Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 14 (2004) 337–341

Carbonic anhydrase inhibitors: X-ray crystallographic structure of the adduct of human isozyme II with the antipsychotic drug sulpiride

Francesco Abbate,[a] Anita Coetzee,[b] Angela Casini,[c] Samuele Ciattini,[d] Andrea Scozzafava[c] and Claudiu T. Supuran[c,*]

[a]*Bruker-AXS s.r.l., Milano, via G. Pascoli 70/3, I-20133 Milan, Italy*
[b]*Bruker-NoniusBV, Oostsingel 209, 2612 Al Delft, The Netherlands*
[c]*Università degli Studi di Firenze, Polo Scientifico, Dipartimento di Chimica, Laboratorio di Chimica Bioinorganica, Via della Lastruccia, 3, Rm. 188, I-50019 Sesto Fiorentino, Firenze, Italy*
[d]*Università degli Studi di Firenze, Polo Scientifico, Centro Interdipartimentale di Cristallografia, Via della Lastruccia, 5, I-50019 Sesto Fiorentino, Firenze, Italy*

Received 2 September 2003; revised 4 November 2003; accepted 6 November 2003

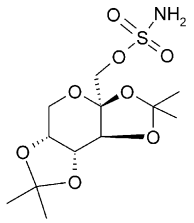
Abstract—The X-ray crystal structure for the adduct of human carbonic anhydrase (hCA) II with sulpiride, a sulfonamide derivative clinically used as antipsychotic drug, has been resolved at a resolution of 1.6 Å. This compound is an effective inhibitor of the physiologically most relevant isozyme hCA II (K_i of 40 nM), being only a moderate or moderate-weak inhibitor of the cytosolic isozyme hCA I (K_i of 1200 nM) and the membrane-bound isozyme hCA IV (K_i of 620 nM). Sulpiride shows CA inhibitory properties of the same magnitude as dichlorophenamide, a clinically used antiglaucoma sulfonamide, or valdecoxib, a COX-2 selective inhibitor recently shown to inhibit CA. The binding of sulpiride to the hCA II active site is similar to that of other sulfonamide inhibitors, considering the interactions of the sulfonamide zinc anchoring group, but differs considerably when the organic scaffold of the molecule is analyzed. Indeed, one unprecedented hydrogen bond involving the imino moiety of the carboxamido group of sulpiride and a water molecule was observed, together with a unique stacking interaction of the *N*-methyl-pyrrolidine ring of the inhibitor and the aromatic ring of Phe 131 of the enzyme active site, which has been observed only recently in another CA–sulfonamide complex.

1. Introduction

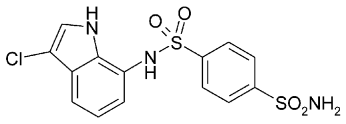
The sulfonamides and their close derivatives such as the sulfamates constitute an important class of drugs, with several types of pharmacological agents possessing antibacterial, anti-carbonic anhydrase, diuretic, hypoglycemic, anticancer, antithyroid or protease inhibitory activity among others.[1–3] A large number of structurally novel sulfonamide/sulfamate derivatives originally designed for a certain specific biological activity, such as the antiepileptic drug topiramate **1**,[4,5] the anticancer derivative indisulam **2**,[6,7] the steroid sulfatase inhibitor EMATE **3**,[8,9] or the COX-2 selective inhibitors celecoxib **4** and valdecoxib **5**[10,11] — all of which possess sulfonamide/sulfamate moieties in their molecule — were recently shown by this group to behave as potent inhibitors of the metallo-enzyme carbonic anhydrase (CA, EC 4.2.1.1).[5,7,9,11] Furthermore, the X-ray crystal structures for the adducts of inhibitors **1**–**5** with the major human CA isozyme, hCA II, have also been reported, being then shown that this originally undesired and unexpected biological activity (i.e., CA inhibition) may be valuable from the pharmacological point of view, leading to potentially novel applications of these compounds/drugs.[5,7,9,11] The high affinity of compounds **1**–**5** for different CA isozymes is primarily due to the presence of the strong zinc binding functions of the sulfonamide/sulfamate type in their molecules, as well as to favorable interactions between their organic scaffold and amino acid residues present within the hCA II active site, leading generally to low nanomolar affinity for this isozyme (more precisely in the range of 5–40 nM).[5–11]

