COX-2 Selective Inhibitors, Carbonic Anhydrase Inhibition and Anticancer Properties of Sulfonamides Belonging to This Class of Pharmacological Agents

Claudiu T. Supuran[1*], Angela Casini[1], Antonio Mastrolorenzo[2] and Andrea Scozzafava[1]

[1]*Università degli Studi di Firenze, Dipartimento di Chimica, Laboratorio di Chimica Bioinorganica, Via della Lastruccia, 3, Rm. 188, I-50019 Sesto Fiorentino (Florence), Italy*

[2]*Università degli Studi di Firenze, Dipartimento di Scienze Dermatologiche, Centro MTS, Via degli Alfani 37, I-50121 Florence, Italy*

Abstract: The sulfonamides constitute an important class of drugs, with several types of pharmacological agents possessing antibacterial, anti-carbonic anhydrase, diuretic, hypoglycaemic, antithyroid, protease inhibitory and anticancer activity among others. A recently developed class of pharmacological agents incorporating primary sulfamoyl moieties in their molecule is constituted by the COX-2 selective inhibitors, with at least two clinically used drugs, celecoxib and valdecoxib. Another drug of this class, rofecoxib, does not contain sulfonamide moieties, but the isosteric and isoelectronic methylsulfone group. It was recently shown that the sulfonamide COX-2 selective inhibitors (but not the methylsulfone ones) also act as nanomolar inhibitors of several isozymes of the metallo-enzyme carbonic anhydrase (CA), some of which are strongly involved in tumourigenesis. In consequence, the potent anticancer effects of the sulfonamide COX-2 selective inhibitors and the much weaker such effects of rofecoxib, reported ultimately by many researchers, may be explained by the contribution of CA inhibition to such processes in addition to COX-2 inhibition.

1. INTRODUCTION

Cyclooxygenases (COXs) catalyze the committed step in the conversion of arachidonic acid to prostaglandins (PGs) and thromboxane, with at least three distinct isozymes, COX-1 - COX-3 isolated up to now [1-3]. The inducible COX-2 was shown to be associated with inflammatory conditions, whereas the constitutive form (COX-1) is responsible for the beneficial effects of the PGs [1,2]. COX-3 has only recently been isolated and little is known presently regarding its physiological/pathological function or its inhibition [3]. Thus, in the last several years COX-2 specific inhibitors have been developed that do not show the gastro-intestinal side-effects of classical non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit both COX-1 and COX-2 isozymes [4-6]. Whereas the anti-inflammatory effects of these new drugs can easily be explained taken into consideration their strong affinity for the above-mentioned COX isozyme [1,2], some recent reports dealing with the strong antitumour activity of several such drugs [7-19] offered only hypothesis for explaining this rather unexpected pharmacological profile. The recent report of the potent inhibition of several carbonic anhydrase (CA, EC 4.2.1.1) isozymes with the sulfonamide COX-2 selective inhibitors, and the determination of the X-ray crystal structures for the adducts of celecoxib and valdecoxib – two clinically used such drugs – with isozyme CA II [20] allow us to interpret the antitumour data of these compounds in an entirely different manner. In this review we shall discuss these new findings, showing that the effective antitumour properties of the sulfonamide type COX-2 selective inhibitors (but not of the methylsulfone ones) may be due to a large extent to their potent inhibition of several CA isozymes involved in tumour growth and tumour angiogenesis.

2. CLINICALLY USED COX-2 SELECTIVE INHIBITORS OF THE COXIB TYPE

A large variety of COX-2 selective inhibitors have been developed in the last years [4,21]. Chemically, they can be divided into the following three structural classes: (i) methanesulfoanilides of the nimesulide **1** or NS-398 **2** type; (ii) tricyclic inhibitors (which in turn may be of the methylsulfone or sulfonamide type). Among the first type of such compounds the most investigated are rofecoxib (Vioxx^{TM}) **3** – a clinically used drug, DuP 697 **4** or SC-58125 **5**, whereas the sulfonamides are represented by the clinically used derivatives celecoxib (Cerebrex^{TM}) **6** and valdecoxib (Bextra^{TM}) **7**; (iii) structurally modified NSAIDs, i.e., inhibitors obtained by using as lead molecules non-selective COX inhibitors. Such compounds include the 2,4,6-trichlorobenzoyl derivative of indomethacine, **8**, or the zomepirac derivative **9** [4,21]. It should be stressed that a very large number of COX-2 selective inhibitors belonging to the three classes mentioned above as well as to other structural types have been reported ultimately and they are extensively reviewed in [21]. It is not our scope to mention in detail all compounds of this type.

