal. ($C_{33}H_{29}N_3O_3$) C, H, N.

1-{4-(Phenyl)-2-[(2-Oxo-2*H*-chromene-3-carbonyl)-amino]-phenyl}-piperazine (11d). Compound **11d** was prepared by a method similar to that described for **9d**. Yield 13%, R_f 0.06 (EtOAc: cHex,2:1). ^{1}H NMR (CD_3OD) δ_H 3.13 (brt, 4H, J = 4.7 Hz, NCH_2CH$_2$NH), 3.00 (brt, 4H, J = 4.7 Hz, NCH$_2$CH_2NH), 7.39−7.51 (m, 8H, Ar*H*), 7.61−7.64 (m, 2H, Ar*H*), 7.75−7.84 (m, 1H, Ar*H*), 7.91 (dd, 1H, J = 1.6 and 8.1 Hz, Ar*H*), 8.80 (brs, 1H, NCH$_2$CH$_2$N*H*·TFA), 8.85 (d, 1H, J = 2.0 Hz, Ar*H*), 9.06 (s, 1H, Ar*H*), 11.50 (brs, 1H, N*H*C(O)). ESI-MS m/z $[M + H]^+ = 426$. Anal. ($C_{28}H_{24}F_3N_3O_5$) C, H, N.

Bace-1 Enzymatic Assay. These experiments have been preformed using BACE1 (β-secretase) FRET Kit assay, from PanVera Corporation (Madison, WI), 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 procedure is as follows: the substrate and enzyme are diluted according to the described protocol into the provided assay buffer (50 mM Tris, pH 7.5, 10% glycerol). Each inhibitor is diluted into DMSO at the desired concentration. The substrate (Rh-EVNLDAEFK-Quencher; 10 μL of the main solution) and each inhibitor (1 μL of the corresponding solution) are introduced in the 96-well flat bottom black polystyrene plate (Corning, NY). The resulting mixtures are gently mixed and 10 μL of the enzyme solution are then added to each well to start the reaction. The reaction mixtures are incubated at 25 °C for 90 min and the spectrofluorescence is monitored at 530−545 nm (excitation wavelength) and 570−590 nm (emission wavelength). The kinetic assays are performed in duplicate for each inhibitor, using BACE1 inhibitor (H-Lys-Thr-Glu-Glu-Ile-Ser-Glu-Val-Asn-(3*S*,4*S*)-Stat-Val-Ala-Glu-Phe-OH, IC_{50} = 30 nM; Calbiochem, Beeston, UK) as reference (negative test, no cleavage), the provided BACE1 Product Standard (Rh-EVNL) as positive test (100% cleavage), and a control test using only the enzyme and the substrate under the same conditions to allow a 15% cleavage of the substrate after 90 min.

Aβ Cell-Based Assay EC_{50}. Inhibitory potencies of each compound on γ-secretase activity were analyzed by cell-based assay. Mouse Neuro2a (N2a) and human embryonic kidney (HEK293) cells were used for Aβ cell-based assays.[28] Cells were plated in 24-well plates and were cultured at confluency. HEK293 cells were further transfected with C100 in pcDNA3.1-Hygro and cultured for 12 h. For compound screening and dose−response testing, compounds were diluted from stock solutions in dimethyl sulfoxide (DMSO) to yield a final concentration equal to 0.1% DMSO in media. After 24 h treatment with compounds, conditioned media were drawn off and analyzed by a sandwich ELISA (BNT77/BA27 and BNT77/BC05) specific for total Aβ.[28,29] Reduction of Aβ production was measured relative to control cells treated with 0.1% DMSO and expressed as a percentage inhibition. Compounds toxicities were measured by MTT assay as previously described.[30]

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Supporting Information Available: Synthetic procedures and table of elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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