* Corresponding author. Tel.: +39-055-457-3005; fax: +39-055-457-3385; e-mail: claudiu.supuran@unifi.it

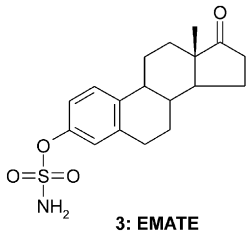
0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.11.014



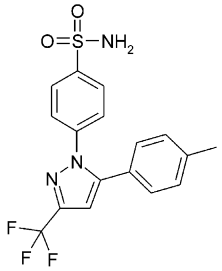
1: Topiramate



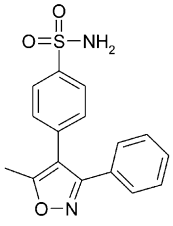
2: Indisulam



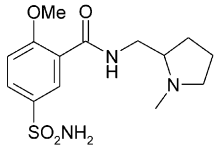
3: EMATE



4:Celecoxib



5: Valdecoxib



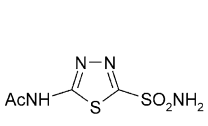
6: Sulpiride

The antipsychotic drug sulpiride **6**, a selective dopamine D2/D3 antagonist,[12,13] also incorporates a primary sulfonamide moiety in its molecule, but its CA inhibitory properties were not investigated up to now. Sulpiride is widely used clinically in the treatment of schizophrenic or depressive disorders, its proposed mechanism of action implying a selective modulation of the dopaminergic system in the mesocorticolimbic area, important for cognitive processing of internal and external cues, related to survival.[12,13] It is presently not known whether the potential CA inhibitory action of this sulfonamide drug may have clinical significance.

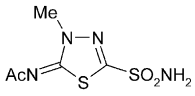
In this paper, we report a detailed CA inhibitory study with sulpiride. Three major human CA (hCA) isozymes, namely hCA I, II and IV were investigated for their interaction with the drug, and the X-ray crystal structure for the adduct of hCA II with sulpiride is also reported here.

2. Chemistry and CA inhibition

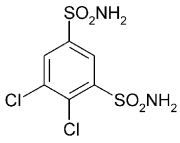
Sulpiride **6** is commercially available. The racemic mixture is used clinically, since both enantiomers possess similar biological activity.[13] Levo-sulpiride has been used on the other hand in our enzymatic and crystallographic experiments reported here, in order to avoid the formation of two complexes with the enzyme. The other sulfonamides/sulfamates used as reference compounds were either commercially available (**1**, **4**–**9**) or prepared as previously reported (**2** and **3**).[6,8] Inhibition data against three CA isozymes with the six compounds **1**–**6** and the standard, clinically used CA inhibitors acetazolamide AZA (**7**), methazolamide MZA (**8**) and dichlorophenamide DCP (**9**) are shown in Table 1.



7: AZA



8: MZA



9: DCP

These data clearly indicate that sulpiride **6** acts as an efficient CA inhibitor against the three investigated isozymes CA I, II, and IV, with potencies comparable to those of reference sulfonamide/sulfamate drugs, or clinically used CA inhibitors as diuretics, antiglaucoma agents, antiepileptics and so on.[1–3] Thus, against the red blood isozyme CA I, **6** behaves as a moderate inhibitor, with an inhibition constant of 1200 nM, exactly as dichlorophenamide **9**, a sulfonamide in clinical use as antiglaucoma agent.[1–3] The other two benzenesulfonamides investigated, celecoxib **4** and valdecoxib **5** are much weaker CA I inhibitors as compared to **6** and **9**, whereas compounds **1**–**3** and **7**, **8** act as more potent CA I inhibitors, with K_i values in the range of 31–250 nM (Table 1). Against the physiologically most relevant and rapid isozyme, CA II, sulpiride **6** shows the same level of inhibition as dichlorophenamide **9** and valdecoxib **5**, with K_i values in the range of 38–43 nM, whereas the other compounds investigated here are more effective inhibitors, with K_i values in the range of 5–21 nM. An intermediate level of inhibition between those of the two cytosolic isozymes discussed above has been observed for sulpiride **6** against the membrane-associated isozyme CA IV, with an inhibition constant of 620 nM. Sulpiride is thus one of the weakest CA IV inhibitors in the small series of compounds investigated here, since the other benzenesulfonamides **4** and **5** showed K_i values in the range of 290–380 nM, whereas the heterocyclic derivatives and the sulfamates (**1**, **2**, **7** and **8**) acted as much stronger CA IV inhibitors with K_i values in the range of 36–70 nM (Table 1).

