Graphical Abstract

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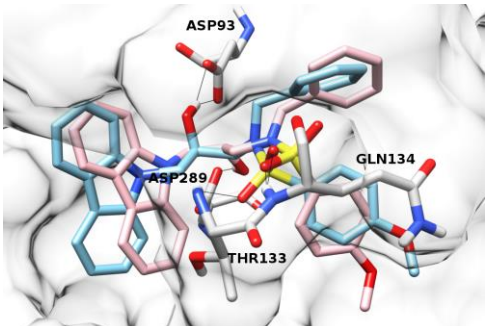

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Sulfonamido-derivatives of unsubstituted carbazoles as BACE1 inhibitors

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ABSTRACT

A novel series of variously substituted *N*-[3-(9*H*-carbazol-9-yl)-2-hydroxypropyl]-arylsulfonamides has been synthesized and assayed for β-Secretase (BACE1) inhibitory activity. BACE1 is a widely recognized drug target for the prevention and treatment of Alzheimer's Disease (AD). The introduction of benzyl substituents on the nitrogen atom of the arylsulfonamide moiety has so far led to the best results, with three derivatives showing IC_{50} values ranging from 1.6 to 1.9 μM. Therefore, a significant improvement over the previously reported series of *N*-carboxamides (displaying IC_{50}'s ≥ 2.5 μM) has been achieved, thus suggesting an active role of the sulfonamido-portion in the inhibition process. Preliminary molecular modeling studies have been carried out to rationalize the observed structure-activity relationships.

Alzheimer's disease (AD) is the most common form of dementia, which occurs predominantly in older people (over 65 years of age). It is a progressive and irreversible neurodegenerative disorder, which compromises cognitive functions (memory, thinking, reasoning) and behavioral skills in such a way as to interfere with daily life and with the fulfillment of the simplest tasks. The main neuropathological features of AD are extracellular senile plaques, essentially made of amyloid β peptide (Aβ),[1] and intracellular neurofibrillary tangles, caused by the aggregation of phosphorylated tau proteins.[2]

The Aβ peptide is generated through proteolytic processing of the amyloid precursor protein (APP) first by β-secretase (BACE1) followed by γ-secretase. In particular, the cleavage operated by BACE1 produces the secreted amino-terminal part of APP (sAPPβ) and the membrane-bound carboxy-terminal fragment, which is 99 amino acids in length (C99). γ-Secretase subsequently cleaves the C99 fragment, releasing the Aβ peptide, which aggregates to form toxic amyloid plaques in the brain.[3] It has been demonstrated that BACE1 knockout (BACE1 -/-) mice are unable to generate C99 and Aβ, are viable,[4] and drastically ameliorate the pathology when crossed with APP transgenic mice (a mouse model of AD).[5] Therefore, BACE1 is an attractive drug target for lowering brain levels of amyloid beta and, consequently, for the treatment or prevention of AD.

Many BACE1-inhibitors studied to date are peptidomimetics and incorporate the hydroxyethylamine (HEA) moiety,[6] which is known to well mimic the transition state of aspartyl proteases (like BACE1) substrates.[7] These compounds have in general high molecular weights and suffer from poor blood brain barrier permeability.[8] So, in recent years, more efforts have been dedicated to the development of non-peptidomimetic BACE1-inhibitors, with the aim of obtaining smaller active molecules and, therefore, more drug-like agents for the treatment of AD.[9-11] Among those, only a few compounds have entered in advanced clinical phases.[11, 12]

We previously reported a series of α-naphthylaminoalcohol derivatives of unsubstituted carbazole showing a BACE1 inhibitory activity in the low μM range.[13] Furthermore, we recently reported that *N*-carboxamido-derivatives are still active against this enzyme (Figure 1).[14]

In this work, we describe the synthesis and the evaluation of BACE1 inhibitory activity of a further series of derivatives, in which the unsubstituted carbazole-methyl carbinol portion was kept constant and the *N*-atom was sulfonylated with different

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groups (**1-24**, Figure 1). A focused screening in the literature of this type of compounds was primarily carried out, and six derivatives were found commercially available (**1-3**, **5**, **11** and **22**, see Table 1).

