Experimental Carbonic Anhydrase Inhibitors for the Treatment of Hypoxic Tumors

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Abstract: Carbonic anhydrase (CA, EC 4.2.1.1) isoforms IX and XII are overexpressed in many hypoxic tumors as a consequence of the hypoxia inducible factor (HIF) activation cascade, being present in limited amounts in normal tissues. These enzymes together with many others are involved in the pH regulation and metabolism of hypoxic cancer cells, and were validated as antitumor targets recently. A multitude of targeting strategies against these enzymes have been proposed and are reviewed in this article. The small molecule inhibitors, small molecule drug conjugates (SMDCs), antibody-drug conjugates (ADACs) or cytokine-drug conjugates but not the monoclonal antibodies against CA IX/XII will be discussed. Relevant synthetic chemistry efforts, coupled with a multitude of preclinical studies, demonstrated that CA IX/XII inhibition leads to the inhibition of growth of primary tumors and metastases and depletes cancer stem cell populations, all factors highly relevant in clinical settings. One small molecule inhibitor, sulfonamide SLC-0111, is the most advanced candidate, having completed Phase I and being now in Phase Ib/II clinical trials for the treatment of advanced hypoxic solid tumors.

Keywords: carbonic anhydrase, hypoxia, inhibitor, small molecule drug conjugates, anticancer drug, SLC-0111

Introduction

Tumor cells have many features which make them different from normal cells, among which are a more acidic pH outside the cell (pHe in the range of 6.5–6.8), connected with a slightly alkaline cytosolic pH (pHi values around 7.2–7.5), as well as lower than normal levels of O₂ (hypoxia): these and other irregularities are known as the Warburg effect, from the name of the German physiologist who discovered them and who was awarded a Nobel prize in 1931, for this and similar breakthrough discoveries related to metabolism. In 2019, another Nobel prize for medicine was awarded to three scientists who explained the Warburg effect at the molecular level: Kaelin, Ratcliffe and Semenza, who discovered the transcription factors involved in tumor oxygen levels sensing, i.e., the hypoxia inducible factors 1 and 2 (HIF-1/2). HIF-1/2 are also involved in the regulation of genes implicated in the metabolism/homeostasis of cancer cells, such as the glucose transporters (GLUT1-4), pH regulation (e.g., carbonic anhydrases, CAs; Na⁺/H⁺ exchangers; vacuolar ATPase, etc.), transport of anions, such as the monocarboxylate transporters (MCTs), the sodium bicarbonate co-transporters, as well as angiogenesis (vascular endothelial growth factor VEGF)6–18 These abnormalities of tumor microenvironment (lower levels of oxygen, disturbed pH balance,
upregulated glucose metabolisms, etc.) were considered as potential opportunities to develop anti-tumor drugs, which might specifically target tumor cells without affecting normal ones, more than one decade ago. In fact, the Warburg effect started to be exploited for diagnostic purposes several decades ago, in the 1980s, through the use of 18F-fluoro-deoxyglucose (FDG) for positron emission tomography (PET) imaging, as this glucose analog is a substrate for several GLUT isoforms and has a rapid uptake in cancer cells, thus making possible precise tumor imaging by means of computer tomography (CT) scans. However, the development of novel antitumor drugs based on this approach was more difficult than originally expected, although already in 2006 Pouysségur, and later Neri and Supuran, proposed various potential drug targets, such as the Na+/H+ co-transporters, Na+/H+ exchangers, anion exchangers (e.g., chloride for bicarbonate exchangers), monocarboxylate transporters (MCTs), vacuolar ATPase as well as several CA isoforms. At present only therapies which target VEGF, such as the monoclonal antibody (Mab) bevacizumab and similar biological drugs (known as anti-angiogenesis therapies), have been highly successful and constitute an important component of antitumor therapies for a variety of cancers. Other proteins whose genes are under the control of HIF-1/2 proved to be less “druggable” than originally thought. There is just one exception, which is constituted by the CA isoforms over-expressed in tumors as a consequence of the HIF-1/2 activation cascade, CA IX and XII, which will be discussed in this article.

