Bioorganic & Medicinal Chemistry Letters 21 (2011) 2515–2520

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Virtual screening-driven identification of human carbonic anhydrase inhibitors incorporating an original, new pharmacophore

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ARTICLE INFO

Article history:
Received 7 January 2011
Revised 10 February 2011
Accepted 12 February 2011
Available online 17 February 2011

Keywords:
Human carbonic anhydrase
CA inhibitors
Virtual screening

ABSTRACT

Combinated ligand- and pharmacophore-based virtual screening approaches were used to discover novel potential pharmacophores acting as carbonic anhydrase (CA, EC 4.2.1.1) inhibitors (CAIs). A free database of commercially available compounds was screened through drug-like filters using a four-point pharmacophore, and followed by docking calculation within the active site of an X-ray structure of isoform CA II. One compound, bearing a trifluoro-dihydroxy-propanone moiety, showed an interesting, selective inhibitory activity in low micromolar range against this isoform versus CA I. The chemical originality of this new pharmacophore can represent an important bioisosteric alternative to the sulfonamido-based functionalities, thus leading to the development of a new class of CAIs.

Carbonic anhydrases (CAs) are an ubiquitary group of metalloenzymes, which have crucial physiological roles in prokaryotes and eukaryotes.[1–3] Such enzymes are efficient catalysts for the reversible hydration of carbon dioxide to bicarbonate, an essential reaction for all living organism. In humans, many CA isoforms are thus involved in several cellular and physiological processes and are consequently implied in a plethora of disease or pathological conditions. To date, one of the main classes of CA inhibitors (CAIs) is constituted by the unsubstituted sulfonamides and their bioisosteres (Fig. 1).

The active site of most CAs contains a zinc ion [Zn(II)] as a metal cofactor, which is essential for catalysis.[1–3] It has been reported that sulphonamide-type inhibitors bind to the Zn(II) ion of the enzyme by substituting the non-protein zinc ligand to generate a tetrahedral adduct.[2] In the course of the last years, several SAR studies combinated with X-ray crystallographic data led to identify a general binding mode of the sulfonamide CAIs (Fig. 2a and b). This can be schematized as a structure bearing three main moieties: (i) the Zinc Binding Function (ZBF), (ii) an aromatic scaffold, and (iii) a high variable tail.

Experimental data indicate that the ZBF is fundamental for the inhibitory activity of the inhibitors acting as zinc binders

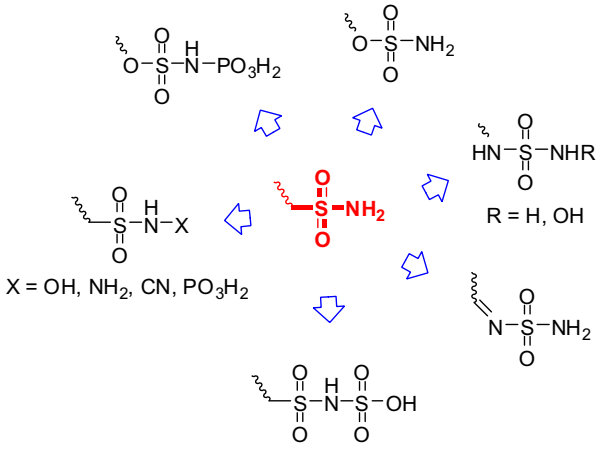

Figure 1. Representative sulfonamido- and sulfonamido-based pharmacophores of CAIs.

(e.g., derivatives belonging to the sulfonamide, sulfamate or sulfamide families, carboxylates).[1–3] Furthermore, the aromatic scaffold ensures the appropriate positioning of the ZBF and stabilizes the enzyme–inhibitor complex interacting with the hydrophobic/hydrophilic residues of the active site. The tail, almost always located in *pseudo*-para or meta position with respect to the ZBF, is

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doi:10.1016/j.bmcl.2011.02.059

