Pergamon

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Bioorganic & Medicinal Chemistry Letters 13 (2003) 863–865

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Docking Studies of Sulphamate Inhibitors of Estrone Sulphatase in Human Carbonic Anhydrase II

Nigel Vicker,[a] Yaikat Ho,[b] James Robinson,[a] Lawrence L. W. Woo,[a] Atul Purohit,[b] Michael J. Reed[b] and Barry V. L. Potter[a,*]

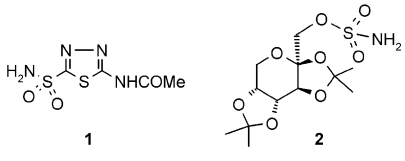
[a]*Medicinal Chemistry, Department of Pharmacy and Pharmacology and Sterix Ltd, University of Bath, Bath BA2 7AY, UK*
[b]*Endocrinology and Metabolic Medicine and Sterix Ltd., Imperial College Faculty of Medicine, St Mary's Hospital, London W2 1NY, UK*

Received 21 August 2002; revised 12 November 2002; accepted 16 December 2002

Abstract—We describe the docking of selected steroidal and non-steroidal estrone sulphatase inhibitors, including the Phase I clinical trial candidate 667COUMATE (**6**), into the active site of human carbonic anhydrase II (hCA II). The docking scores are compared with the inhibition of hCA II and show good correlation with biological activity.

Carbonic anhydrase II (CA II; E. C. 4. 2. 1. 1) is a zinc containing metalloenzyme that catalyses the reversible hydration of CO_2 to HCO_3^-.[1,2] The human enzyme, hCA II, is a 29.3 kDa protein containing a catalytic zinc unit with tetrahedral coordination to three histidine groups and a water molecule which is displaced by the anionic form of an inhibitor on binding.[3] The use of CA inhibitors and their therapeutic potential has recently been reviewed.[2] Sulphonamide inhibitors of hCA II such as acetazolamide (**1**) have been used clinically for some time in ocular diseases like glaucoma where increased intraocular pressure can damage the optic nerve, and it has been shown that acetazolamide (**1**) fails to decrease intraocular pressure in patients with carbonic anhydrase deficiency.[4] Topiramate (**2**),[5] an anti-convulsant agent and RWJ-37497 (**3**), a related sugar sulphamate, have also been shown to be potent inhibitors of hCA II,[3] although the anti-convulsant activity exhibited by (**2**) and (**3**) is thought to be due to a number of biological effects.[5,6]

The crystal structure of hCA II with (**3**) co-crystallised, reported recently, showed that the ligand was intact and not irreversibly bound.[3] Reversible inhibition of hCA II by these agents is observed, followed by a therapeutic effect unrelated to hCA II inhibition.[5,6] These facts indicate that reversible inhibition of cytosolic hCA II may be a mechanism of delivering an agent with a potent inhibitory effect on a different target.



1 2 3

Observations from immunohistochemical measurements that show CA II is highly expressed in several tumours, including pancreatic and gastric carcinomas and malignant brain tumours.[7–9] Sulfonamides and sulfonylated derivatives as anticancer agents and the connection between carbonic anhydrase and cancer has recently been reviewed.[10] Acetazolamide (**1**) suppresses the invasion of renal cancer cells in vitro and the presence of cytosolic hCA II in these cell lines has been shown by immunocytochemical and Western blotting techniques.[11] In addition, Teicher has shown acetazolamide (**1**) produces additive tumour growth delays in vivo.[12] The extracellular pH in solid tumours is acidic[13] and the intracellular/extracellular pH gradient is controlled by ion transport proteins[13,14] and carbonic anhydrases.[15,16] A reversible inhibitor bound to hCA II as its anion would, in an acidic environment, be released. Therefore, it is possible that an anti-cancer agent reversibly bound to hCA II targeting a tumour could be delivered to its site of action.