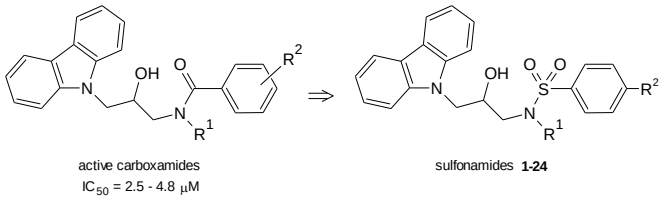
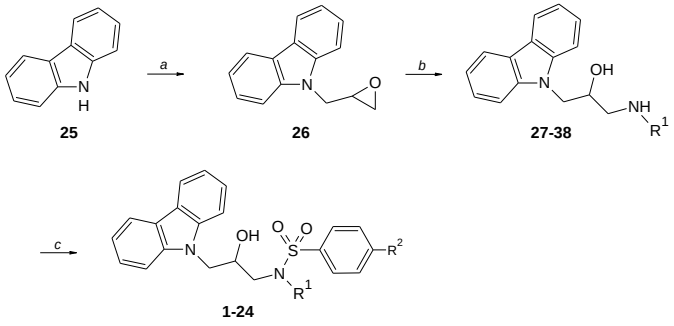


Figure 1. General structure of already reported *N*-[3-(9*H*-carbazol-9-yl)-2-hydroxypropyl]-arylcarboxamides and novel variously substituted *N*-[3-(9*H*-carbazol-9-yl)-2-hydroxypropyl]-arylsulfonamides (**1-24**, see also Table 1).

These sulfonamido-derivatives were synthesized as shown in Scheme 1. Commercially available carbazole **25** was alkylated with epichlorohydrin (2.5 eq., added dropwise at 0 °C) in the presence of KOH (1.2 eq.) in DMF, affording epoxide **26**. Reaction of this intermediate with the appropriate amine (2.0 eq.) in EtOH afforded aminoalcohols **27-38**. These derivatives were then treated with variously substituted aryl-sulfonyl-chlorides (1.1 eq.) in the presence of PS-DIEA (1.2 eq.) and DMAP (catalytic amount) in CH_2Cl_2, obtaining final compounds **1-24**.



Scheme 1. Reagents and conditions: a) epichlorohydr in, KOH, DMF, 0 °C, 5 h, 50%; b) R^1-NH_2, EtOH, 65 °C, overnight, 40-79%; c) $ArSO_2Cl$, PS-DIEA, DMAP, CH_2Cl_2, r. t., overnight, 13-86%.

The inhibitory activity of the newly synthesized compounds towards BACE1 was determined by a previously reported fluorescence-based assay[15] and the results are shown in Table 1.

The introduction of a simple phenyl substituent on the sulfonamide nitrogen (R^1), together with the presence of an unsubstituted or substituted aryl sulfonamide (compounds **1-4**, IC_{50} ranging from 2.4 to 3.0 μM), leads to a BACE1-inhibitory activity comparable to that of some of the most active compounds included in the previous series of *N*-carboxamides (IC_{50} = 2.5 μM).[14] When the sulfonamide aromatic ring is unsubstituted (R^2 = H) and the phenyl on the sulfonamide nitrogen is substituted in position 4, the activity worsens slightly (compounds **7** and **8**), significantly (compound **5**) or is completely lost (compounds **6** and **9**). The presence of a benzyl substituent on the sulfonamide nitrogen causes an appreciable increase in the inhibitory activity, except in one case (compound **11**); compounds **10**, **12** and **13** proved to be the most potent inhibitors of this series, with IC_{50} values ranging from 1.6 to 1.9 μM. The *para*-substitution of the benzyl group (leaving the aryl sulfonamide unsubstituted) generally causes a decrement in the activity (compounds **14-16**). If a phenethyl group is introduced on the sulfonamide nitrogen, regardless the presence of substituents in position 4 of the aryl sulfonamide moiety (compounds **17-20**), the activity is preserved or slightly decreased respect to the most active compounds of the previous series of *N*-carboxamides (IC_{50} = 2.5 μM).[14] The activity generally decreases when R^1 is a completely aliphatic group, such as a cyclohexyl (compounds **21-24**). Regarding to the substituent on the sulfonamide aromatic ring, when R^2 = H and R^1 = phenyl, 4-substituted phenyl, 4-substitutedbenzyl, phenethyl or cyclohexyl, the BACE1 inhibitory activity worsens slightly (compounds **1**, **7-8**, **15-17** and **21**) or is lost (compounds **6**, **9** and **14**). When R^2 = Me, the activity is preserved (compounds **2**, **11** and **18**) or slightly worsened (compounds **22**). When the phenyl ring of the sulfonamide is substituted with an alogen atom (Cl in this case) or a methoxy group, the activity is almost preserved (compounds **3** and **4**), improved (compounds **12** and **13**) or worsened (compounds **19**, **23**, **20** and **24**). In general, the BACE1 inhibitory activity of this kind of molecules is mostly influenced by the substituent on the sulfonamide nitrogen (R^1) and the *N*-benzyl-substituted compounds are the most active (except **11**) regardless the substitution on the 4-position of the sulfonamide phenyl ring (R^2).