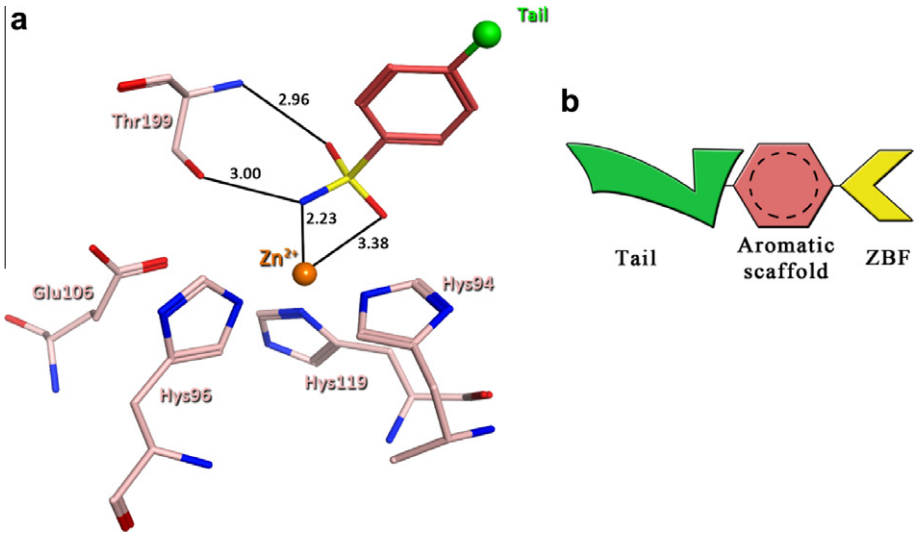

Figure 2. (a) Classical sulfonamides' binding mode; (b) Schematic representation of generic sulfonamido-based CAIs.

responsible for secondary interactions that confer better stability and high affinity to the enzyme–ligand complex.[1] As more CAIs are entering clinical trials, due to both the toxicity and relative non-specificity of the sulfonamido-like functionality, it is important to develop diverse chemical classes of selective inhibitors, especially isozyme-specific compounds, bearing different ZBF.

In this context, together with other traditional strategies, computational approaches demonstrated their utility in the modern drug discovery of novel and potent CAIs.[4] Among them, virtual screening (VS) is one of the most interesting computational tools for rapid discovery of novel and original chemical entities provided of potential activity.[5,6] This method is used with the goal to detect molecules in waste compound libraries, in order to increase the hit rate in subsequent biological assays.[7,8]

The present study was aimed on searching for new potential and original pharmacophoric motifs for CAIs by using a VS strategy, to be submitted to further chemical optimization. On these bases, combinated pharmacophore- and ligand-based VS approach was performed by using MOE (Molecular Operative Environment) platform.[9]

The outline of the experimental strategy was the following: first, the construction of a suitable pharmacophore model; second, the virtual screening of free database by means of docking procedures; third, the study of the poses on CA II enzyme model of the most interesting selected compounds. After these bioinformatic analyses, we validated the results obtained by performing CA I/II catalytic activities in in vitro enzyme assays employing a stopped-flow instrument for assaying the CA-catalyzed CO_2 hydration activity.[10]

In particular, after a careful analysis of RCSB Protein Data Bank, 34 available high-resolution X-ray crystallographic structures of CA II in complex with different sulfonamides were selected and, to retain the common structural features into a putative pharmacophoric region, an alignment procedure was performed[11] (Fig. 3).

Therefore, a suitable four-point pharmacophore was derived; it consisted by (a) the classical ZBF as metal ligator, (b) two H-bond acceptor functions, and (c) an aromatic hydrophobic region (Fig. 4).

Among all selected systems, the ligand–protein complex with pdb code 2NNO appeared a suitable CA II enzyme model (complexed with the 4-carboxyethyl benzene-sulfonamide)[12] to be used as a target structure for preliminary docking studies. Prior to the execution of the VS experiment, the pharmacophore model and the docking settings were tested using a training database containing previously reported 566 CAIs, having a K_i ranging from 10^{-3} to 10^{-10} M. Therefore, the docking procedure was tested by cross-docking all the ligands. The pharmacophore/docking model recognized 417 compounds, which represent ~75% of the database. Noting the heterogeneity of the structures in the database and the fact that the four-point pharmacophore was developed with a limited number of highly active CAIs, we considered this a reasonable result and we used this model as filter in the initial stage of the VS procedure. So, the free ZINC[13] lead-like database containing 972,608 of commercially available compounds was screened in a hierarchical fashion, using fast 2D filters, 3D pharmacophore searches, and protein–ligand docking.[14] This database was built by filtering the main database ZINC after selection of structures that satisfied good drug-likeness criteria ($\log P \leqslant 3.5$; molecular weight $\leqslant$350 and rotatable bounds $\leqslant$7), to avoid a pre-filtering operation like Lipinski's rules. Furthermore, a washing procedure was performed in order to remove possible incongruous structures (i.e., presence of wrong bound valence, incorrect charges, presence of transition elements). The first stage of screening was constituted by an one-step procedure that analyzed each structure by performing a conformational analysis coupled to a pharmacophore search. In the second stage, the resulting database of 37,398 hits was treated to remove the structures containing S atoms reducing it to 4603 hits. A docking evaluation was then performed using FlexX,[15] thus resulting in a library of 688 poses related to 29 structures.

