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Novel peptidomimetics as BACE-1 inhibitors: Synthesis, molecular modeling, and biological studies

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ABSTRACT

Aiming at identifying new scaffolds for BACE-1 inhibition devoid of the pharmacokinetic drawbacks of peptide-like structures, we investigated a series of novel peptidomimetics based on a 1,4-benzodiazepine (BDZ) core **1a–h** and their seco-analogues **2a–d**. We herein discuss synthesis, molecular modeling and in vitro studies which, starting from **1a**, led to the seco-analogues (*R*)-**2c** and (*S*)-**2d** endowed with BACE-1 inhibition properties in the micromolar range both on the isolated enzyme and in cellular studies. These data can encourage to pursue these analogues as hits for the development of a new series of BACE-1 inhibitors active on whole-cells.

Alzheimer's disease (AD), one of the most prevalent neurodegenerative disorders among the elderly, is pathologically characterized by the extracellular accumulation of amyloid-β (Aβ) plaques and by intracellular neurofibrillary tangles. Considerable evidence indicates a central role of the Aβ peptide and its aggregation in the pathogenesis of AD.[1] High levels of soluble Aβ peptides correlate to cognitive decline in AD[2,3] and the original amyloid cascade hypothesis has evolved to propose that soluble oligomeric Aβ assemblies precede deposition and are the proximal cause of synaptic dysfunction and early impairment in AD.[4] Aβ40 and Aβ42[5] are the two major isoforms of Aβ found in AD brains. Although the Aβ40 is the most abundant isoform, Aβ42 is enriched in AD brains as it progressively accumulates into extracellular senile plaques.[4] The proteolytic enzyme β-secretase (BACE-1) catalyzes the rate-limiting step of the Aβ generation by cleaving the Met671–Asp672 peptide bond of amyloid precursor protein (APP) at the extracellular space.[6,7] Currently available therapies for AD only treat disease symptoms and do not address the underlying disease processes.[8] On the basis of a number of seminal in vitro and in vivo studies the aspartic protease BACE-1 has been recognized as a relevant drug target for the development of AD disease-modifying therapies.[9] BACE-1 potent inhibitors have been produced by academic and industrial research.[10] However very few of them fulfill the requirements for in vivo biological and clinical studies and only recently, orally available highly efficient BACE-1 inhibitors became available.[11] To identify new scaffolds for BACE-1 inhibition to overcome the well-known pharmacokinetic drawbacks of peptide-like structures, we decided to search for novel peptidomimetic compounds based on the C_8/C_9-substituted 1,4-benzodiazepine (BDZ) structural system or on the seco-1,4-BDZ scaffold, represented by the core structures **1** and **2** depicted in Figure 1.

The BDZ system has not been explored to date for developing BACE-1 inhibitors, and the BDZ system is one of the most important privileged pharmacogenic structures for drug discovery endowed with a high degree of druggability. This prompted us to develop compounds **1a–h**. Furthermore, we recently discovered a versatile synthetic protocol for the synthesis of C_8 and C_9 modified

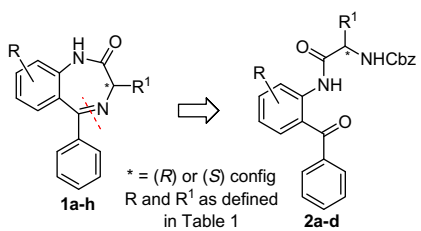

Figure 1. Title compounds: benzodiazepines **1a–h** and seco-analogues **2a–d**.

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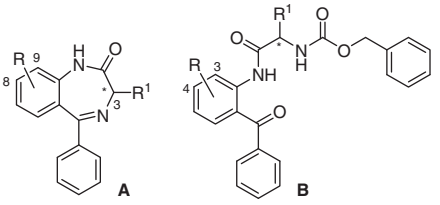
1,4-BDZs which gave us the opportunity to investigate BDZs bearing at these positions protonatable and non-protonatable functions.[12] Thus, we started our research program with the synthesis and evaluation of a series of BDZs and their seco-analogues (**1** and **2**, Fig. 1). In particular the compounds were functionalized with a piperazine group at C_9 and/or with the hydroxyethylamine (HEA) moiety introduced at various positions of the BDZ scaffold of **1** and of the seco-BDZs **2** (see Table 1).