A molecular docking study of the sulfonamide derivatives in BACE1 active site was conducted to give an interpretation of the structure-activity relationship at a molecular level. First, BACE1 holo crystal structures were retrieved from the Protein Data Bank, resulting in 302 ligand-BACE1 complexes. The preliminary operations of our work were devoted to the identification of the best protein structure and docking protocol to use in the subsequent docking calculations.

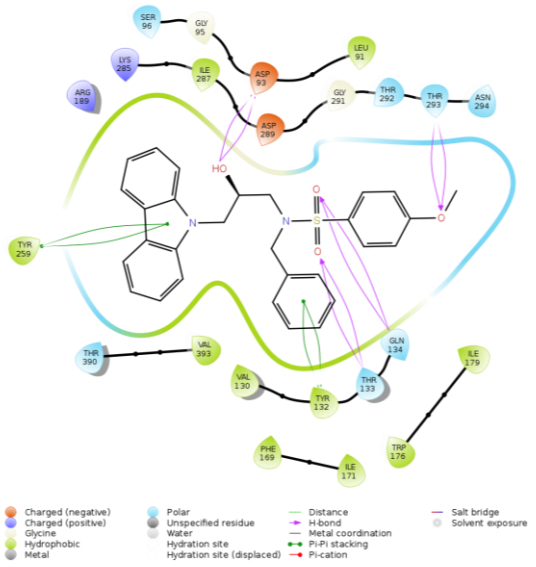
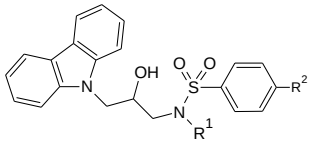


Figure 2. Schematic representation of the principal interactions resulting from the docking study of compound **13**.

According to a previously validated protocol,[16] crystal structures were filtered according to chemical similarity of their co-crystallized ligand to compound **13**, chosen as representative of the series of inhibitors herein described because of its highest potency (IC_{50} = 1.6 μM). MACCS Tanimoto similarity was computed exploiting RDKit[17] functionalities, and the 6 structures characterized by highest similarity values were selected (PDB ID: 2WF2,[18] 2WF3,[18] 2WF4,[19] 2VNN,[20] 2VKM,[21] 4FCO, with the addition of structure 1W51[22] used in a previous study[14]). The selected structures were subjected to a docking benchmark study

to evaluate the performance of different docking protocols and scoring functions in the self-docking exercise. The co-crystallized ligands were prepared by adding hydrogens using the Protonate3D tool of MOE,[23] while the proteins were prepared by exploiting the Protein-preparation tool and the Protonate3D of the same software suite.

Table 1. Structures and BACE1 inhibitory activities of novel variously sustituted arylsulfonamides **1-24**.