One should stress that in the last 5 years, three compounds of those mentioned above became available clinically – rofecoxib **3**, celecoxib **6** and valdecoxib **7** – and that they tend to replace the classical NSAIDs for many therapeutic uses [1, 5-19, 22].

*Address correspondence to this author at the Università degli Studi di Firenze, Dipartimento di Chimica, Laboratorio di Chimica Bioinorganica, Via della Lastruccia, 3, Rm. 188, I-50019 Sesto Fiorentino (Florence), Italy; Tel: +39-055-4573005; Fax: +39-055-4573385; E-mail: claudiu.supuran@unifi.it

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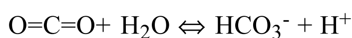
3. CARBONIC ANHYDRASES AND THEIR INHIBITION BY SULFONAMIDES

The carbonic anhydrases (CAs, EC 4.2.1.1) are ubiquitous metallo-enzymes, present in prokaryotes and eukaryotes, being encoded by three distinct, evolutionarily unrelated gene families: the α-CAs (present in vertebrates, *Bacteria*, algae and cytoplasm of green plants), the β-CAs (predominantly in *Bacteria*, algae and chloroplasts of both mono- as well as dicotyledons) and the γ-CAs (mainly in *Archaea* and some *Bacteria*), respectively [23-26]. In higher vertebrates, including humans, 14 different α-CA isozymes or CA-related proteins (CARP) were described, with very different subcellular localization and tissue distribution [23-26]. Basically, there are several cytosolic forms (CA I-III, CA VII), four membrane-bound isozymes (CA IV, CA IX, CA XII and CA XIV), one mitochondrial form (CA V), as well as a secreted CA isozyme, CA VI, together with three acatalytic forms, (isozymes CARP VIII, X and XI) [23-26].

These enzymes catalyze a very simple but fundamental physiological reaction, the interconversion between carbon dioxide and the bicarbonate ion:



$$O{=}C{=}O + H_2O \Leftrightarrow HCO_3^- + H^+$$

Since CO_2 is generated in high amounts in all living organisms, CAs are involved in crucial physiological processes connected with respiration and transport of CO_2/bicarbonate between metabolising tissues and lungs, pH and CO_2 homeostasis, electrolyte secretion in a variety of tissues/organs, biosynthetic reactions (such as gluconeogenesis, lipogenesis and ureagenesis), bone resorption, calcification, tumourigenicity, and many other physiologic or pathologic processes [23-26].

The general shape of a representative belonging to these proteins – hCA II – containing one polypeptide chain of around 260 amino acid residues (molecular weight of 30 kDa) mainly folded as β-pleated sheets, and a catalytical metal ion are shown in Fig. **1**. The Zn(II) ion of CAs is essential for catalysis [23-28]. X-ray crystallographic data showed that the metal ion is situated at the bottom of a 15 Å deep active site cleft (Figs. 1 and 2), being coordinated by three histidine residues (His 94, His 96 and His 119) and a water molecule/hydroxide ion [23-28]. The zinc-bound water is also engaged in hydrogen bond interactions with the hydroxyl moiety of Thr 199, which in turn is bridged to the carboxylate moiety of Glu 106; these interactions enhance the nucleophilicity of the zinc-bound water molecule, and orient the substrate (CO_2) in a favourable location for the

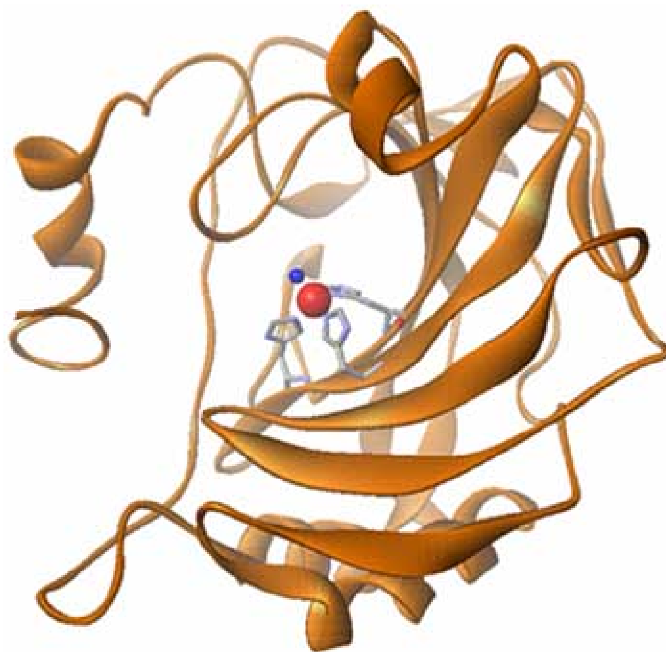
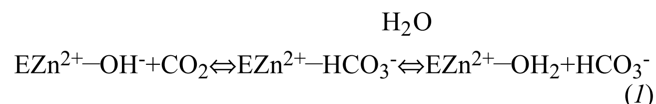
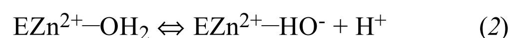