Finally, a refinement docking procedure using AutoDock 4.2.1 was carried out,[16] and a unique original structure (**1**) was identified (Fig. 5).

According to docking results, the amino acid residues Thr200 and Thr199, located near the catalytic metal ion, are involved in the binding of compound **1** (Fig. 6).

These residues were shown to be very important for the activity of CA and for the binding of substrates/inhibitors, such as sulfamide, sulfamic acid and a host of more complex primary sulfonamides/sulfamates.[1–3] The estimated free binding energy values (ΔGbind) of the docked positions, expressed in kcal/mol, indicated favourable interactions and tight binding of **1** with key aminoacid residues on the CA II active site. In particular, with ΔGbind = −8.84 kcal/mol (Table 1), **1** shared better energy results than the other 28 selected compounds (data not shown).

We also found that **1** is involved in complexation with the Zn^{2+} ion, thus revealing the already observed accommodation for

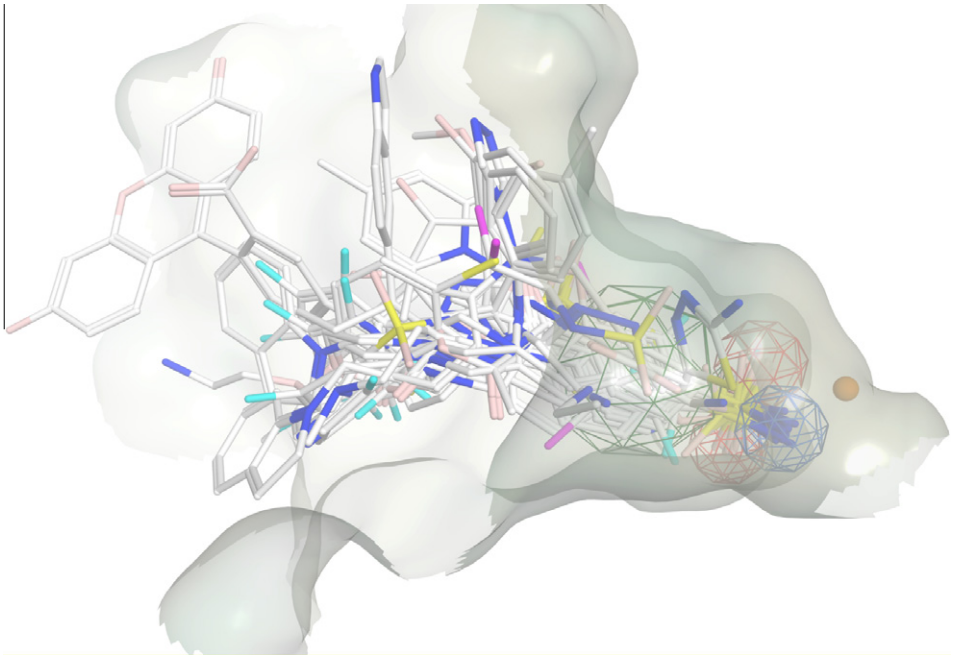

Figure 3. Snapshot of the catalytic site with all 34 ligands used for the development of the pharmacophore model.