Table 1. CA inhibition data with standard inhibitors and the derivatives **1**–**6**[a]

Inhibitor	IC_{50} (nM)[b]			
	hCA I[c]	hCA II[c]	b/hCA IV[d]	
1	250	5	54	(bCAIV)
2	31	15	65	(bCAIV)
3	37	10	nt	
4	50,000	21	290	(bCAIV)
5	54,000	43	340	(bCAIV)
6[e]	1200	40	620	(hCAIV)
AZA	250	12	70	(bCAIV)
MZA	50	14	36	(bCAIV)
DCP	1200	38	380	(bCAIV)

nt, not tested.
[a] Inhibitors were incubated with enzymes for 15 min prior to assay.
[b] Errors in the range of 5–10% of the reported value, from three determinations.
[c] Human cloned isozymes, esterase assay method.[14]
[d] Isolated from bovine lung microsomes, esterase assay method.[14]
[e] Human cloned isozymes, CO_2 hydration stopped-flow method.[15]

3. Crystallography

The hCA II-**6** adduct obtained by co-crystallization, was subjected to detailed X-ray crystallography.[16] Software developed by Bruker Nonius was used, as follows: Proteum package for data acquisition and manipulation; Cosmo for strategy calculation; Saint+ for integration and processing; ProScale for Scaling, and SHELXTL for solution and refinement.[17] Other Software used: Xfit for view and fit the structure and Protcheck to check the final structure.[18] The soaked crystal was isomorphous to the native enzyme, being mono-clinic $P2_1$ with the following cell parameters: $a = 42.18$, $b = 41.38$, $c = 72.09$, $\beta = 104.4°$. The Fourier maps 2Fo–Fc and Fo–Fc were then calculated, where Fc and phases were obtained from the native hCA II model from which all the water molecules have been omitted. The difference Fourier maps after the first refinement cycle, before the assignment of water molecules, already showed clear evidence for the presence of the inhibitor bound within the active site. Dataset was 93% complete, with a redundancy of ca. 2, giving an overall Rsym of 4.79% for data up to 1.6 Å. The crystal clearly diffracted further, but due to time constraints was only collected up to 1.6 Å. The last refinement cycle yielded a final R factor of 0.21 ($R_{free} = 0.23$) with a final temperature factor of the inhibitor atoms ranging between 8.5 and 24.0 Å^2. The final number of water molecules was 194 and the final rmsd from ideal geometry for bond lengths and angles were 0.01 Å and 1.8°, respectively. The statistics of data collection and refinement are shown in Table 2. A final refinement resolution of 1.6 Å has been achieved.

The structure refinement allowed us to observe the spatial arrangement of the inhibitor within the active site of the enzyme (Fig. 1), whereas the electron density map of sulpiride bound to hCA II is shown in Figure 2. The schematic, detailed representation of the interactions of **6** with the metal ion and amino acid residues/water molecules present in the hCA II active site are shown in Figure 3.