Compounds **1a**,**b** were prepared as described in Scheme 1 following a synthetic procedure previously described for the preparation of analogues **1c**,**d**.[12] The L- or D-Cbz-protected phenylglycines were activated by means of hexachloroacetone (HCA) and triphenylphosphine, and then treated with the piperazino-substituted aminobenzophenone **3**[12] to give compound **2a**. After deprotection and cyclization (*R*)- and (*S*)-**1a** were obtained. Treatment of (*S*)-**1a** with HCl gave the amine (*S*)-**1b**.

The synthesis of the BDZ scaffold necessary for the preparation of the C_8-hydroxyethylamino-BDZs was performed starting from the benzophenone **4**[12] (Scheme 2) which was coupled with Cbz or Fmoc protected phenylglycine and successively exposed to tributylvinyl tin[13] to give **5a**,**b**. These latter compounds were oxidized in the presence of *m*-chloroperbenzoic acid (*m*-CPBA). The epoxide ring **6a** (obtained as a mixture of two diasteroeisomers) was subjected to a nucleophilic ring opening reaction by exposure to *N*-Boc-piperazine. The resulting hydroxyethylamino-derivative **2b** was obtained in good yield. When **6a** was treated with benzylamine in the presence of lithium perchlorate[14] both regioisomers were obtained (**2c** and **2d**). Deprotection of *N*-Cbz **2b** followed by cyclization furnished compound **1e**. Starting from **2c**,**d,** deprotection of the *N*-Cbz under different reaction conditions was unsuccessful, so the corresponding *N*-Fmoc protected derivatives **2e**,**f** were prepared. Deprotection of the Fmoc-group in the presence of diethylamine was achieved and the intermediates were cyclized to **1f**,**g**. Scheme 3 reports the synthesis of **1h**. Starting from the commercially available aminobenzophenone **7**, coupling with L-Cbz-Met followed by a *m*-CPBA oxidation provided the sulfoxide (*S*)**-8**. The sulfoxide was then submitted to a thermal elimination reaction by refluxing in xylene ((*S*)**-9**). The olefin **9** was oxidized to the corresponding epoxide **10** (mixture of two diastereoisomers) which was reacted with *N*-Boc-piperazine. After *N*-Cbz and *N*-Boc deprotection and cyclization, **1h** was obtained.

The BDZs **1a**–**h** and the seco-analogues **2a**–**d**, bearing or not the HEA substructure, were tested against the isolated BACE-1 enzyme in fluorescence-based assays (Table 1). Analytical data for the tested compounds are given as Supplementary Information (SI). HEA fragment is a peptide cleavage transition state mimetic, which found multiple applications in targeting aspartyl proteases.[15] Consequently, to improve solubility, the piperazine moiety, present in **1a**–**d** (**1a**, Log*S* = −4.77, **1b**, Log*S* = −1.83, calculated at pH 7, ACD/Labs V12.0, Toronto, Canada), was combined to the HEA fragment (**1e**,**h**) (**1e**, Log*S* = −4.37, **1h**, Log*S* = 0.44). To evaluate the effect of the piperazine ring we synthesized analogues **1f**,**g** exclusively bearing the HEA moiety. In the BDZs series only **1a**, **1g** and **1h** showed inhibition of BACE-1 in the micromolar range (Table 1). Starting from **1a** we also investigated the stereoselectivity of interaction and we observed that (*R*)-**1a** and (*S*)-**1a** showed a comparable potency. Removal of the carbamoyl moiety from **1a** dramatically reduced potency (**1b**). Moreover, tethering the phenyl ring at C_3 of **1a** by one or two methylenes led to inactive analogues (**1c** and **1d**). The BDZ core structure was also decorated at C_8 or C_3 with HEA functionalities bearing or not the piperazine system (**1e**–**h**). Compounds **1e**,**f** present a substitution at the HEA-carbon atom bearing the OH, while the HEA fragment of **1g**,**h** was substituted on the carbon atom close to the nitrogen. As regard to **1b** and **1f**, when only the piperazine (**1b**) or the HEA group (**1f**) is present, neither