Compd	R^1	R^2	IC_{50}[a] (μM)	$logBB_{pred}$[b]
1	Ph	H	3.0	-0.58
2	Ph	CH_3	2.6	-0.57
3	Ph	Cl	2.9	-0.61
4	Ph	OCH_3	2.4	-0.64
5	4-CH_3-Ph	H	7.1	-0.57
6	4-Cl-Ph	H	> 10	-0.61
7	4-CF_3-Ph	H	3.6	-0.60
8	4-NO_2-Ph	H	3.8	-0.79
9	4-F-Ph	H	> 10	-0.63
10	Bn	H	1.9	-0.61
11	Bn	CH_3	2.7	-0.61
12	Bn	Cl	1.7	-0.65
13	Bn	OCH_3	1.6	-0.67
14	4-CH_3-Bn	H	> 10	-0.61
15	4-Cl-Bn	H	2.7	-0.65
16	4-OCH_3-Bn	H	5.7	-0.68
17	Phenethyl	H	2.8	-0.60
18	Phenethyl	CH_3	2.5	-0.59
19	Phenethyl	Cl	3.8	-0.63
20	Phenethyl	OCH_3	3.0	-0.66
21	Cyclohexyl	H	4.1	-0.61
22	Cyclohexyl	CH_3	3.9	-0.60
23	Cyclohexyl	Cl	6.5	-0.65
24	Cyclohexyl	OCH_3	3.2	-0.67

[a] IC_{50} measurements were performed as reported in Ref. 15. Data represent mean values for at least three separate experiments. Standard errors are not shown for the sake of clarity and were never higher than 15% of the means.

[b] Predicted blood-brain barrier permeation ($logBB_{pred}$ = log[Brain]/[Blood]).[28]

DockBench tool[24] was employed to automatically perform the docking benchmark, and, after the analysis of the benchmark results, the protein 2WF4 with the Gold-goldscore[25] protocol were chosen because of the high performance in DockBench Protocol Score.

The structures of the sulfonamide derivatives (stereoisomer *R* and *S* of each compound) were constructed by using the MOE builder function, the starting conformation was initially generated exploiting Corina[26] and then minimized using PM3 theory. The docking calculations were performed for each compound, limiting the conformational search within a 20 Å radius sphere centered on the center of mass of the co-crystallized ligand in the corresponding complex.

Both *R* and *S* stereoisomers find a good accommodation within the active site of BACE1, with the (2-hydroxypropyl)sulfonamide portion protected behind the flap region and the benzylic and carbazolic moieties pointing toward two hydrophobic clefts positioned on left and right. The interaction established by compound **13** are depicted in Figure 2 using the ligand interaction diagram as implemented in the Schrodinger suite.[27] In detail, the predicted binding mode of compound **13** is reported in Figure 3, panel A. The benzylic moiety attached to the sulfonamide nitrogen is inserted into a hydrophobic pocket defined by Leu91, Ile179, Trp176, Ile171, and Phe169. Also, the carbazole portion leans on a hydrophobic portion of the protein, characterized by Tyr259, Ile287, and Val393. The sulfonamide function acts as a hydrogen bond acceptor with Gln134 positioned in the flap region. The hydroxyl groups of both *R* and *S* stereoisomers are involved in a hydrogen bond with one of the two catalytic aspartates: in particular, the *R*-enantiomer interacts with Asp93, while the *S* enantiomer with Asp289. Moreover, both enantiomers are stabilized by a further hydrogen bond between the methoxy group in R^2 and Thr293. The pose of compound **13** (Figure 3, panel B) is similar to that displayed by the crystallographic binding mode of the co-crystallized ligand (PDB ID: 2WF4; ligand ID: ZY4) within the protein conformation used in our docking calculations. The (2-hydroxypropyl)sulfonamide of compound **13** resembles the *gem*-diol group of the (2,2-dihydroxypropyl)amide moiety of the crystallographic compound: here the amide carbonyl group makes a hydrogen bond with Gln134 and the two hydroxyls are engaged as donors in two hydrogen bonds with Asp93.