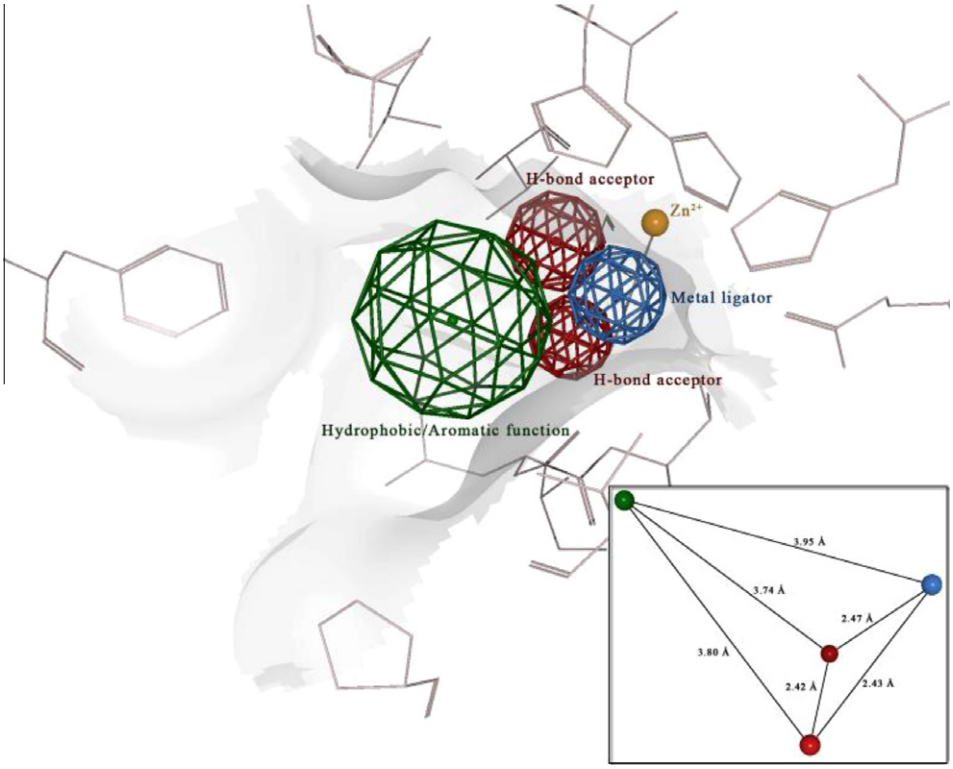

Figure 4. Pharmacophore model represented into the active site. Schematic view and distance geometries of the pharmacophore functions (rectangular insert).

sulfonamide-containing compounds within the CA active site (Fig. 6). Presumably, the dihydroxy-keto moiety is potentially able to establish a coordination bond with Zn^{2+} ion through the deprotonated dihydroxy system. In fact, the electron-withdrawing character of the trifluoro-group present on the pharmacophore fragment might contribute in enhancing the acidity of geminal diol, thus favouring its metal chelating properties (carboxylates, which contain a rather 'similar pharmacophore, COO^{-}', were found bound to the Zn(II) ion within several CA isoforms active sites[3c,d]). Furthermore, the software estimated a putative inhibition constant of 0.33 μM (Table 1), that resulted approximately in the same order of magnitude as the experimentally obtained data (see below). Then, we performed calculation of electronic density with the aim of understanding how structural changes can affect substrate charge distributions. Figure 7 displays the three-dimensional molecular electrostatic potential (MEP) surfaces for monodeproto-

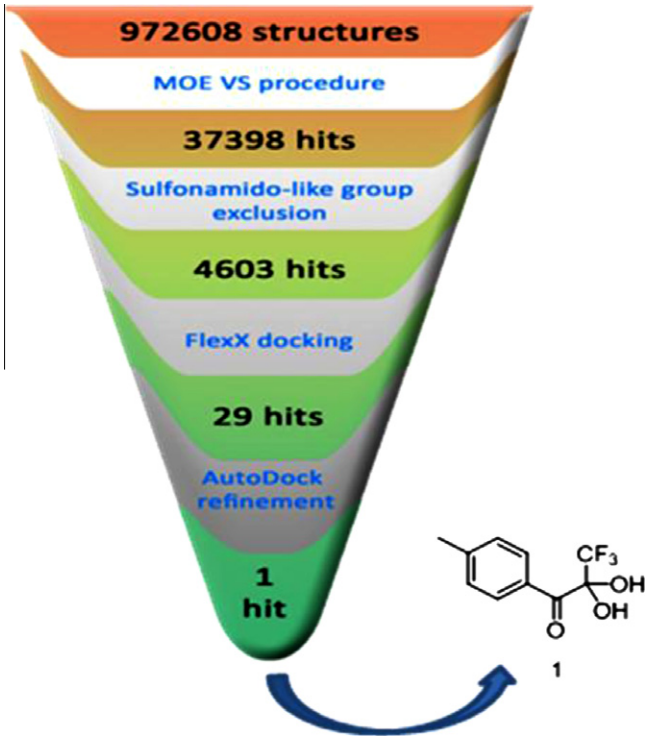

Figure 5. Schematization of the VS workflow.