*Corresponding author. Tel.: +44-1225-826639; fax: +44-1225-826114; e-mail: b.v.l.potter@bath.ac.uk

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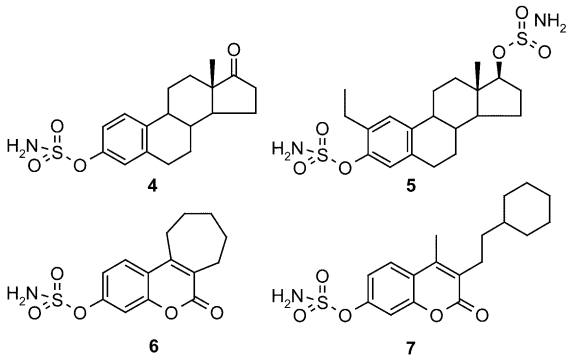
doi:10.1016/S0960-894X(03)00009-X

A number of novel steroidal and non-steroidal sulphamates, for example estrone 3-*O*-sulphamate, EMATE (**4**), the 2-ethylestradiol bis-sulphamate (**5**), 667-COUMATE (**6**) and the related compound (**7**) have been synthesised by our group as potential anti-cancer agents.[17,18] In particular, (**6**) is a Phase I clinical trial candidate.



These compounds act by inhibiting the enzyme estrone sulphatase (STS), which is a key therapeutic target for estrogen dependent tumours.[18] In a recent patent evaluation on compounds for the treatment of estrogen-dependent illness it has been suggested that a general class of STS inhibitors may possess an additional mechanism of antitumour action related to CA inhibition.[19] Since sulphamates have recently been shown to inhibit hCA II[3,5,19] we decided to evaluate the hCA II inhibitory activity of our potent STS inhibitors **4**–**7**. This would give the first report of the hCA II inhibitory activity of potent STS inhibitors.

Potent sulphonamide inhibitors of hCA II have been identified by Klebe, by virtual screening of databases with a derived pharmacophore using UNITY and docking using FlexS.[20–22] To broaden the knowledge in this area we decided to investigate the docking of compounds **4**–**7** in hCA II to see if our STS inhibitors would fit into the active site of hCA II and thereby be predicted to be good hCA II inhibitors. Here we describe the docking studies of selected sulphamate STS inhibitors into two crystal structures of hCA II and compare the in silico docking scores with the measured in vitro inhibition of hCA II.

The crystal structures chosen for the docking studies were from human sources and have Protein DataBank (PDB) codes 2CBA[23] with 1.54 Å resolution and 1EOU[3] with 2.10 Å resolution with hCA II in complex with (**3**). The PDB files were used as deposited. The docking studies on **1**, **3**–**7** in hCA II were performed using FlexX[24] as a fast, flexible algorithm for docking small ligands into binding sites, using an incremental construction algorithm that actually builds the ligands into the binding site. FlexX has a scoring function, an enhanced version by Böhm used to estimate the binding energy and rank potential binding affinities for the interaction of the ligands with the protein.[25,26] A large negative score may be predictive of high affinity. Compounds were docked using FlexX in Sybyl version 6.8, no torsions were included, formal charges were assigned and the FlexX Score function was used for scoring.

The docking studies shown in Figures 1 and 2 depict how our potent non-steroidal and steroidal STS inhibitors bind to hCA II showing the sulphamate group in the proximity of the zinc and hydrophobic residues in the region of the lipophilic templates. Figure 1 shows 667-COUMATE (**6**) highlighted in green docked into the active site of 1EOU with the coordinated zinc and selected residues highlighted. The sulphamate moiety of (**6**) is clearly in the coordination region of the zinc unit. From this docking study hydrophobic interactions with (**6**) and residues Phe131, Leu141 and Leu198 would be predicted to improve binding.

In a similar fashion, the docking of EMATE (**4**) highlighted in green docked into 1EOU is depicted in Figure 2.

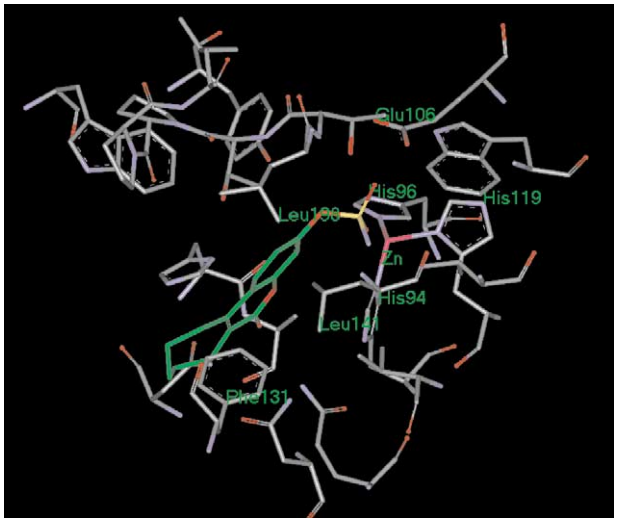


Figure 1.