Table 1
BACE-1 inhibition activity of compounds **1a**–**h** and **2a**–**d** as IC_{50} (μM)[a]



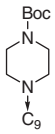
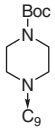
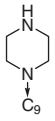
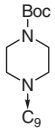
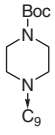
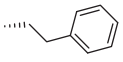
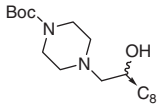
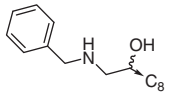
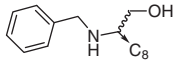
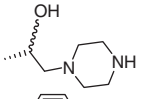
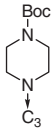
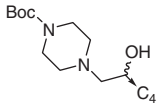
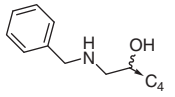
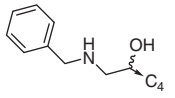
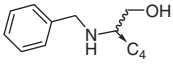
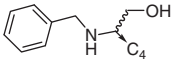
Compd	A/B	R	R^1	IC_{50}[b] (μM)
(*R*)-**1a**	A			43
(*S*)-**1a**	A			35
(*S*)-**1b**	A			NA[c]
(*S*)-**1c**	A			NA[c]
(*S*)-**1d**	A			NA[c]
(*R*)-**1e**	A			NA[c]
(*R*)-**1f**	A			NA[c]
(*R*)-**1g**	A			10.9
(*S*)-**1h**	A	—		12.5
(*S*)-**2a**	B			NA[c]
(*R*)-**2b**	B			4.9
(*S*)-**2c**	B			2.4
(*R*)-**2c**	B			2.6

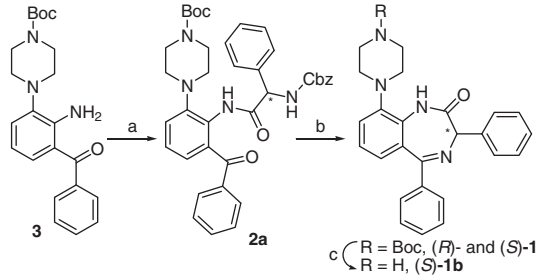
Table 1 (*continued*)

Compd	A/B	R	R^1	IC_{50}[b] (μM)
(*S*)-**2d**	B			4.1
(*R*)-**2d**	B			4.2

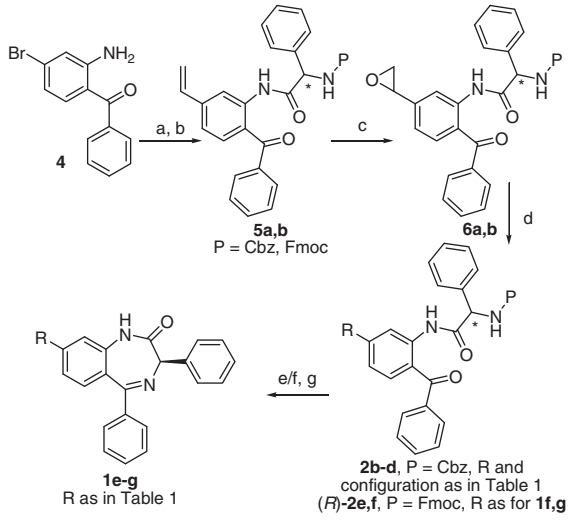
[a] Tests were performed on the isolated BACE-1 enzyme as previously described,[16] experimental details are given as SI.

[b] Each value is the mean of at least three experiments (all SD are within 10% of the mean).

[c] NA not active at 100 μM.