nated inhibitors $\mathbf{1}^-$ and $\mathbf{2}^-$ (the sulfonamide-related compound), which were generated from ab initio DFT method (B3LYP/6-311G). The most nucleophilic regions (negative electronic potential) are shown in red, while the electrophilic regions (positive/neutral electrostatic potential) are shown in blue/green. A more intense region of negative electrostatic potential around the 2-hydroxy-3-oxo-2-olate group of $\mathbf{1}^-$ and the monodeprotonated sulfonamide moiety of $\mathbf{2}^-$ were observed. These results can further explain the similar disposition of these inhibitors within the active site, particularly the interaction with the Zn^{2+} ion as observed.

To assess the biological activity, compound **1** was purchased by Aurora Fine Chemicals Ltd, and then tested for the ability to inhibit CA I and II isoforms catalytic activities.[17] Interestingly, **1** showed

Table 1
Docking results for compound **1**

Compound	f_{occ}[a]	M.B.E.[b]	E.F.E.B[c]	E.I.C., K_i[d]
1	5/5	−7.62	−8.84	0.33 μM

[a] f_{occ} = Number of distinct conformational clusters found, out of 100 runs/Number of multi-member conformational clusters found, out of 100 runs.
[b] M.B.E. = Mean binding energy.
[c] E.F.E.B = Estimated free energy of binding.
[d] E.I.C., K_i = Estimated inhibition constant, K_i.

anti-CA II activity in the low micromolar concentration range (K_i CA II = 9 μM, Table 2), as predicted by the docking studies. Since this value falls into the average hit potency values for all VS studies (range from ~4 to ~19 μM),[6] it fulfills the criteria for a useful hit, which must exceed a specific potency threshold against the target (e.g., <10 μM inhibition).[4] Finally, Table 2 reports the inhibition results of **1** compared to the classical CAI Acetazolamide, 5-acetamido-1,3,4-thiadiazole-2-sulfonamide (AZA). Although compound **1** was less potent than reference compound towards CA II (K_i CA II = 9 μm and K_i CA II = 0.012 μM, for **1** and AZA, respectively), it resulted approximately 45-fold more active in inhibiting the human CA II isoform with respect to CA I (K_i CA II = 9 μm vs K_i CA I = 410 μM), thus demonstrating similar specificity toward CA II isoform as compared to reference compound (K_i ratios = 45 and 75, for **1** and AZA, respectively).

Summary: One of the main goal in the CA field is the development of new CAIs that combine adequate inhibitory activity and selectivity with lower toxic effect than classical CAIs. In this context, the identification of new structures capable to interact with the CA zinc metal cofactor without bear the sulfonamido group can be a suitable strategy. The work presented here reports the identification of an original ZBF, obtained by means of combinated pharmacophore- and docking-based VS approaches. A free database of commercially available compounds was screened through drug-like filters by a four-point pharmacophore of CA II inhibitors and by docking within the active site of a CA II model structure. One of them (**1**), that bear a trifluoro-dihydroxy-propanone moiety, showed an interesting inhibitory activity in low micromolar concentration range towards human CA II isoform. Analysis of the docking pose provided structural insights into the binding mode of compound **1** within the enzymatic active site. Due to its chemical originality, this compound can be used as a lead