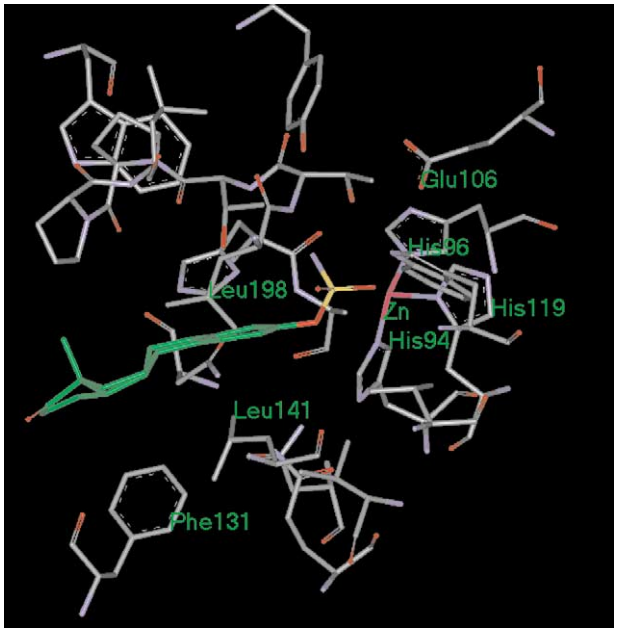


Figure 2.

Compounds for biological evaluation were prepared according to published procedures;[17,18] acetazolamide was purchased from the Sigma-Aldrich Chemical Co. Ltd. The inhibition of hCA II was determined using a modified colorimetric assay as described by Armstrong et al.[27] which quantifies inhibition of CAII-catalysed hydrolysis of *p*-nitrophenyl acetate to *p*-nitrophenol. Measurements were performed in quadruplicate in 96-well plates and IC_{50} values calculated using Graphpad Prism 3 and acetazolamide (**1**) was used as a positive control. The results of the docking studies compared to the measured inhibition of hCA II of our STS inhibitors are depicted in Table 1.

Table 1. hCA II inhibition compared with docking scores for compounds **1**, **3**–**7**

Compd	Inhibition of hCA II (IC_{50}, nM)[a]	FlexX score[b] using 1EOU	FlexX score[b] using 2CBA
1	13.7	−21.7	−21.0
3	36[c]	−22.7	
4	9.0	−13.3	−13.6
5	290	−9.5	−11.9
6	17.0	−23.2	−25.1
7	15.0	−19.9	−20.1

[a]Values are mean of four measurements with <5% variation.
[b]Large negative scores correspond to high predicted affinities.
[c]Reported value ref 3.

In general, the larger the negative score the higher the predicted affinity and the greater the inhibitory potency of the ligand. The compounds chosen for this study all gave negative docking scores in correlation with their rank order of potency and with the least potent compound **5** having the least negative score. Compounds **1**, **4**, **6** and **7** all have IC_{50} values <20 nM and all have large negative docking scores which would lead one to predict these compounds as being potent inhibitors of hCA II. Compound (**5**) is a bis sulphamate and may bind to hCA II with either sulphamate coordinated to the zinc, this may explain why the docking score does not correlate so well with the observed potency, as the score represents one binding orientation. In general, the docked sulphamates with scores more negative than minus 10 gave good inhibition of hCA II. As a preliminary selection step to identify novel STS sulphamate inhibitors of hCA II, compounds that dock with a score below −10 would be predicted to be good hCA II inhibitors.

In summary, we have shown that selected potent sulphamate inhibitors of estrone sulphatase (STS) are also potent and reversible inhibitors vide infra of human carbonic anhydrase II. Docking studies and scoring of these potent STS inhibitors on crystal structures of hCA II would lead to the prediction that these compounds have high affinity for hCA II. This was confirmed by biological evaluation. These studies suggest that binding to hCA II may be in part a mechanism of delivery of such anti-cancer agents to their site of action.