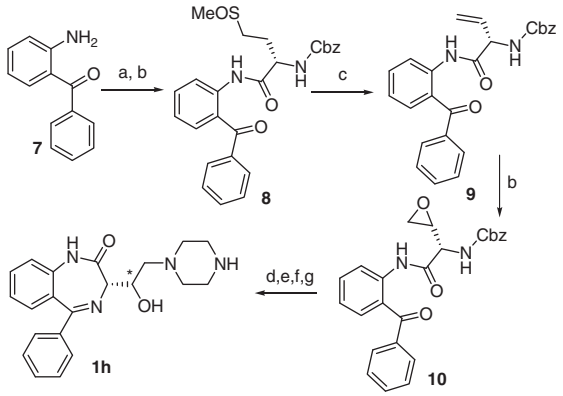


Scheme 1. Reagents and conditions: (a) L- or D-Cbz-Phenyl-Gly, HCA, PPh_3, DCM, −10 °C, 30′ then rt, 5′, 95%; (b) Pd/C 10%, 1,4-cyclohexadiene, EtOH, reflux, 24 h, 93%; (c) AcCl, MeOH, 60 °C, 30′, 99%.



Scheme 2. Reagents and conditions: (a) L- or D-Cbz or Fmoc-Phenyl-Gly, HCA, PPh_3, DCM, −10 °C, 30′, then rt, 5′, 83% for Cbz-derivative and 88% for Fmoc-derivative; (b) $Pd(PPh_3)_4$, tributyl(vinyl)tin, dry toluene, reflux, 1 h, 55% for **5a**, 60% for **5b**; (c) *m*-CPBA, DCM, 0 °C, 2 h, 28% for **6a**, 53% for **6b**; (d) *N*-Boc-piperazine, EtOH, reflux 24 h, 64% for **2b**, $LiClO_4$, benzylamine, MeCN, rt, 1 h, 21–42% for **2c**–**f**; (e) Pd/C 10%, 1,4-cyclohexadiene, EtOH, reflux, 24 h, 99%; (f) Et_2NH, DCM, rt, 5 h, 99%; (g) EtOH, reflux, 24 h, 20–70%.

the protonated nitrogen nor the hydroxyl group are suitably close to any of the catalytic aspartic residues to engage an interaction, in contrast with **1g** and **1h** which regain a certain activity against the enzyme (with IC_{50} values of about 10 μM). Several seco-analogues of BDZs (**2a**–**d**, Table 1) were also tested for activity and, with the exception of (*S*)-**2a**, they were found more potent inhibitors than their BDZ counterparts. Accordingly, the intermediates **2b** and **2c** showed a micromolar activity against the enzyme, being more potent than the BDZs **1e**,**f**. Furthermore, as already observed with **1a**,



Scheme 3. Reagents and conditions: (a) L-Cbz-Met, HCA, PPh_3, DCM, −10 °C, 30′, then rt, 5′, 56%; (b) *m*-CPBA, DCM, 0 °C, 2 h, 62% for **8**, 87% (76:24 *dr*) for **10**; (c) xylene, 200 °C, sealed tube, 3 h, 45%; (d) *N*-Boc-piperazine, EtOH, reflux, 24 h, 56%; (e) Pd/C 10%, 1,4-cyclohexadiene, EtOH, reflux, 24 h, 99%; (f) AcCl, dry MeOH, 60 °C, 30′, 99%; (g) EtOH, reflux, 24 h, 57%.

the configuration at the aminoacidic carbon did not influence activity ((*R*)-**2c**,**d** vs (*S*)-**2c**,**d**). The seco-analogue **2d** again proved to be more potent than the BDZs counterpart **1g**.

This structure–activity relationship (SAR) analysis was rationalized at the molecular level by means of docking studies (AutoDock4.2 program) performed with our ligands into the BACE-1 active site. BACE-1 is a structurally challenging protein target, which displays a pronounced induced-fit upon ligand interaction. Indeed, a detailed comparison of the available X-ray structures suggests that the flap region (residues 68–74) of the enzyme undergoes a massive rearrangement upon ligand binding, as well as the 10s-loop (residues 9–14).[17,18] Thus, we selected for docking studies five X-ray structures representative for the enzyme flexibility. Particularly, we chose 1XN3 (PDB code) as representative of the flap region open conformation, while 1FKN, 1W51 and 1TQF were chosen to represent the closed, the open and the outlier conformation of the 10s-loop.[17,18] These four structures, together with the 2G94, also account for side chains flexibility of key active site residues (L30, R128, K224, R235, R307). Docking of our ligands using each of the five BACE-1 X-ray structures provided similar results. However, results obtained using 1W51 and 1FKN better suited with the experimentally determined IC_{50}. Thus, we will herein discuss the poses found in 1W51 (highly similar to those found in 1FKN).