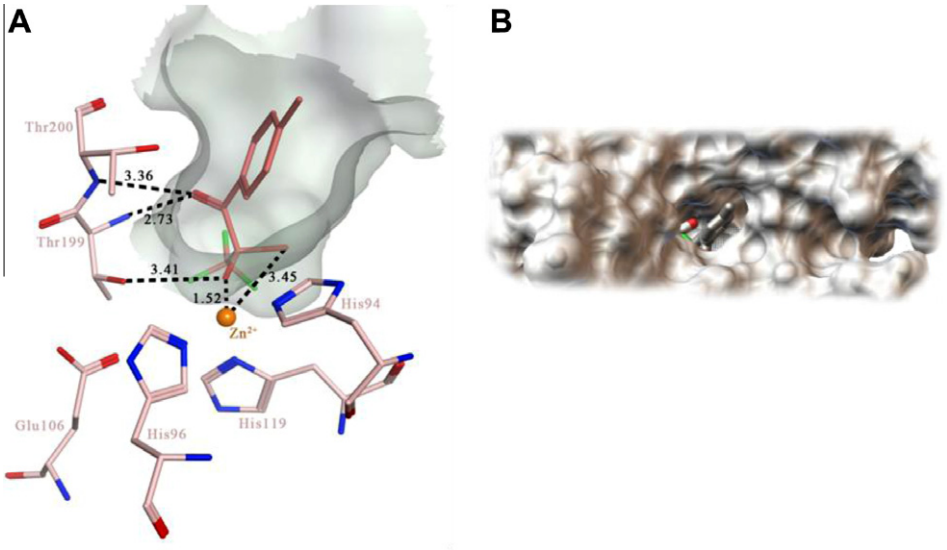

Figure 6. (A) Proposed binding mode of compound **1**. Dashed lines highlight hydrogen and metal transition ligands. (B) Compound **1** located in the active site tunnel.

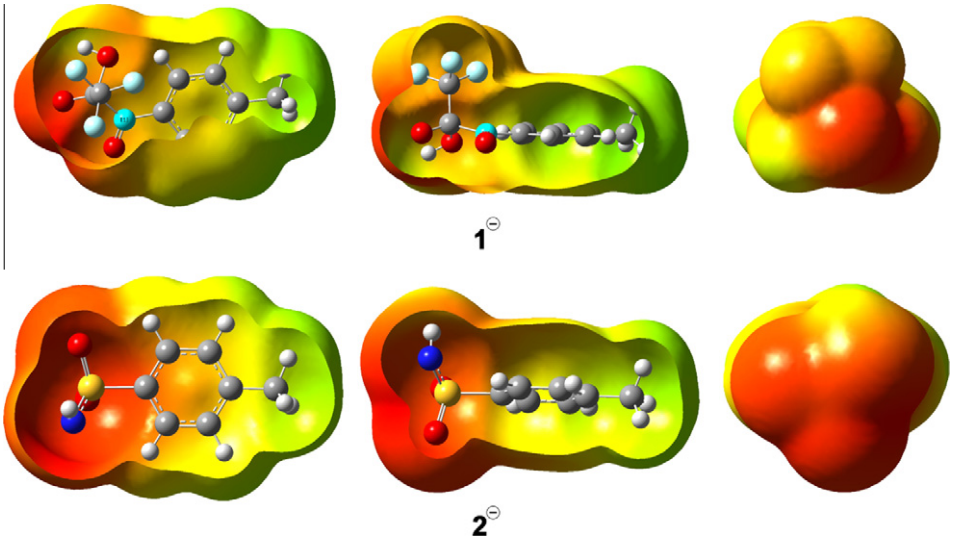

Figure 7. MEPs derived from B3LYP/6-311G calculations for $\mathbf{1}^-$ and from B3LYP/6-311+G(d) $\mathbf{2}^-$ (the sulfonamide-related inhibitor). The increase of negative charges comes from the blue/green (positive/neutral) to red (negative).

Table 2
Inhibition activities of compound **1** in comparison the reference CA inhibitor acetazolamide (AZA)

Compound	K_i (μM)[a]		K_i ratios K_i hCA I/K_i hCA II
	hCA I	hCA II	
1	410	9.0	45
AZA	0.90	0.012	75

[a] Errors in the range of 5–10% of the shown data, from three different assays, by a CO_2 hydration stopped-flow assay.

for further development. Work is in progress to extend this virtual screening method in terms of scoring functions, databases, and target structures, as well as on synthesizing a set of derivatives.