Acknowledgements

We thank Sterix Ltd for financial support of this work and Drs. M. Leese and D. Ganeshapillai for supplying compounds for preliminary biological evaluation.

References and Notes

1. Lindskog, S. *Pharmacol. Ther.* **1997**, *74*, 1.
2. Supuran, C. T.; Scozzafava, A. *Expert Opin. Ther. Pat.* **2002**, *12*, 217.
3. Racacha, R.; Costanzo, M. J.; Maryanoff, B. E.; Chattopadhyay, D. *Biochem. J.* **2002**, *361*, 437.
4. Krupin, T.; Sly, W. S.; Dodgson, S. J. *Amer. J. Opthamol.* **1985**, *99*, 396.
5. Masereel, B.; Rolin, S.; Abbate, F.; Scozzafava, A.; Supuran, C. T. *J. Med. Chem.* **2002**, *45*, 312.
6. Maryanoff, B. E.; Costanzo, M. J.; Nortey, S. O.; Greco, M. N.; Shank, R. P.; Schupsky, J. J.; Ortegon, M. P.; Vaught, J. L. *J. Med. Chem.* **1998**, *41*, 1315.
7. Parkkila, A. K.; Herva, R.; Parkkila, S.; Rajaniemi, H. *Histochem. J.* **1995**, *27*, 974.
8. Pastorekova, S.; Parkkila, S.; Parkkila, A. K.; Opavsky, R.; Zelink, V.; Saarnio, J.; Pastorek, J. *Gastroenterology* **1997**, *112*, 398.
9. Parkkila, S.; Parkkila, A. K.; Jovonen, T.; Lehto, V. P.; Rajaniemi, H. *Histochem. J.* **1995**, *27*, 133.
10. Casini, A.; Scozzafava, A.; Mastrolorenzo, A.; Supuran, C. T. *Curr. Cancer. Drug. Targets* **2002**, *2*, 55.
11. Parkkila, S.; Rajaniemi, H.; Parkkila, A. K.; Kivela, J.; Waheed, A.; Pastorekova, S.; Pastorek, J.; Sly, W. S. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 2220.
12. Teicher, B. A.; Liu, S. D.; Holden, S. A.; Herman, T. S. *Anticancer Res.* **1993**, *13*, 1549.
13. Webb, S. D.; Sherratt, J. A.; Fish, R. G. *J. Theor. Biol.* **1999**, *196*, 237.
14. Montcourrier, P.; Silver, I.; Farnoud, R.; Bird, I.; Rochefort, H. *Clin. Exp. Metastasis* **1998**, *15*, 382.
15. Lee, A. H.; Tannock, I. F. *Cancer Res.* **1998**, *58*, 1901.
16. Alvarez, L.; Fanjul, M.; Carter, N.; Hollande, E. *J. Histochem. Cytochem.* **2001**, *49*, 1045.
17. Howarth, N. M.; Purohit, A.; Reed, M. J.; Potter, B. V. L. *J. Med. Chem.* **1994**, *37*, 219.
18. Woo, L. W.; Purohit, A.; Malini, B.; Reed, M. J.; Potter, B. V. L. *Chem. Biol.* **2000**, *7*, 773.
19. *Exp. Opin. Ther. Pat.* **2000,** *10*, 1771.
20. Grüneberg, S.; Wendt, B.; Klebe, G. *Angew. Chem., Int. Ed.* **2001**, *40*, 389.
21. UNITY *Chemical Information Software*, Version 4. 1. 1, Tripos, Inc.: St Louis, MO, 2000.
22. Lemmen, C.; Lengauer, T.; Klebe, G. *J. Med. Chem.* **1998**, *41*, 4502.
23. Hakansson, K.; Carlsson, M.; Svensson, L. A.; Liljas, A. *J. Mol. Biol.* **1992**, *227*, 1192.
24. Rarey, M.; Kramer, B.; Lengauer, T.; Klebe, G. *J. Mol. Biol.* **1996**, *261*, 470.
25. Klebe, G. *J. Mol. Biol.* **1994**, *237*, 583.
26. Böhm, H.-J. *J. Comput. Aided Mol. Des.* **1994**, *8*, 243.
27. Armstrong, J. M.; Myers, D. V.; Verpoorte, J. A.; Edsall, J. T. *J. Biol. Chem.* **1966**, *241*, 5137.