Analysis of the docking structure of (*S*)-**1a** into the BACE-1 enzyme revealed that the BDZ scaffold lies under the flap region (Fig. 2, and SI for experimental details). The R substituent present on the BDZ core (*N*-Boc piperazine) occupies the S2′ pocket with no H-bonding to the Asp residues (D32 and D228) at the catalytic

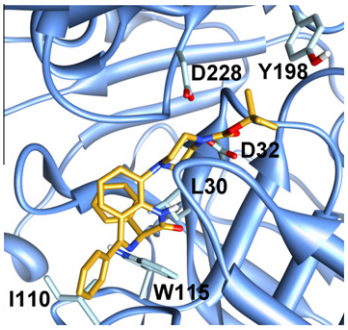


Figure 2. Docked structure of (*S*)-**1a** into the BACE-1 (PDB code: 1W51). Ligands carbon atoms are displayed in golden, and key binding site residues as cyan sticks.

site. The two phenyl rings at C_3 (R^1, Table 1) and C_5 positions lean forward the S1 and the S3 cavities, respectively, establishing hydrophobic contacts with L30, I110 and W115 side chains. These interactions may account for the micromolar inhibitory potency of (*S*)-**1a**. In line with the experimentally determined IC_{50}, a similar docking outcome was obtained with the isomer (*R*)-**1a**. Consistent with the binding pose of **1a**, and in line with the IC_{50}, the tethering of the phenyl ring at C_3 by one or two methylenes (**1c** and **1d**) and the introduction of an hydroxyethyl group (**1e**), resulted in a severe clash with the S1 pocket wall (due to the BDZ scaffold rigidity), and in a clash in the S2′ pocket respectively. It must be noted that in compounds **1b** and **1f**, neither the protonated nitrogen (**1b**) nor the hydroxyl group (**1f**) lay close enough to any of the Asp residues at the catalytic site for engaging productive interactions. Contrastingly, for **1g** and **1h** a more favorable spatial arrangement of the HEA portion may account for the micromolar BACE-1 inhibition potency. In line with the experimental IC_{50} values docking results obtained with **1a** suggested that the major flexibility of the seco-analogues, would allow the molecules to better fit within BACE-1 active site. Accordingly, when the most active compound (*S*)-**2c** was docked within the enzyme (Fig. 3a), H-bonding interactions with the catalytic Asp residues were detected (Fig. 3b). Furthermore, the benzylamine group of (*S*)-**2c** occupies the S2′ pocket, establishing a T-shaped interaction with the Y198 side chain and hydrophobic contacts with I226, T329, S35, and Y71. The *o*-disubstituted phenyl ring, lying under the flap region, establishes a π–π stacking with Y71 side chain and hydrophobic contacts with F108 and I118. The phenone moiety of (*S*)-**2c** enters the S1 cavity, surrounded by a L30 and I110, engaging a T-shaped interaction with W115, while the R_1 substituent (phenyl ring) plunges into the S3 pocket where no tight interactions are visible. The benzylcarbamate branch stretches out over the S3 pocket where an H-bond is detected between T232 backbone NH and the carbamate carbonyl oxygen; R235 side chain establishes a cation-π interaction with the aromatic ring. The experimentally observed lack of stereoselectivity of interaction ((*S*)-**2c** vs (*R*)-**2c**, Table 1) was justified by the superimposable binding poses obtained for both enantiomers. Also the seco-analogues (*R*)-**2b**, (*R*)- and (*S*)-**2d** share the binding mode and enzyme inhibition potency in the low micromolar range with compound **2c** (as already observed in the BDZ series). Our proposed binding mode could also explain the loss of activity of compound (*S*)-**2a**, where the piperazine moiety at the C_3 faces the active site floor and gives a massive clash.