Acknowledgments

M.S. is grateful to Fondazione Banco di Sardegna and to University of Sassari for their partial financial support. The work in CS's group was supported by Metoxia (European 7th framework programme). N.P. and M.S. thank the Master & Back Program of Regione Autonoma della Sardegna.

References and notes

1. Alterio, V.; Di Fiore, A.; D'Ambrosio, K.; Supuran, C. T.; De Simone, G. X-ray Crystallography of CA Inhibitors and Its Importance in Drug Design. In *Drug Design of Zinc-Enzyme Inhibitors: Functional, Structural, and Disease Applications*; Supuran, C. T., Winum, J. Y., Eds.; Wiley: Hoboken, 2009; pp 73–138.
2. (a) Supuran, C. T. *Nat. Rev. Drug Disc.* **2008**, *7*, 168; (b) Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3467; (c) Innocenti, A.; Vullo, D.; Scozzafava, A.; Casey, J. R.; Supuran, C. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 573; (d) Santos, M. A.; Marques, S.; Vullo, D.; Innocenti, A.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1538.
3. Supuran, C. T. *Curr. Pharm. Des.* **2010**, *16*, 3233.
4. Barchi, J. J., Jr.; Neamati, N. *Curr. Top. Med. Chem.* **2002**, *2*, 211.
5. (a) Köppen, H. *Curr. Opin. Drug. Discov. Dev.* **2009**, *12*, 397; (b) McInnes, C. *Curr. Opin. Chem. Biol.* **2007**, *11*, 494; (c)*Virtual Screening for Bioactive Molecules*; Böhm, H.-J., Schneider, G., Eds.; Wiley: Weinheim, Germany, 2000; (c) Sotriffer, C.; Klebe, G.; Stahl, M.; Böhm, H.-J. *Docking and Scoring Functions/Virtual Screening* In *Burger's Medicinal Chemistry and Drug Discovery*; Wiley, 2003; Vol. 1, pp 281–333; (d)*Virtual Screening in Drug Discovery*; Alvarez, J., Shoichet, B., Eds.; CRC Press: Boca Raton, Florida, USA, 2005.
6. Ripphausen, P.; Nisius, B.; Peltason, L.; Bajorath, J. *J. Med. Chem.* **2010**, *53*, 8461.
7. Jorgensen, W. L. *Acc. Chem. Res.* **2009**, *42*, 724.
8. Thiry, A.; Ledecq, M.; Cecchi, A.; Frederick, R.; Dogné, J. M.; Supuran, C. T.; Wouters, J.; Masereel, B. *Bioorg. Med. Chem.* **2009**, *17*, 553.
9. Molecular Operating Environment (MOE 2009.10). Chemical Computing Group, Inc. Montreal, Quebec, Canada.
10. Khalifah, R. G. *J. Biol. Chem.* **1971**, *246*, 2561.
11. *Pharmacophore creation*. A group of 34 deposited CA II-inhibitor crystallographic complexes were selected (PDB IDS: 2NNO, 2NNS, 2EU2, 2NNG, 2AW1, 1OQ5, 1ZFQ, 2HL4, 1ZFK, 1CIL, 2EU3, 1ZGE, 1Z9Y, 1ZH9, 2F14, 1ZGF, 1XQ0, 1G52, 1I9Q, 2HD6, 1G54, 1BCD, 1CNX, 1I8Z, 1IF4, 1G53, 1IF8, 1TTM, 1IF7, 1I90, 1I91, 1IF5, 1IF9, 1ZE8). Each complex was recorded at a maximum resolution of 2 Å, and was formed by a sulfonamide CA inhibitor with activity against CA II in the range of 10^{-7}–10^{-10} M. The solvent was removed and the receptor sequences were aligned and superimposed considering the binding pockets residues (RMSD = 0.25). The pharmacophore model was created using the PPCH_ALL (Planar-Polarity-Charge-Hydrophobicity) scheme. Consensus parameters: Tolerance = 1.1; Threshold = 50%. As spatial constraints were created a volume exclusion feature using the binding pocket of pdb 2NNO complex.
12. Srivastava, D. K.; Jude, K. M.; Banerjee, A. L.; Haldar, M.; Manokaran, S.; Kooren, J.; Mallik, S.; Christianson, D. W. *J. Am. Chem. Soc.* **2007**, *129*, 5528.
13. Irwin, J. J.; Shoichet, B. K. *J. Chem. Inf. Model.* **2005**, *45*, 177.
14. *Virtual screening procedures*. The ZINC lead-like database was washed using the appropriate function implemented in MOE. Next, the database compounds were filtered with the docking function of MOE (parameters: derived four-point pharmacophore; Placement = Alpha Triangle; Rescoring = London dG). The hits were partitioned into two different databases (containing and non-containing sulfo groups). The structures without S groups were filtered by FlexX docking program (all parameters were kept as default).
15. FlexX 3.0. BioSolveIT GmbH, Sankt Augustin, Germany. http://www.biosolveit.de.
16. *AutoDock procedures*. Docking studies were carried out following a previously used procedure.[18,19] Model compound **1** was constructed with standard bond lengths and angles from the fragment database with MacroModel 6.0[20] using a Silicon Graphics O2 workstation running on IRIX 6.3. Sybyl 6.3 (2001)[21] was used as graphic platform. The atomic charges were assigned using the Gasteiger–Marsili method.[22] Representative minimum energy conformations of **1** was optimized using the ab initio quantum chemistry program GAUSSIAN 03 W using DFT B3LYP method and 6-311G as basis set.[23] Docking calculations were performed on HP 8100 Workstation, Intel i7-870 processor with S.O. Linux Ubuntu 10.10. The X-ray crystal structure of CA isoform II (pdb 2ILI) was retrieved from the Brookhaven Data Bank, and used for docking studies. Docking was performed with AutoDock version 4.2[24] using the empirical free energy function and the Lamarckian protocol.[25] The atomic charges for the protein were assigned using the Kollman United method.[26] Mass-centered grid maps were generated with 80 grid points for every direction and with 0.375 Angstroms spacing by the AutoGrid program for the whole protein target. Random starting position on the entire protein surface, random orientations and torsions were used for the ligands. The distance-dependent dielectric permittivity of Mehler and Solmajer was used for the calculation of the electrostatic grid-maps. One hundred independent docking runs were carried out for each ligand. The cluster analyses were computed with a cluster tolerance by less than 1.5 Å in positional root-mean-square deviation with AutoDock Tools.
17. *Enzymatic evaluation*. An applied photophysics stopped-flow instrument has been used for assaying the CA-catalyzed CO_2 hydration activity.[10] Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 10–20 mM Hepes (pH 7.5) as buffer,