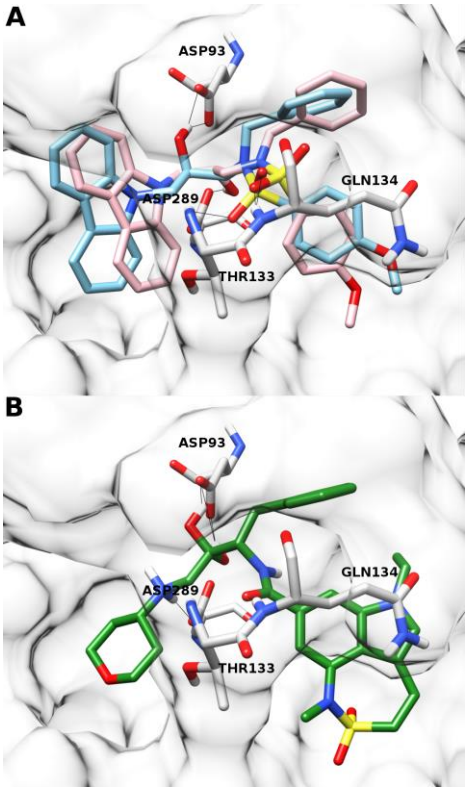


Figure 3. (A) Docking results of compound **13** in BACE1. Both (*R*) and (*S*)-stereoisomer are respectively shown in cyan and pink. (B) The obtained docking result is compared to the crystallographic binding mode of ligand ZY4 within the BACE1 conformation selected for docking studies (PDB ID: 2WF4).

This makes plausible the binding of both the stereoisomers of compound **13** to the BACE1 binding site. A binding mode similar to that of compound **13** was obtained by docking simulations for almost all the other sulfonamide derivatives, as reported in Video-S1 (Supplementary Material).

All the BACE1 inhibitors are meant to have central nervous system (CNS) activity, so they are expected to cross the blood-brain barrier (BBB). Thus, the logBB was computed with the Stardrop software[23] for all compounds, to estimate their capability to distribute from blood to the CNS. The logBB predicted values, being higher than -0.8 in all cases, fall under the -1 limit for passing the blood-brain barrier, so they seem to satisfy the CNS permeability expectation. Compound **8**, which is characterized by the presence of a nitrophenyl substituent, shows the highest value of this series (logBB = -0.79), due to the relatively high polarity of the NO_2 group, thus making its putative BBB permeation less promising than those of the other analogues.

In conclusion, we have synthesized a series of BACE1 inhibitors possessing a *N*-[3-(9*H*-carbazol-9-yl)-2-hydroxypropyl]-arylsulfonamido structure. Among the 24 derivatives, 21 active analogues were found, with three highly active compounds (IC_{50} values ranging from 1.6 to 1.9 μM). The docking study showed that both enantiomers of the most active compound of this series (**13**) find a good accommodation within the active site of BACE1; a similar binding mode was obtained by docking simulations of almost all the other sulfonamide derivatives, as reported in Video-S1 (Supplementary Material). Moreover, the predicted logBB values of all compounds (ranging from -0.57 to -0.79) indicate satisfactory BBB permeabilities.