Validation of CA IX/XII as Antitumor Drug Targets

The CAIs (EC 4.2.1.1) are a superfamily of metalloenzymes widespread in all life kingdoms, that catalyze the conversion of CO₂ to bicarbonate. As the hydration of CO₂ generates a proton, whereas the reversed reaction, i.e., bicarbonate dehydration consumes one, these enzymes are involved primarily in pH regulation in many cells, tissues and organisms, but also in several metabolic processes. The field of CAIs and their inhibitors with pharmacological applications has been reviewed extensively and will be not discussed in detail here. It should however be mentioned that CA inhibitors (CAIs) of the sulfonamide/sulfamate type have been known for decades and are still in clinical use as diuretics, anti-glaucoma, anti-epileptic and anti-obesity drugs. More recent applications of these as well as newer pharmacological agents belonging to the CAIs, demonstrated their potential in the management of neuropathic pain, cerebral ischemia, rheumatoid arthritis, or as anti-infective agents (for the treatment of bacterial, fungal and protozoan infections). However, the use of CAIs as antitumor agents constituted the subject of very intense research over the last two decades, with significant progress being achieved, and these derivatives will be reviewed here.

Two of the 15 known human (b) CA isoforms, hCA IX and XII are predominantly found in tumor cells and show a rather limited diffusion in normal cells. Both isoforms are multi-domain trans-membrane proteins with an extracellular CA domain, and were demonstrated to participate in the rather complex machinery of pH regulation, which as mentioned above, is dysregulated in cancer cells due to the activation of HIF-1/2. It should also be noted that these two enzymes are just a part of the complex network of proteins/processes which regulate pH and metabolism in tumor cells, most of which were mentioned in the Introduction. However, proof-of-concept studies from Pastorekova’s laboratories demonstrated that hCA IX (but presumably hCA XII has a similar role) participates significantly in the extracellular acidification of tumor cells, with the concomitant alkalization of the cytosol.

Essential for the validation were the fluorescent sulfonamides 1 and 2 incorporating fluorescein thiourea moieties which unlike the classical, clinically used sulfonamide acetazolamide 3 (Figure 1) showed a slightly selective inhibition of the transmembrane isoforms hCA IX/XII over the cytosolic enzymes hCA I and II, and were also membrane-impermeant due to the presence of the carboxylate moieties. The use of these inhibitors demonstrated on one hand the significant effect of catalytically active hCA IX in the extracellular acidification but also the fact that the inhibitor effectively binds to the enzyme only in hypoxia and not in normoxia, which was considered as a very promising finding. Indeed, inhibition of hCA IX with such compounds reverted the acidification of extracellular pH in cell cultures, with an almost normalization of the parameter which from 6.5 (pHe of the tumor cells) returned to a more normal physiological value of 7.2–7.3 (after the use of inhibitors such as 1 and 2). Furthermore, the fact that the fluorescent inhibitors were observed bound only in hypoxia,
constituted the first evidence that targeting hCA IX/XII may also have applications for imaging, not only for the treatment of hypoxic tumors. Indeed, intense research was thereafter initiated in several laboratories for finding more effective, drug-like anti-tumor CAIs.

**Antitumor CAIs Developed in Zurich**

The first CAIs which were reported to show antitumor effects *in vivo*, were compounds 4 and 5 discovered by Neri’s group in 2009 (Figure 2). Over the years, this group made significant contributions to the field, with many interesting approaches being proposed for targeting CA IX by scientists from this laboratory. Derivatives 4 and 5 incorporated the acetazolamide scaffold as binding moiety to the enzyme zinc ion, and tails of the fluorescein carboxamide type in 4, similar to 1 and 2 reported earlier, as well as the albumin-binding moiety in 5. Both derivatives showed effective in vitro and in vivo binding to hCA IX, and 5 was also active in vivo, in a SK-RC-52 xenograft model of cancer.

Neri’s group also reported the first DNA-encoded chemical libraries for obtaining tight binding CA IX inhibitors. This approach, apart from being highly innovative, also afforded sub-micromolar bis-sulfonamide CAIs, some of which have been prepared without the DNA-tag. They showed accumulation within the hypoxic tumor tissue and effective in vivo anti-tumor action. Subsequently, another pioneering approach has been proposed by the same group: small molecule drug conjugates (SMDCs) targeting CA IX and incorporating toxin payloads such as the cytotoxic, DNA-binding agents duocarmycins or the tubulin inhibitor mertansine (also known as DM1, compound 6 in Figure 2). Such SMDCs again incorporated the acetazolamide scaffold for targeting CA IX, to which a water-solubilizing tetrapeptide fragment has been attached by using click chemistry of the alkyn-azide cycloaddition type. The peptide fragment contained the Cys terminal amino acid residue, which reacts with the SH group of mertansine, forming a disulfide bond by which the drug conjugate has been obtained (derivative 7 in Figure 2). The SMDC 7 showed effective antitumor effects in subcutaneous SK-RC-52 (renal cell carcinoma tumors) xenograft models.