and 20 mM Na_2SO_4 (for maintaining a constant ionic strength), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10–100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor, at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (10 mM) were prepared in distilled-deionized water, and dilutions up to 0.01 nM were achieved thereafter with distilled-deionized water. Inhibitor and enzyme solutions were preincubated together for 15 min (data not showed) and for 6 h at room temperature prior to the assay, to allow formation of the E–I complex. The inhibition constants were obtained by nonlinear least-squares methods using PRISM 3, whereas the kinetic parameters for the uninhibited enzymes from Lineweaver–Burk plots, as reported previously,[8] represent the means from at least three different determinations.

18. Sechi, M.; Angotzi, G.; Dallocchio, R.; Dessì, A.; Carta, F.; Sannia, L.; Mariani, A.; Fiori, S.; Sanchez, T.; Movsessian, L.; Plasencia, C.; Neamati, N. *Antiviral Chem. Chemother.* **2004**, *15*, 67.
19. Sechi, M.; Sannia, L.; Carta, F.; Palomba, M.; Dallocchio, R.; Dessì, A.; Derudas, M.; Zawahir, Z.; Neamati, N. *Antiviral Chem. Chemother.* **2005**, *16*, 41.
20. Macromodel, version 6.0; Columbia University: New York, USA, 1997.
21. SYBYL, version 6.3; Tripos Inc.: St. Louis, MO., USA, 1996.
22. Gasteiger, J.; Marsili, M. *Tetrahedron* **1980**, *36*, 3219.
23. GAUSSIAN 03, Revision E.01. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, Jr., J. A.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. Gaussian, Inc., Wallingford CT, 2004.
24. Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J. *J. Comput. Chem.* **2009**, *16*, 2785.
25. Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. *J. Comput. Chem.* **1998**, *19*, 1639.
26. Sanner, M. F. *J. Mol. Graphics Modell.* **1999**, *17*, 57.