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References and notes

1. Hardy, J.; Selkoe, D. J. *Science* **2002**, *297*, 353.
2. Gendron T. F. and Petrucelli L. *Mol. Neurodegener.* **2009**, *4*, 13.
3. Kandalepas, P. C. and Vassar, R. *J. Neurochem.* **2012**, *120*, 55.
4. (a) Luo, Y.; Bolon, B.; Kahn, S.; Bennett, B. D.; Babu-Khan, S.; Denis, P.; Fan, W.; Kha, H.; Zhang, J.; Gong, Y.; Martin, L.; Louis, J.-C.; Yan, Q.; Richards, W. G.; Citron, M.; Vassar, R. *Nat. Neurosci.* **2001**, *4*, 231−232. (b) Roberds, S. L.; Anderson, J.; Basi, G.; Bienkowski, M. J.; Branstetter, D. G.; Chen, K. S.; Freedman, S. B.; Frigon, N. L.; Games, D.; Hu, K.; Johnson-Wood, K.; Kappenman, K. E.; Kawabe, T. T.; Kola, I.; Kuehn, R.; Lee, M.; Liu, W.; Motter, R.; Nichols, N. F.; Power, M.; Robertson, D. W.; Schenk, D.; Schoor, M.; Shopp, G. M.; Shuck, M. E.; Sinha, S.; Svensson, K. A.; Tatsuno, G.; Tintrup, H.; Wijsman, J.; Wright, S.; McConlogue, L. *Hum. Mol. Genet.* **2001**, *10*, 1317.
5. McConlogue, L.; Buttini, M.; Anderson, J. P.; Brigham, E. F.; Chen, K. S.; Freedman, S. B.; Games, D.; Johnson-Wood, K.; Lee, M.; Zeller, M.; Liu, W.; Motter, R.; Sinha, S. J. *Biol. Chem.* **2007**, *282*, 26326.
6. (a) Kumar, A. B.; Anderson, J. M.; Melendez, A. L.; Manetsch, R. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4740. (b) De Strooper, B.; Vassar, R.; Golde, T. *Nat. Rev. Neurol.* **2010**, *6*, 99. (c) Ghosh, A. K.; Gemma, S.; Tang, *J. Neurotherapeutics* **2008**, *5*, 399. (d) Hills, I. D.; Vacca, J. P. *Curr. Opin. Drug Discov.* Devel. **2007**, *10*, 383.
7. (a) Konvalinka, J.; Brynda, J.; Sedlacek, J.; Fabry, M. *J. Med. Chem.* **2002**, *45*, 1432. (b) Ghosh, A. K.; Fidanze, S. *J. Org. Chem.* **1998**, *63*, 6146. (c) Tucker, T. J.; Lumma, W. C.; Payne, L. S.; Wai, J. M.; Desolms, S. J.; Giuliani, E. A.; Darke, P. L.; Heimbach, J. C.; Zugay, J. A.; Schleif, W. A.; Quintero, J. C.; Emini, E. A.; Huff, J. R.; Anderson, P. S. *J. Med. Chem.* **1992**, *35*, 2525.
8. Yuan, J.; Venkatraman, S.; Y. Zheng, Y.; B.M. McKeever, B. M.; L.W. Dillard, L. W.; S.B. Singh, S. B. *J. Med. Chem.* **2013**, *56*, 4156-4180.
9. Chiriano, G.; De Simone, A.; Mancini, F.; Perez, D. I.; Cavalli, A.; Bolognesi, M. L.; Legname, G.; Martinez, A.; Andrisano, V.; Carloni, P.; Roberti, M. Eur. *J. Med. Chem.* **2012**, *48*, 206-213.
10. Butler, C. R.; Ogilvie, K.; Martinez-Alsina, L.; G. Barreiro, G.; Beck, E. M.; Nolan, C. E.; Atchison, K.; Benvenuti, E.; Buzon, L.; Doran, S.; Gonzales, C.; Helal, C. J.; Hou, X.; Hsu, M.- H.; Johnson, E. F.; Lapham, K.; Lanyon, L.; Parris, K.; O'Neill, B. T.; Riddell, D.; Robshaw, A.; Vajdos, F.; Brodney, M. A. *J. Med. Chem.* **2017**, *60*, 386-402.
11. Scott, J. D.; Li, S. W.; Brunskill, A. P. J.; Chen, X.; Cox, K.; Cumming, J. N.; Forman, M.; Gilbert, E. J.; Hodgson, R. A.; Hyde, L. A.; Jiang, Q.; Iserloh, U.; Kazakevich, I.; Kuvelkar, R.; Mei, H.; Meredith, J.; Misiaszek, J.; Orth, P.; Rossiter, L. M.; Slater, M.; Stone, J.; Strickland, C. O.; Voigt, J. H.; Wang, G.; Wang, H.; Wu, Y.; Greenlee, W. J.; Parker, E. M.; Kennedy, M. E.; Stamford, A. W. *J. Med. Chem.* **2016**, *59*, 10435-10450.
12. Ghosh, A. K.; Brindisi, M.; Tang, J. *J. Neurochem.* **2012**, *120*, 71-83.