Fig. (1). Human isozyme CA II (hCA II) backbone folding, zinc ion and its ligands (three histidine residues and a water molecule).

nucleophilic attack (Fig. **3**) [23-28]. This nucleophile attacks the CO_2 molecule bound in a hydrophobic pocket in its neighbourhood (the elusive substrate-binding site comprises residues Val 121, Val 143 and Leu 198 in the case of the human isozyme CA II (Fig. 3B), leading to the formation of bicarbonate coordinated to Zn(II) (Fig. **3C**). The bicarbonate ion is then displaced by a water molecule and liberated into solution, leading to the acid form of the enzyme, with water coordinated to Zn(II) (Fig. **3D**), which is catalytically inactive [23-28]. In order to regenerate the basic form A, a proton transfer reaction from the active site to the environment takes place, which may be assisted either by active site residues (such as His 64 – the proton shuttle in isozymes I, II, IV, VII and IX among others, see Fig. **2** for isozyme II)) or by buffers present in the medium. The process may be schematically represented by equations (*1*) and (*2*) below:



$$EZn^{2+}\!-\!OH^- + CO_2 \Leftrightarrow EZn^{2+}\!-\!HCO_3^- \xrightarrow{H_2O} EZn^{2+}\!-\!OH_2 + HCO_3^- \quad (1)$$



$$EZn^{2+}\!-\!OH_2 \Leftrightarrow EZn^{2+}\!-\!HO^- + H^+ \quad (2)$$

The generally high activity of most CA isozymes as well as their abundance in different target tissues in higher vertebrates is a critically important factor for obtaining

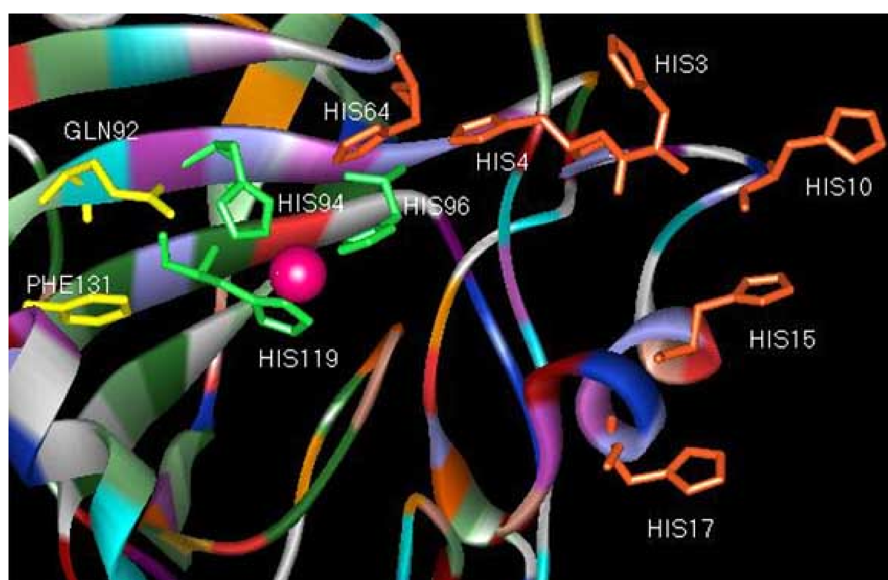


Fig. (2). Details of hCA II active site. The Zn(II) ion (central pink sphere) and its three histidine ligands (in green, His 94, His 96, His 119) are shown. The histidine residues (His 64, His 4, His 3, His 17, His 15 and His 10) involved in proton transfer reactions between the active site and the reaction medium are also evidenced. The figure was generated from the X-ray coordinates reported by Briganti *et al.* (PDB entry 4TST) [28].