The same approach has been thereafter explored and enriched for acetazolamide/benzenesulfonamide CAIs serving as selective delivery vehicles for radionuclides ($^{99m}$Tc), antibodies, dipeptide-linked renal cell carcinoma-targeting agents, cytokines (such as IL-2) and other small molecule toxins (e.g., auristatin methyl ester, cryptophycin) as drug conjugates. All these approaches were highly successful in leading to SMDCs, antibody-drug conjugates (ADACs) or cytokine-drug conjugates with an enhanced antitumor effect compared with the parent molecules from which they were obtained. The ETH–Neri group approach is definitely one of the most effective and innovative, in generating small molecules or conjugates with a very interesting pharmacological profile for targeting CA IX.

**The Maastricht–Montpellier–Tampere–Florence Approach**

Significant contributions to the discovery of interesting CA IX/XII inhibitors were made by Winum’s group in Montpellier, in collaboration with Lambin’s group in Maastricht, Parkkila’s group in Tampere and our group in Florence. Only the most relevant papers for the present review will be considered here. In fact many other contributions were achieved by these researchers in other fields connected to CAIs, which have been reviewed elsewhere.
A very interesting idea from Winum’s laboratory was that of coupling the nitroazole chemotypes, present in many radio/chemosensitizing agents, with CAIs belonging to the sulfonamide, sulfamate or sulfamide type. Compounds prepared by this approach (derivatives 8–11, Figure 3) incorporated 2- or 5-nitroimidazoles and a range of aromatic scaffolds on which the zinc-binding groups (ZBGs) responsible for binding to CA were appended, particularly the primary sulfonamide, sulfamate and sulfamide moieties (Figure 3). The binding of some of these compounds (e.g., 10 and 11) to hCA II, an off-target isoform, was also investigated by means of X-ray crystallography, proving interesting interactions between the inhibitor scaffold and the enzyme active site.

Effects on hypoxia-induced extracellular acidification in the presence of compounds such as 10 and 11 (which are low nanomolar hCA IX/XII inhibitors) was evaluated in vitro, in HT29 and HeLa cancer cells, whereas HT-29 tumor bearing mice were treated with the compounds alone or in combination with radiation. One compound, sulfamide 11, markedly enhanced sensitization towards radio- and chemotherapy, when administered alone or in
combination with the anticancer agent doxorubicin, thus proving the usefulness of this approach.\(^74,75\) Furthermore, these compounds were shown to be non-toxic \textit{in vivo} in several animal models\(^77,78\) whereas some newer congeners were also reported recently.\(^79\)

**University of Manchester–University of Florence Sulfamate CAIs**

A series of aromatic sulfamates, highly effective as hCA IX/XII inhibitors, was reported by Williams’ group in Manchester in collaboration with our group.\(^80,81\) These derivatives, of types 12–15 (Figure 4), incorporate the sulfamate ZBG (present also in derivative 10 discussed above) and most of them possess aromatic-ureido (12) or thiureido (13) tails. The sulfamate fluorescent derivative 14, structurally similar to the early sulfonamide derivatives 1 and 2 which allowed the validation of CA IX as an antitumor drug target, was also reported in the same study. In this rather large series of congeneric ureas/thioureas 12 and 13, the compound which underwent a thorough investigation \textit{in vitro} and \textit{in vivo} was 15, also known as S4.\(^80,81\) This compound was a highly effective \textit{in vitro} hCA IX (Ki of 7 nM) and hCA XII (Ki of 2 nM) inhibitor, and in an orthotopic MDA-MB-231 breast carcinoma tumor model in mice showed a significant reduction of the primary tumor and metastases growth at a dose of 10 mg/kg.\(^80\) Furthermore, S4 was also active in a small cell lung cancer (SCLC) animal model, in mice, both alone and in combination with cisplatin, through synergistic, hypoxia-specific targeting.\(^81\)