13. Asso, V.; Ghilardi, E.; Bertini, S.; Digiacomo, M.; Granchi, C.; Minutolo, F.; Rapposelli, S.; Bortolato, A.; Moro, S.; Macchia, M. *ChemMedChem* **2008**, *3*,1530.
14. Bertini, S.; Asso, V.; Ghilardi, E.; Granchi, C.; Manera, C.; Minutolo, F.; Saccomanni, G.; Bortolato, A.; Mason, J.; Moro, S.; Macchia, M. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6657.
15. Porcari, V.; Magnoni, L.; Terstappen, G. C.; Fecke, W.; *Assay Drug Dev. Technol.* **2005**, *3*, 287.
16. Salmaso, V.; Sturlese, M.; Cuzzolin, A.; Moro, S. J Comput Aided Mol Des 2016, 30, 773–789.
17. RDKit: Open-source cheminformatics.
18. Charrier, N.; Clarke, B.; Demont, E.; Dingwall, C.; Dunsdon, R.; Hawkins, J.; Hubbard, J.; Hussain, I.; Maile, G.; Matico, R.; Mosley, J.; Naylor, A.; O'Brien, A.; Redshaw, S.; Rowland, P.; Soleil, V.; Smith, K. J.; Sweitzer, S.; Theobald, P.; Vesey, D.; Walter, D. S.; Wayne, G. Bioorg Med Chem Lett 2009, 19, 3669–3673.
19. Charrier, N.; Clarke, B.; Cutler, L.; Demont, E.; Dingwall, C.; Dunsdon, R.; Hawkins, J.; Howes, C.; Hubbard, J.; Hussain, I.; Maile, G.; Matico, R.; Mosley, J.; Naylor, A.; O'Brien, A.; Redshaw, S.; Rowland, P.; Soleil, V.; Smith, K. J.; Sweitzer, S.; Theobald, P.; Vesey, D.; Walter, D. S.; Wayne, G. Bioorg Med Chem Lett 2009, 19, 3674–3678.
20. Charrier, N.; Clarke, B.; Cutler, L.; Demont, E.; Dingwall, C.; Dunsdon, R.; East, P.; Hawkins, J.; Howes, C.; Hussain, I.; Jeffrey, P.; Maile, G.; Matico, R.; Mosley, J.; Naylor, A.; O'Brien, A.; Redshaw, S.; Rowland, P.; Soleil, V.; Smith, K. J.; Sweitzer, S.; Theobald, P.; Vesey, D.; Walter, D. S.; Wayne, G. J Med Chem 2008, 51, 3313–3317.
21. Ghosh, A. K.; Kumaragurubaran, N.; Hong, L.; Kulkarni, S.; Xu, X.; Miller, H. B.; Reddy, D. S.; Weerasena, V.; Turner, R.; Chang, W.; Koelsch, G.; Tang, J. Bioorg Med Chem Lett 2008, 18, 1031–1036.
22. Patel, S.; Vuillard, L.; Cleasby, A.; Murray, C. W.; Yon, J. J Mol Biol 2004, 343, 407–416.
23. Chemical Computing Group (CCG) Inc. Molecular Operating Environment (MOE); Chemical Computing Group: 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2016.
24. Cuzzolin, A.; Sturlese, M.; Malvacio, I.; Ciancetta, A.; Moro, S. Molecules 2015, 20, 9977–9993.
25. Verdonk, M. L.; Cole, J. C.; Hartshorn, M. J.; Murray, C. W.; Taylor, R. D. Proteins 2003, 52, 609–623.
26. Molecular Networks GmbH CORINA; Germany.
27. Schrödinger, LLC Schrödinger Release 2017-1: Maestro; New York, NY, 2017.
28. StarDrop. Optibrium Ltd, 7221 Cambridge Research Park, Beach Drive, Cambridge, CB25 9TL, UK. http://www.optibrium.com/.

Supplementary Material

All experimental procedures and Video S1 are available as supplementary material.

Video-S1 Caption. The file contains an Animation of the energetically most favorable pose of all the compounds. The ligands are colored in cyan while the protein more relevant residues are colored in gold. The background of the poses shows a heat map corresponding to the contribution of the most relevant residues belonging to the binding site. The electrostatic interaction energy (kcal/mol) and a scoring estimating hydrophobic interactions (Arbitrary Unit, AU) were computed with MOE suite and their values are coded into colorimetric metrics from blue (negative value) to green palette (positive values).