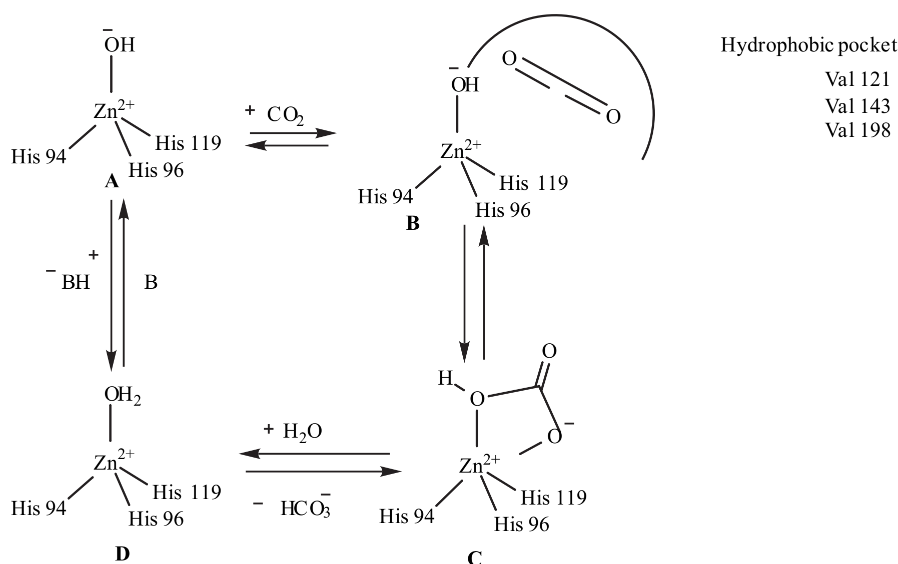


Fig. (3). Schematic representation of the catalytic mechanism for the CA catalyzed CO_2 hydration. The hypothesized hydrophobic pocket for the binding of substrate(s) is shown schematically at step **B**.

inhibitors with clinical applications, since a very high degree (> 99.9 %) of inhibition is needed in order to observe a pharmacological response [23-25].

Two main classes of CA inhibitors (CAIs) are known: the metal complexing anions, and the unsubstituted sulfonamides, which bind to the Zn(II) ion of the enzyme either by substituting the non-protein zinc ligand or add to the metal coordination sphere, generating trigonal-bipyramidal species [23-28]. Sulfonamides, which are the most important CAIs, bind in a tetrahedral geometry of the Zn(II) ion, in deprotonated state, with the nitrogen atom of the sulfonamide moiety coordinated to Zn(II) [29].

X-ray crystallographic structures are available for many adducts of sulfonamide inhibitors with isozymes CA I, II and IV [23-25, 29-32]. In all these adducts, the deprotonated sulfonamide is coordinated to the Zn(II) ion of the enzyme, and its NH moiety participates in a hydrogen bond with the Oγ of Thr 199, which in turn is engaged in another hydrogen bond to the carboxylate group of Glu 106 [23-25, 29-32]. One of the oxygen atoms of the SO_2NH moiety also participates in a hydrogen bond with the backbone NH moiety of Thr 199 [15-17]. Four systemic sulfonamide drugs have been used clinically mainly as antiglaucoma agents, diuretics, antiepileptics or for the management of other neuromuscular disorders: acetazolamide (**10**), methazolamide (**11**), ethoxzolamide (**12**), and dichlorophenamide (**13**) [23-25]. Systemic inhibitors are useful as antiglaucoma drugs in reducing elevated intraocular pressure (IOP) characteristic to this disease, as they represent the most efficient physiological treatment of glaucoma, since by inhibiting the ciliary process enzymes (the sulfonamide susceptible isozymes CA II and CA IV), a reduced rate of bicarbonate and aqueous humour secretion is achieved, which leads to a 25 – 30 % decrease of IOP, but the inhibition of various CA isozymes present in other tissues than the eye leads to an entire range of side effects [23-25]. In addition to these systemically acting inhibitors, the clinical antiglaucoma armamentarium comprises two new drugs, dorzolamide (**14**), and brinzolamide (**15**), which show much less side effects as compared to the first drugs mentioned above, but which also basically inhibit all the physiologically relevant CA isozymes [23-25].