**Brisbane–Florence Glycomimetic CAIs**

The collaboration between Poulsen’s group in Brisbane, Australia and the Florence group led to a large number of highly effective hCA IX/XII inhibitors belonging mainly to the sulfonamide, sulfamate and sulfamide classes.\(^82–90\)

Many such compounds, among which an example of sulfamides is presented in Figure 5, incorporate sugar moieties and they were termed as glycomimetics. The
presence of sugars is beneficial both for the interaction with the enzyme, as many of these CAIs are highly effective inhibitors,\(^{82-90}\) but also for their physico-chemical and pharmacological properties, as the presence of the sugar moieties enhances water solubility and bioavailability. The drug design was attentively performed, as most of these compounds incorporate three fragments: the CA inhibitory fragment, belonging to the aromatic sulfonamide, aromatic/aliphatic sulfamate and sulfamide, linked through a triazole unit to the sugar fragment, which can be with free OH or acylated moieties. A range of sugars (mono-, di- and tri-saccharides), as well as their acetylated, propionylated or butylated esters were used for generating a remarkable number of CAIs. Furthermore, the chemical diversity was enhanced also by using various position of the sugar where the other two elements were appended (compare for example 16 and 22 in Figure 5). These compounds were prepared by so-called click-tailing, using the cycloaddition reaction between azides and alkynes, also known as “click chemistry.”\(^{82-90}\) In some cases, saccharin\(^{90}\) derivatives were also obtained with sugar clicked tails, based on the early finding from our and Klebe’s laboratory\(^ {91}\) that saccharin is an effective CA IX inhibitor and
binds to the metal ion from the CA active site as anion, through the secondary sulfonamide nitrogen atom. Some of these compounds showed effective CA IX/XII inhibition in vitro, and some of them were shown to interfere with cell proliferation and to induce cell apoptosis in T-cell lymphomas expressing CA XII.88

In another study, gallium-68 radiolabeled sulfonamides targeting CA IX were reported, which were investigated in a mouse xenograft HT29 tumor model.89 An accumulation of radioactivity within the tumor and a low uptake in blood, with clearing into the urine has been observed, which makes them of interest as imaging agents. It should be mentioned that this approach was in fact reported earlier by the Vancouver group.92–95

Another research line opened by the Poulsen–Supuran collaboration was on the natural product sulfonamide Psammaplin C, 23 (Figure 6), which was discovered in 1991 in the marine sponge Pseudoceratina purpurea.96 The derivative comprises a bromotyrosine-oxime functionality as well as a functionalized aminoethyl-sulfonamide fragment, being one of the very few natural products to incorporate such a moiety. Psammaplin C was shown to be a low nanomolar hCA IX inhibitor (Ki of 12.3 nM) and a subnanomolar hCA XII inhibitor (Ki of 0.79 nM) and its X-ray crystal structure in complex with hCA II (Ki of 88 nM) was also resolved.96 In a subsequent study, it has been demonstrated that combining Psammaplin C (as well as some of its synthetic derivatives), with temozolomide, a clinically used chemotherapeutic agent, reversed multidrug resistance and significantly increased survival in an animal model of glioblastoma, a highly aggressive brain tumor.97

**Vancouver–Florence – The Winning Approach**

The collaboration between Dedhar’s and Supuran’s groups started in 2010, and led shortly thereafter to the identification of a series of ureido-substituted benzenesulfonamides acting as CA IX/XII-selective in vitro CAIs, but also showing very promising in vivo antitumor/antimetastatic effects.98,99

Benzenesulfonamides incorporating carboxamido- and secondary sulfonamide linkers were in fact known for many years,29,32–34 and, although showing potent inhibitory action, no relevant selectivity for isoforms of interest was ever evidenced. Thus, it was rather surprising that the presence of ureido linkers, as in compounds 24 and 25 (Figure 7) as well as many of their congeners, led to many highly isoform-selective CAIs.98,100 This was explained when McKenna’s group reported the X-ray crystal structure of several of these derivatives bound to hCA II.98,100 It was observed that due to the flexibility conferred by the ureido linker, the tails of such sulfonamides were able to bind in very different regions of the active site, towards its

![Figure 6: The natural product sulfonamide Psammaplin C, 23.](image1)