BACE-1 is a transmembrane protein anchored to the luminal side of the intracellular compartment. Since some BACE-1 inhibitors active in isolated enzyme-assays fail when tested in cellular assays, we engaged the most interesting analogues of the series ((*S*)-**1g**, (*S*)-**2c**, (*R*)-**2c**, and (*S*)-**2d**) in functional cell-based assays. One assay used HEK293 cells expressing APP construct containing a modified BACE cleavage sequence, NFEV and a K612V mutation which prevents processing by α-secretase.[19] The other assay employs SHSY5Y cells expressing APP NFEV and the wild-type α-cleavage site. The compounds were tested to determine their IC_{50} against the generation of sAPPβ (direct functional read out for BACE-1 activity). The determined IC_{50} values ranged from 2.6 μM to >22 μM in HEK293 cells and from 1.2 μM to >22 μM in the SHSY5Y cell line. In more detail, (*S*)-**1g** (IC_{50} >22 μM in HEK293, and IC_{50} >11 μM in SHSY5Y) was in general less potent than the seco-compounds. Indeed (*R*)-**2c** and (*S*)-**2d** were found the most interesting of the series showing IC_{50} values in both cell lines ranging from 1.2 μM to 2.6 μM. Activities on cell based assays are in line with the observed potencies on the isolated enzyme thus suggesting that these scaffolds could be new hits for the development BACE-1 inhibitors active on whole-cell assays.

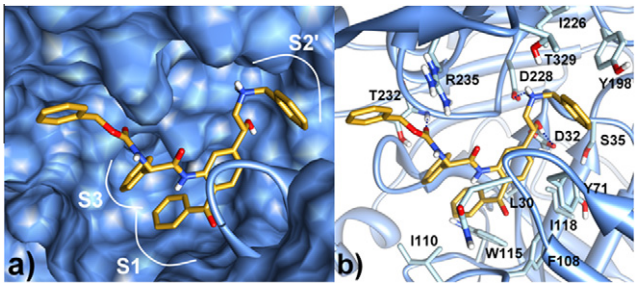


Figure 3. Docked structure of (*S*)-**2c** into the BACE-1 (PDB code: 1W51), where a portion of the binding site is visible as cornflower surface. (a) Detailed interaction mode of (*S*)-**2c** with BACE-1 active site residues; (b) ligands carbon atoms are displayed in golden, and key binding site residues as cyan sticks. H-Bonds are shown as dark-blue dotted lines.

In summary, we described a new series of BACE-1 inhibitors based on a BDZ or a seco-BDZ structure. These compounds represent the first benzodiazepine compounds disclosed as BACE-1 inhibitors. As shown in Table 1, the seco-analogues proved to be slightly more potent than BDZs and through docking studies we have been able to clarify why the BDZ system opening led to an improvement of the inhibitory potency. Nevertheless, optimization of the inhibitors is necessary to obtain IC_{50}s in the nanomolar range and rational modifications of the BDZ scaffold will be guided by the docking studies here presented. Taken together our molecular modeling studies traced out the experimentally determined SAR data, explained the higher potency of the seco-BDZs intermediates, and highlighted the optimization strategy which is currently ongoing in our laboratories.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.11.011.