As may be seen from these figures, the ionized sulfonamide moiety of **6** has replaced the hydroxyl ion/water molecule coordinated to Zn(II) in the native enzyme (Zn–N distance of 1.83 Å), as in other hCA II–sulfonamide complexes for which the X-ray structures have been reported (Fig. 3).[19–22] An important note is that the Zn–N bond is appreciably shortened in this complex as this distance is usually around 1.95–2.10 Å.[19–22] This shortening may be considered as a first factor favoring the good affinity of **6** for hCA II. The Zn(II) ion remains in its stable tetrahedral geometry, being coordinated, in addition to the sulfonamidate nitrogen of **6**, by the imidazolic nitrogens of His 94, His 96 and His 119. The proton attached to the sulfonamidate nitrogen atom of the inhibitor also makes a hydrogen bond with the hydroxyl group of Thr 199, which in turn accepts a hydrogen bond from the carboxylic group of Glu 106 (Fig. 3). One of the oxygen atoms of the coordinated sulfonamidate moiety makes a hydrogen bond with the backbone amide of Thr 199 (O–H distance of 2.79 Å), whereas the other one is semi-coordinated to the catalytic Zn(II) ion (O–Zn distance of 3.15 Å). These interactions are generally seen in all complexes of hCA II with sulfonamide/sulfamate inhibitors.[19–22] The benzenesulfonamide part of **6** lies in the hydrophobic part of the active site cleft, where it makes van der Waals interactions with the side chains of Ile 91, Val 121, Val 143, Leu 198 and Pro 202, whereas the methoxy group is oriented towards the hydrophilic part of it, making van der Waals contacts with residues Gln 92, Ser 197 and Thr 200 (Fig. 3). The NH group of the carboxamido moiety of sulpiride is then involved in one hydrogen bond (of 3.19 Å) with a water molecule not previously evidenced in any other sulfonamide–hCA II

Table 2. Statistics of data collection and refinement for the hCAII-sulpiride adduct

	Sulpiride–hCA II complex
Resolution range (Å)	35–1.6
Space group	$P2_1$
Unit cell (Å, ° for β)	$a = 42.18$, $b = 41.38$, $c = 72.09$, $\beta = 104.4$
No. of unique reflections	30,046
Completeness (%)	93.0
R_{sym} (%)	4.79
Refined residues	261
Refined water molecules	194
Resolution range in refinement (Å)	30–1.6
R_{cryst} ($F_o > 4\sigma$ F_o; F_o)	21.3, 19.2
R_{free} ($F_o > 4\sigma$ F_o)	23.0
Rms deviations	
Bond lengths (Å)	0.01
Bond angles (°)	1.8
Average B value (Å^2)	19.6
Ramachandran plot	
Most favored (%)	89.4
Additionally allowed (%)	10.1
Generously allowed (%)	0.5
Disallowed (%)	0.0

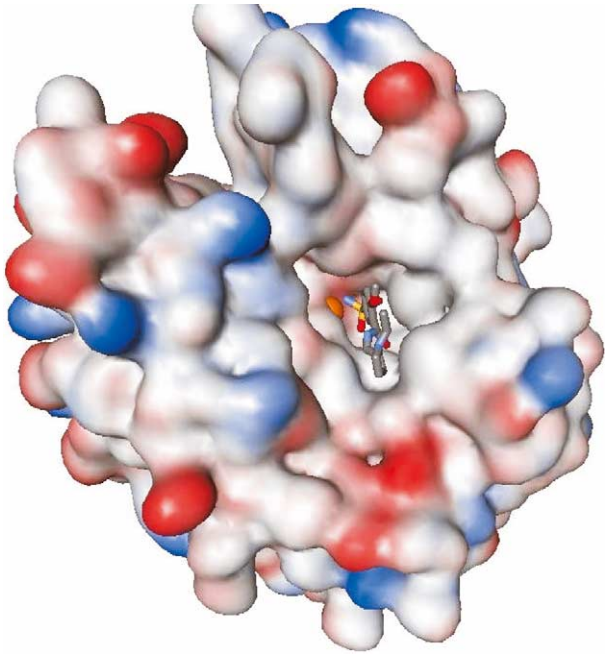


Figure 1. Binding of sulpiride **6** within the hCA II active site (CPK color coding).