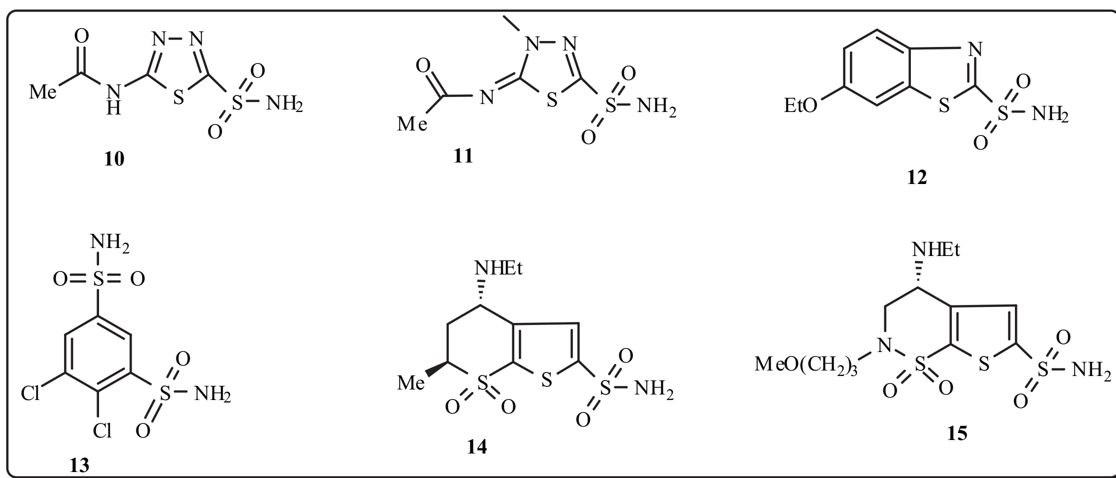


Table 1. CA inhibition Data with Standard, Clinically Used Sulfonamide Inhibitors and COX-2 Selective Inhibitors. Inhibitors were Incubated with Enzymes for 15 Minutes Prior to Assay [20]

Inhibitor	K_I (nM)*			
	hCA I [a]	hCA II [a]	bCA IV [b]	hCA IX[c]
Celecoxib	50000	21	290	16
Valdecoxib	54000	43	340	27
Acetazolamide	250	12	70	25
Methazolamide	50	14	36	27
Dorzolamide	50000	9	43	52
Dichlorophenamide	1200	38	380	50
SC 58125	>100 µM	>100 µM	>100 µM	>100 µM

* Errors in the range of 5-10 % of the reported value, from three determinations.
[a] Human cloned isozymes, esterase assay method, [b] Isolated from bovine lung microsomes, esterase assay method; [c] Human cloned isozyme, CO_2 hydrase assay method [20].

A large number of novel types of CAIs have been reported in the last years. Most of them were developed as candidate topically acting antiglaucoma drugs, but other applications were also envisaged, such as the design of CAIs with anticancer properties, anti-osteoporosis agents, novel antiepileptics, etc. [23-25, 32-34]

4. CARBONIC ANHYDRASE INHIBITION BY COXIBS INCORPORATING SULFAMOYL GROUPS

As mentioned earlier, some recently developed, clinically used coxibs, such as celecoxib **6** and valdecoxib **7**, contain primary sulfamoyl moieties in their molecule. Only recently it was reported that these compounds, in contrast to the structurally related derivatives incorporating methylsulfone groups (such as SC 58125, **5**), act as potent inhibitors of several CA isozymes, with affinity for some of them of the same order of magnitude as those of clinically used CAIs (Table 1).

As seen form data of Table 1, celecoxib **6** and valdecoxib **7** act as potent inhibitors of isozymes II and IX, possessing affinities in the same range as the clinically used CAIs acetazolamide, methazolamide and dichlorophenamide. On the other hand, these two coxibs act as much weaker inhibitors against isozymes I and IV as compared to the classical CAIs. It is also important to note that the structurally related sulfone COX-2 inhibitor SC 58125 is not at all an inhibitor of the investigated CA isozymes [20].

In order to understand why the sulfonamide COX-2 selective inhibitors possess such a high affinity for some CA isozymes, the X-ray crystal structures for adducts of hCA II with **6** and **7** have been determined [20]. In Fig. **4** the schematic interactions between the enzyme (hCA II) and celecoxib **6** bound to it are shown, as determined crystallographically at a resolution of 1.5 Å [20].

As seen in Fig. **4**, the ionised sulfonamide moiety of **6** has replaced the hydroxyl ion/water molecule coordinated to Zn(II) in the native enzyme (Zn-N distance of 2.0 Å), as in other hCA II-sulfonamide complexes for which the X-ray structures have been reported [29-32]. The Zn(II) ion remained 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