References and notes

1. Citron, M. *Nat. Rev. Drug Discov.* **2010**, *9*, 387.
2. McLean, C. A.; Cherny, R. A.; Fraser, F. W.; Fuller, S. J.; Smith, M. J.; Beyreuther, K.; Bush, A. I.; Masters, C. L. *Ann. Neurol.* **1999**, *46*, 860.
3. Naslund, J.; Haroutunian, V.; Mohs, R.; Davis, K. L.; Davies, P.; Greengard, P.; Buxbaum, J. D. *JAMA* **2000**, *283*, 1571.
4. Walsh, D. M.; Selkoe, D. J. *J. Neurochem.* **2007**, *101*, 1172.
5. Haass, C.; Hung, A. Y.; Schlossmacher, M. G.; Oltersdorf, T.; Teplow, D. B.; Selkoe, D. J. *Ann. N.Y. Acad. Sci.* **1993**, *695*, 109.
6. Yan, R.; Bienkowski, M. J.; Shuck, M. E.; Miao, H.; Tory, M. C.; Pauley, A. M.; Brashier, J. R.; Stratman, N. C.; Mathews, W. R.; Buhl, A. E.; Carter, D. B.; Tomasselli, A. G.; Parodi, L. A.; Heinrikson, R. L.; Gurney, M. E. *Nature* **1999**, *402*, 533.
7. Vassar, R.; Bennett, B. D.; Babu-Khan, S.; Kahn, 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.; Biere, A. L.; Curran, E.; Burgess, T.; Louis, J. C.; Collins, F.; Treanor, J.; Rogers, G.; Citron, M. *Science* **1999**, *286*, 735.
8. Sadowski, M.; Wisniewski, T. *Curr. Pharm. Des.* **1943**, *2007*, 13.
9. De Strooper, B. *Physiol. Rev.* **2010**, *90*, 465.
10. Ghosh, A. K.; Brindisi, M.; Tang, J. *J. Neurochem.* **2012**, *120*, 71.
11. Sankaranarayanan, S.; Holahan, M. A.; Colussi, D.; Crouthamel, M. C.; Devanarayan, V.; Ellis, J.; Espeseth, A.; Gates, A. T.; Graham, S. L.; Gregro, A. R.; Hazuda, D.; Hochman, J. H.; Holloway, K.; Jin, L.; Kahana, J.; Lai, M. T.; Lineberger, J.; McGaughey, G.; Moore, K. P.; Nantermet, P.; Pietrak, B.; Price, E. A.; Rajapakse, H.; Stauffer, S.; Steinbeiser, M. A.; Seabrook, G.; Selnick, H. G.; Shi, X. P.; Stanton, M. G.; Swestock, J.; Tugusheva, K.; Tyler, K. X.; Vacca, J. P.; Wong, J.; Wu, G.; Xu, M.; Cook, J. J.; Simon, A. J. *J. Pharmacol. Exp. Ther.* **2009**, *328*, 131.

12. Butini, S.; Gabellieri, E.; Huleatt, P. B.; Campiani, G.; Franceschini, S.; Brindisi, M.; Ros, S.; Coccone, S. S.; Fiorini, I.; Novellino, E.; Giorgi, G.; Gemma, S. *J. Org. Chem.* **2008**, *73*, 8458.
13. Krolski, M. E.; Renaldo, A. F.; Rudisill, D. E.; Stille, J. K. *J. Org. Chem.* **1988**, *53*, 1170.
14. Chini, M.; Crotti, P.; Macchia, F. *J. Org. Chem.* **1991**, *56*, 5939.
15. Zhu, Z. *Trends Pharmacol. Sci.* **2012**.
16. Porcari, V.; Magnoni, L.; Terstappen, G. C.; Fecke, W. *Assay Drug Dev. Technol.* **2005**, *3*, 287.
17. Cosconati, S.; Marinelli, L.; Di Leva, F. S.; La Pietra, V.; De Simone, A.; Mancini, F.; Andrisano, V.; Novellino, E.; Goodsell, D. S.; Olson, A. J. *J. Chem. Inf. Model.* **2012**.
18. Limongelli, V.; Marinelli, L.; Cosconati, S.; Braun, H. A.; Schmidt, B.; Novellino, E. *ChemMedChem* **2007**, *2*, 667.
19. Pietrak, B. L.; Crouthamel, M. C.; Tugusheva, K.; Lineberger, J. E.; Xu, M.; DiMuzio, J. M.; Steele, T.; Espeseth, A. S.; Stachel, S. J.; Coburn, C. A.; Graham, S. L.; Vacca, J. P.; Shi, X. P.; Simon, A. J.; Hazuda, D. J.; Lai, M. T. *Anal. Biochem.* **2005**, *342*, 144.