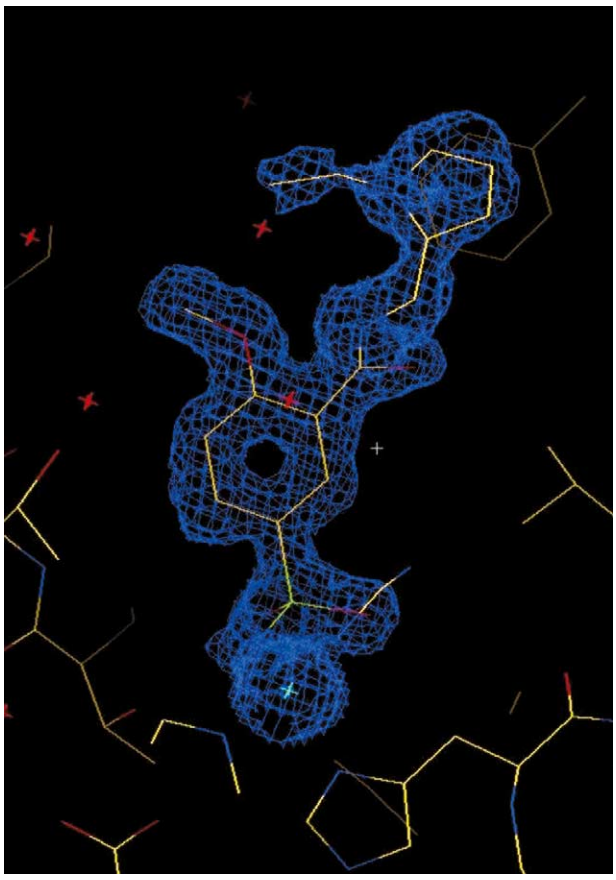


Figure 2. Electron density map of sulpiride **6** bound within hCA II active site.

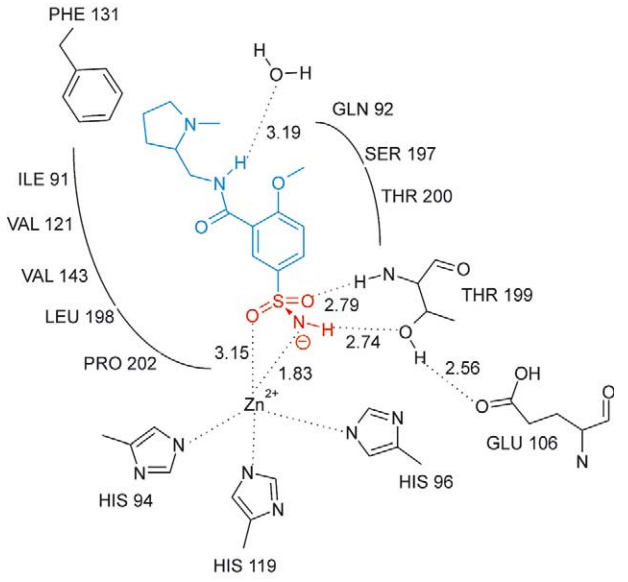


Figure 3. Detailed schematic representation of inhibitor **6** binding within the hCA II active site (figures represent distances in Å).

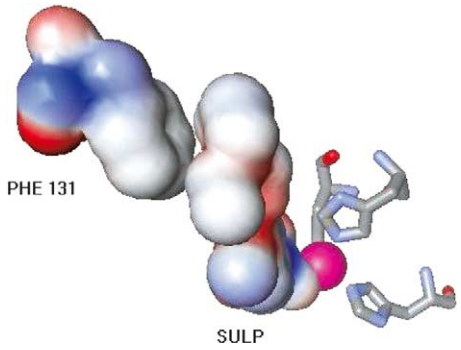


Figure 4. Stacking interaction between the phenyl ring of Phe 131 and the *N*-methyl-pyrrolidine ring of sulpiride bound to hCA II. The Zn(II) ion (magenta sphere) and its three ligands (His 94, 96 and 119) are also evidenced.

complex, whereas the CO group of the same moiety does not participate in any hydrogen bonds, a situation similar with that of another benzene–carboxamide inhibitor for which the X-ray structure has recently been reported by us.[22] Also the nitrogen atom of the *N*-methyl-pyrrolidine moiety does not make hydrogen bonds with amino acid residues of the active site. But a rarely evidenced interaction in other CA–sulfonamide complexes has as protagonist just this moiety: indeed, the *N*-methyl-pyrrolidine ring of sulpiride participates in a strong and very favored stacking interaction with the phenyl ring of Phe 131, a moiety known to be critical for the binding of inhibitors possessing cyclic side chains (Fig. 4).[21] This was recently shown to be the case in the complex of the perfluorobenzoyl analogue of methazolamide in complex with hCA II, in which the same stacking has been evidenced between the perfluorophenyl ring of the inhibitor and Phe 131.[21]