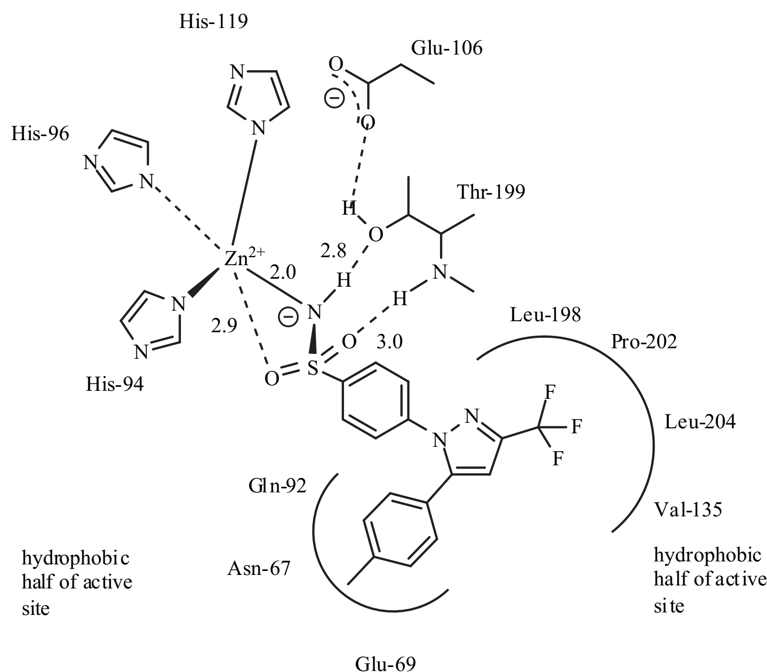


Fig. (4). Schematic drawing of the interactions between hCA II and celecoxib **6**. Hydrogen bonds formed between enzyme and the inhibitor are shown as dotted lines. Bond lengths are given in Å. Residues with a distance less than 4 Å around the inhibitor are schematically drawn. They form a hydrophobic subsite (Leu-198, Pro-202, Leu-204, Val-135, and Phe-131) and a second more hydrophilic subsite (Gln-92, Asn-67 and Glu-69) [20].

group of Thr 199, which in turn accepts a hydrogen bond from the carboxylic group of Glu 106. 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 3.0 Å), whereas the other one is semi-coordinated to the catalytic Zn(II) ion (O-Zn distance of 2.9 Å). These interactions are generally seen in all complexes of hCA II with sulfonamide inhibitors [29-32]. The benzenesulfonamide part of **6** lies in the hydrophobic part of the active site cleft, where it makes van der Waals contacts with the side chains of Leu 198 and Pro 202, whereas the *p*-tolyl group is oriented towards the hydrophilic part of it, making van der Waals contacts with residues Gln 92, Asn 67 and Glu 69 (Fig. **4**). The trifluoromethyl group of the inhibitor is also oriented towards the hydrophobic part of the active site, interacting with residues Leu 198, Pro 202, Leu 204 and Val 135, whereas the pyrazole heteroatoms do not make any hydrogen bonds or other types of interactions with amino acid residues of the active site.

5. ANTITUMOR ACTIVITY OF DUAL COX-2/CARBONIC ANHYDRASE INHIBITORS

The connections between CA and cancer are multiple [23,24,26]. Thus, some CA isozymes are predominantly found in cancer cells, lacking from their normal counterparts [23,35]. Acetazolamide, one of the best-studied, classical CAI used clinically, was shown to function as a modulator in anticancer therapies, in combination with different cytotoxic agents, such as alkylating agents, nucleoside analogues, platinum derivatives, etc [35]. It was hypothesised that the anticancer effects of acetazolamide (alone or in combination with such drugs) might be due to the acidification of the intratumoural environment ensued after CA inhibition, although other mechanisms of action of this drug were not excluded [23,24,35]. Chegwidden, Spencer and Supuran [35] then showed that the antitumour effects of CAIs are probably due to a reduced provision of bicarbonate for nucleotide synthesis (HCO_3^- is the substrate of carbamoyl phosphate synthetase II) as a consequence of CA inhibition. Since different isozymes such as CA I, II, IV, IX and XII were recently shown to be present and probably involved in other types of proliferative conditions, it appeared of great interest to further explore the connections between CAs and tumours. The development of CAIs that also show potent tumour cell growth inhibitory properties was recently reported by our group [36-39]. Such compounds were in fact discovered in a large screening program of sulfonamide CAIs, in collaboration with NIH [36-39]. Several hundred aromatic/heterocyclic sulfonamides were assessed *in vitro* as potential inhibitors of growth of a multitude of tumour cell lines, such as leukaemia, non-small cell lung cancer, ovarian, melanoma, colon, CNS, renal, prostate and breast cancers. The active compounds (most of them nanomolar inhibitors of CA II and CA IV), belonged both to the aromatic, as well as to the heterocyclic sulfonamide classes, and showed GI_{50} values (molarity of inhibitor producing a 50 % inhibition of tumour cell growth after 48 h exposure to the drug) in the micromolar-nanomolar range [36-39].

On the same research line, Parkkila's and Pastorek's groups investigated the effect of acetazolamide, a potent CAI, on the invasive capacity of four renal carcinoma cell lines (Caki-1, Caki-2, ACHN, and A-498) [40]. It was found that 10 μM acetazolamide inhibited the relative invasion rate of these cell lines between 18-74%. The Caki-2 and ACHN cell lines displayed the highest responsiveness, and their responses clearly depended on the acetazolamide concentration in the culture medium. Immunocytochemical and Western blotting results identified the presence of CA isoenzyme II in the cytoplasm of all four cell lines and CA XII on the plasma membrane in three of four cell lines. Because acetazolamide alone reduced invasiveness of these cancer cells *in vitro*, it was concluded that the CAs overexpressed in renal cancer cells contribute to invasiveness [40] (other CAIs have not been investigated for this effect yet). This valuable study constituted a proof-of-concept demonstration that CA inhibitors may be used in the management of tumours that overexpress one or more CA isozymes.