4. Conclusions

The antipsychotic drug sulpiride behaves as an efficient CA inhibitor, mainly against CA II, being less effective an inhibitor of isozymes I and IV. Indeed, against the physiologically most relevant isozyme hCA II, sulpiride shows a K_i of 40 nM, being on the other hand only a moderate or moderate-weak inhibitor of the cytosolic isozyme hCA I (K_i of 1200 nM) and the membrane-bound isozyme hCA IV (K_i of 620 nM). Sulpiride shows CA inhibitory properties of the same magnitude as dichlorophenamide, a clinically used antiglaucoma sulfonamide or valdecoxib, a COX-2 selective inhibitor recently showed to inhibit CA. The binding of sulpiride to hCA II allowed us to observe the usual interactions of the sulfonamide anchor with the zinc ion and the gate-keeping residues Thr 199 and Glu 106, whereas the benzene ring primarily interacts with the hydrophobic half of the active site. The methoxy moiety lies towards the hydrophilic part of the active site, whereas the *N*-methyl-pyrrolidine ring participates in a strong stacking interaction with Phe 131. It is not known actually whether this relatively powerful binding of sulpiride to different CA isozymes has consequences from the pharmacological point of view, or whether some of its biological activity is due just to inhibition of physiologically relevant CAs (it is for example reported that sulpiride is one of the few antipsychotic drugs not producing obesity,[23] a feature also shared by the antiepileptic topiramate **1** and considered to be due to inhibition of the mitochondrial isozyme CA V[24]). It must be anyhow stressed that at clinically used dosa-

ges[12] of this drug, at least CA II should be significantly inhibited and it would be important to know whether this phenomenon has any pharmacological/physiological consequences for the patients undergoing antipsychotic therapy with sulpiride.[25]

References and notes

1. (a) Scozzafava, A.; Owa, T.; Mastrolorenzo, A.; Supuran, C. T. *Curr. Med. Chem.* **2003**, *10*, 925. (b) Casini, A.; Scozzafava, A.; Mastrolorenzo, A.; Supuran, C. T. *Curr. Cancer Drug Targets* **2002**, *2*, 55. (c) Supuran, C. T.; Casini, A.; Scozzafava, A. *Med. Res. Rev.* **2003**, *23*, 535.
2. (a) Supuran, C. T.; Scozzafava, A.; Casini, A. *Med. Res. Rev.* **2003**, *23*, 146. (b) Supuran, C. T.; Scozzafava, A. *Curr. Med. Chem. Imm. Endoc. Metab. Agents* **2001**, *1*, 61.
3. (a) Supuran, C. T.; Scozzafava, A. *Exp. Opin. Ther. Pat.* **2002**, *12*, 217. (b) Supuran, C. T.; Scozzafava, A. *Exp. Opin. Ther. Pat.* **2000**, *10*, 575.
4. Maryanoff, B. E.; Costanzo, M. J.; Nortey, S. O.; Greco, M. N.; Shank, R. P.; Shupsky, J. J.; Ortegon, M. P.; Vaught, J. L. *J. Med. Chem.* **1998**, *41*, 1315.
5. Casini, A.; Antel, J.; Abbate, F.; Scozzafava, A.; David, S.; Waldeck, H.; Schäfer, S.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 841.
6. (a) Owa, T.; Yoshino, H.; Okauchi, T.; Yoshimatsu, K.; Ozawa, Y.; Sugi, N. H.; Nagasu, T.; Koyanagi, N.; Kitoh, K. *J. Med. Chem.* **1999**, *42*, 3789. (b) Supuran, C. T. *Exp. Opin. Invest. Drugs* **2003**, *12*, 283.
7. Abbate, F.; Casini, A.; Owa, T.; Scozzafava, A.; Supuran, C.T. *Bioorg. Med. Chem. Lett.*, in press.
8. (a) Woo, L. W. L.; Howarth, N. M.; Purohit, A.; Hejaz, H. A. M.; Reed, M. J.; Potter, B. V. L. *J. Med. Chem.* **1998**, *41*, 1068. (b) Malini, B.; Purohit, A.; Ganeshapillai, D.; Woo, L. W. L; Potter, B. V. L.; Reed, M. J. *J. Steroid Biochem. Mol. Biol.* **2000**, *75*, 253.
9. Abbate, F.; Winum, J. -Y.; Potter, B. V. L.; Casini, A.; Montero, J.-L.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.*, in press.
10. (a) Talley, J. J. *Exp. Opin. Ther. Pat.* **1997**, *7*, 55. (b) Penning, T. D.; Talley, J. J.; Bertenshaw, S. R.; Carter, J. S.; Collins, P. W.; Docter, S.; Graneto, M. J.; Lee, L. F.; Malecha, J. W.; Miyashiro, J. M.; Rogers, R. S.; Rogier, D. J.; Yu, S. S.; Anderson, G. D.; Burton, E. G.; Cogburn, J. N.; Gregory, S. A.; Koboldt, C. M.; Perkins, W. E.; Seibert, K.; Veenhuizen, A. W.; Zhang, Y. Y.; Isakson, P. C. *J. Med. Chem.* **1997**, *40*, 1347.
11. Weber, A.; Casini, A.; Heine, A.; Kuhn, D.; Supuran, C. T.; Scozzafava, A.; Klebe, G. *J. Med. Chem.*, in press.
12. (a) Pani, L. *Curr. Med. Res. Opin.* **2002**, *18* (Suppl.), 3. (b) Pani, L.; Gessa, G. L. *Mol. Psychiatry* **2002**, *7*, 247.
13. (a) Muller, M. J.; Hartter, S.; Kohler, D.; Hiemke, C. *Pharmacopsychiatry* **2001**, *34*, 27. (b) Drago, F.; Arezzi, A.; Virzi, A. *Eur. Neuropsychopharmacol.* **2000**, *10*, 437. (c) Ferreri, M.; Florent, C.; Gerard, D. *Encephale* **2000**, *26*, 58.
14. Pocker, Y.; Stone, J. T. *Biochemistry* **1967**, *6*, 668.
15. Khalifah, R. G. *J. Biol. Chem.* **1971**, *246*, 2561.
16. The hCA II-**6** complex data were collected at the Bruker-Nonius facility in Delft (The Netherlands). The sample was measured on a prototype microfocus rotating anode generator (0.1×1 mm focus), running at 2.4 kW. This is 90% of the capacity of the Bruker Nonius Microstar. Montel optics were used, with a 0.3 mm collimator and a ProteumR (Smart6000) detector. An exposure time of 60 s/frame and a frame width of 0.3° have been employed. The acquisition and the manipulation of the data was achieved with the Proteum package and Cosmo for strategy calculation, while Saint+ was used for integration and processing, ProScale for Scaling and SHELXTL for solution and refinement of the structure. The size of the crystal was 0.125×0.125×0.125 mm.
17. Proteum package, Cosmo, Saint+, ProScale and SHELXTL from Bruker Nonius Company.
18. Jones, T. A.; Cowan, S. W.; Kjeldgaard, M. *Acta Crystallogr.* **1991**, *A47*, 110.
19. Gruneberg, S.; Wendt, B.; Klebe, G. *Angew. Chem., Int. Ed.* **2001**, *40*, 389.
20. Abbate, F.; Supuran, C. T.; Scozzafava, A.; Orioli, P.; Stubbs, M.; Klebe, G. *J. Med. Chem.* **2002**, *45*, 3583.
21. Abbate, F.; Casini, A.; Scozzafava, A.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2003**, *18*, 303.
22. Casini, A.; Abbate, F.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2763.
23. (a) Baptista, T.; Araujo de Baptista, E.; Ying Kin, N. M.; Beaulieu, S.; Walker, D.; Joober, R.; Lalonde, J.; Richard, D. *Brain Res.* **2002**, *957*, 144. (b) Baptista, T.; Lacruz, A.; Paez, X.; Hernandez, L.; Beaulieu, S. *Eur. J. Pharmacol.* **2002**, *447*, 91.
24. (a) Hebebrand, J.; Antel, J.; Preuschoff, U.; David, S.; Sann, H.; Weske, M. WO Patent 2002, 07821. (b) Supuran, C. T. *Exp. Opin. Ther. Pat.* **2003**, *13*, 1545.
25. The coordinates of the hCA II – sulpiride adduct are available immediately from claudiu.supuran@unifi.it