In a series of important studies [41-48] Parkilla's, Pastorek's and Harris' groups showed the value of various CA isozymes (mainly CA IX and XII) and hypoxia as markers of tumour progression in different organs and tissues. CA IX, a transmembrane protein with a suggested function in maintaining the acid-base balance and intercellular communication, was shown to be expressed in the basolateral plasma membrane of normal biliary epithelial cells, but not in hepatocytes. Pastorek's group recently showed that in the biliary epithelial tumours, immunostaining for CA IX was mainly localised at the basolateral surface of the epithelial cells, like in normal mucosa [41]. All non-invasive dysplastic lesions and 57% of invasive lesions of gall-bladder expressed this isozyme. In liver, 78% of cholangiocellular malignant lesions showed a positive reaction for CA IX, whereas only 33% of hepatocellular carcinomas showed a weak immunoreaction [41]. The conclusions was that abnormal expression of CA IX may be linked to malignant transformation of hepatobiliary cells, this enzyme being a promising marker for biliary differentiation in hepatobiliary neoplasms [41]. In another study [42], the same group examined the expression of this enzyme in non-small cell lung cancers. Of 107 cases analysed, 39 (36.4%) had strong membrane/cytoplasmic expression of CA IX and were grouped as positive. The staining was confined around areas of necrosis, and a significant association of CA IX expression with the extent of necrosis was noted. Nevertheless, 38 of 74 cases with focal or extensive necrosis did not express this enzyme [42]. A direct association of CA IX expression with epidermal growth factor receptor, c-erbB-2, and MUC1 expression was also noted. Survival analysis showed that CA IX expression is related to poor prognosis. Multivariate analysis also revealed that CA IX expression was a significant prognostic factor independent of angiogenesis. It was concluded that CA IX is an important molecule in non-small cell lung cancer, the up-regulation of which occurs in highly hypoxic/necrotic regions of the tumours. The expression of CA IX was linked to the expression of a constellation of proteins involved in angiogenesis, apoptosis inhibition, and cell-cell adhesion disruption, which explained the strong association of this enzyme with poor clinical outcome [42].

A similar study wa then reported in a cohort of patients with invasive breast cancer [43]. The majority of patients

were treated with adjuvant hormonal or chemotherapy. The frequency of CA IX expression, its association with recognised prognostic factors, and the relationship with outcome was evaluated by univariate and multivariate statistical analyses. CA IX expression was present in 49 (48%) of 103 cases. The level of CA IX expression was found to be significantly associated with tumor necrosis, higher grade, and negative estrogen receptor status [43]. CA IX expression was associated with worse relapse-free survival and overall survival in an unselected cohort of patients with invasive breast carcinoma. The potential role of CA IX as a marker of hypoxia within breast carcinomas was also indicated by a significant association with necrosis [43].

There is increasing evidence that hypoxia-regulated gene expression influences tumour aggressiveness, contributing to the poorer outcome of patients with hypoxic tumours [44]. The role of the transcriptional complex hypoxia-inducible factor-1 as an important mediator of hypoxia-regulated gene expression is one of the best-documented pathways. Recently, it has emerged that certain tumour-associated CAs can be added to the list of known hypoxia-inducible factor-responsive genes [42-44]. In a recent study it was proved that the tumour-associated CA IX is correlated with the level of hypoxia in human cervical tumours [44]. There was found a significant positive correlation between the level of tumour hypoxia (HP5) and the extent of CA IX expression. A retrospective study of 130 squamous cell cervical carcinomas demonstrated that a semiquantitative immunohistochemical analysis of CA IX expression in tumour biopsies is a significant and independent prognostic indicator of overall survival and metastasis-free survival after radiation therapy [44]. These studies provide clinical evidence that CA IX expression is upregulated in hypoxic human cervical tumours and is associated with a poor prognosis. CA IX may act as an intrinsic marker of tumour hypoxia and poor outcome after radiation therapy. The level of CA IX expression may be used to aid in the selection of patients who would benefit most from hypoxia-modification therapies or bio-reductive drugs [44].

CA12, the gene encoding isozyme XII, has also been identified as a hypoxia-inducible gene [45]. The expression of CA IX and CA XII in relation to necrosis and early breast tumor progression in 68 cases of ductal carcinoma has been recently examined [45]. CA IX expression was rare in normal epithelium and benign lesions, but was present focally in ductal carcinomas (50% of cases) and in associated invasive carcinomas (29%). In comparison, CA XII was frequently expressed in normal breast tissues (89%), in ductal carcinomas (84%), and in invasive breast lesions (71%). Neither CA IX nor CA XII expression was associated with regional or overall proliferation. Assessment of mammographic calcification showed that CA XII expression was associated with the absence of calcification. These results demonstrate that induction of CA IX and CA XII occurs in regions adjacent to necrosis in these tumours [45]. Furthermore, these data suggested that proliferation status does not influence expression of either CA in breast tissues, that hypoxia may be the dominant factor in the regulation of CA IX, and that factors related to differentiation, as determined by tumour grade, dominated the regulation of CA XII. The existence of differential regulation and associations with an aggressive phenotype may be important in the development of selective inhibitors of CAs useful to prevent/treat tumor invasion [45].

In another study the expression and localisation of CA IX in head and neck squamous cell carcinoma (HNSCC) was examined and related to the location of tumour microvessels, angiogenesis, necrosis, and stage [46]. CA IX was induced by hypoxia in three HNSCC cell lines and overexpressed in HNSCC tumour tissue. Overexpression was localised to the perinecrotic area of the tumour on immunostaining, and the percentage area of the tumour expressing CA IX was significantly higher with more tumor necrosis and advanced stage. CA IX was overexpressed in HNSCC because of hypoxia and may be a potential biomarker for hypoxia in this tumour. Overexpression may help to maintain the intracellular pH, giving tumour cells a survival advantage and enhancing resistance to radiotherapy and chemotherapy. CA IX is considered thus a potential target for future therapy in HNSCC [46].

In a recent study the localisation of isozymes CA I, II, IX and XII in normal large intestine and in colorectal tumours has been investigated [47]. While the normal mucosa of the large intestine showed high expression for CA I and II, the intensity of the immunostaining for both isozymes decreased in benign lesions and was very weak in malignant tumours. The reciprocal pattern of expression observed for these cytoplasmic isozymes and transmembrane CA IX and XII in intestinal tissue specimens supported the suggestion that CA IX and XII may be functionally involved in tumour progression to malignancy and/or in invasion [47]. While CA I and II were shown to be prominent in normal colorectal mucosa, playing a role in regulation of pH homeostasis and water and ion transport, loss of expression of these cytoplasmic isozymes was shown to consistently accompany progression to malignant transformation [47]. In another study, the presence of CA XII along the human nephron and collecting duct, together with its cellular and subcellular localisation have been investigated [48]. CA XII has been revealed to be present in the basolateral plasma membrane of the epithelial cells in the thick ascending limb of Henle and distal convoluted tubules, and in the principal cells of the collecting ducts. A weak basolateral signal was also detected in the epithelium of the proximal convoluted tubules. In addition to the normal kidney specimens, this immunohistochemical study included 31 renal tumours [48]. CA XII showed moderate or strong plasma membrane-associated expression in most oncocytomas and clear-cell carcinomas. The segmental, cellular, and subcellular distribution of CA XII along the human nephron and collecting duct suggested that it may be one of the key enzymes involved in normal renal physiology, particularly in the regulation of water homeostasis [48]. High expression of CA XII in some renal carcinomas may contribute to its role in von Hippel-Lindau carcinogenesis [48].

Breast carcinoma is the most frequent cancer in women and is the second leading cause of death [49]. Choroid metastasis of breast carcinoma can be found either at presentation or in remission, being also frequently encountered in disseminated breast cancer with multiple organ metastasis. It has recently been proposed that the oedema-reducing effect of acetazolamide might be used for fluid removal from the retina to the choroid. In a 40-year-old

female patient on adjuvant chemotherapy for breast cancer with an isolated choroid metastasis, clinical and radiological remission was achieved after orbital radiotherapy, chemotherapy and acetazolamide treatment [68]. Thus, acetazolamide may possess another, slightly explored up to now, beneficial clinical use in patients with choroid metastasis [49].

Thus, the fact that the sulfonamide coxibs (celecoxib and valdecoxib) and to a much lower degree rofecoxib may have a potent antitumour activity [5-19] may now be explained by a dual mechanism of action: in addition to COX-2 inhibition, these compounds also interfere with the activity of CA isozymes critical for the development and invasion of cancer cells, such as CA II, IX and XII [23,24,26]. Indeed, data of table 1 show that the coxibs **6** and **7** act as nanomolar inhibitors against isozymes II and IX, which are among the most active and highly distributed in different tissues, including tumorus [50]. In conclusion, CA inhibition may constitute an important mechanism of antitumour action of some coxibs such as celecoxib and valdecoxib. The COX-2 selective inhibitors in clinical use may have a more complex pharmacology as initially thought [20].

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