![Figure 7: Ureido-sulfonamides 24 and 25 and 7-glycosyl-substituted coumarins 26, 27.](image2)
entrance, which is the most variable part of the different CA isoforms. This in fact may explain the observed selectivity of many ureido sulfonamides to diverse CA isoforms. For example 25 (later known as SLC-0111, Figure 7) had a Ki of 4.5 nM for hCA XII, of 45 nM for hCA IX and > 400 nM for all other 10 catalytically active human isoforms. Initial in vivo experiments were done with the nitro-derivative 24 and thereafter with SLC-0111 25, 98,99 as well as the coumarin CAIs 26 and 27, 101 in orthotopic breast cancer models expressing CA IX (the 4T1 cell line) or without any CA IX expression (67NR cell line). A marked decrease of the primary tumor growth was observed only for the 4T1 line, irrespective of the CAI used (all these compounds 24–27 are low nanomolar CA IX/XII inhibitors and poorly inhibit the cytosolic off-target isoforms CA I and II). Furthermore, mice harboring the 4T1 tumors also spontaneously develop lung metastases. After treatment with the CAIs of type 24–27 a strong reduction in the formation of the lung metastases or even their total lack (at higher doses of inhibitor) was also observed proving that the inhibitors are effective in inhibiting the growth of the primary tumors and metastases. 98–101

Regarding the coumarin CAIs, it should be mentioned that they were discovered in 2009 by a collaboration with Poulsen’s group in Brisbane, 102 and they represented a completely new CAI chemotype with an unprecedented inhibition mechanism. The coumarins act as prodrug inhibitors, being hydrolyzed by the esterase CA activity at the lactone ring, and the formed hydroxyl-cinnamic acid thereafter binds at the entrance of the active site cavity, obstructing its entrance. 102,103

Apart the dual effect on the primary tumors and metastases, subsequent work from Dedhar’s group 104,105 with CAIs of the type mentioned above as well as genetic depletion of CA IX with small hairpin RNAs, showed a third beneficial effect of this class of compounds: depletion of the cancer stem cells population, which is considered a clinically significant phenomenon. In another paper from the same group 106 it has also been shown that CA IX activates a matrix metalloproteinase isoform (MMP-14) for initiating the invasion which is essential for tumor cell migration from the primary tumor to other organs for the formation of metastases. Similar effects were thereafter observed with structurally related sulfonamides to SLC-0111, which possess the sulfonamide moiety in meta to the ureido functionality, 107 proving that the antitumor/antimetastatic effects are a general feature of the potent, CA IX/XII – selective compounds.

Subsequent studies from the Vancouver group evidenced the synergistic effects of SLC-0111 in combination with other anticancer drugs in various animal models, such as pancreatic cancer (combination with gemcitabine); 108 combination with immune check point inhibitors; 109 combination with temozolomide for glioblastoma treatment, 110 etc.

Other research groups apart from the Vancouver one were also active in employing SLC-0111, alone or in combination with other agents/procedures, observing interesting antitumor effects. They include the observation that interference with pH regulation in hepatocellular carcinomas, by using CA IX inhibitors, has a potent antitumor effect, 111 which was also evident when CA IX inhibitors were administered together with radiation, 112 proton pump inhibitors (lansoprazole, omeprazole), 113 cisplatin, 81 antimetabolites, 114 apurinic/apyrimidinic endonuclease 1 (APE1) inhibitors, 115 histone deacetylase inhibitors, 116 etc. Another study showed the lack of endothelial toxicity of SLC-0111, 117 whereas 24 (also known as U104) was shown to reduce prostate cancer 118 and breast cancer 119 cell growth by research groups which did not participate in the discovery of these drugs. Indeed, SLC-0111 was proved to be useful in diverse biomedical studies not related to carcinogenesis, when selective CAIs were needed, such as the demonstration that CA IX is involved in the pH regulation of pulmonary microvascular endothelial cells, 120,121 or in proving that CA VB is involved in mitochondrial biogenesis and production of lactate by human Sertoli cells. 122

SLC-0111 entered in Phase I clinical studies as an antitumor/antimetastatic agent in 2014 and the quite positive report of the study was recently published. 123 In 2017 a Phase Ib/II study was initiated which is still in progress, for assessing the efficacy of SLC-0111 in combination with other therapeutic agents for the management of pancreatic cancer (ClinicalTrials.gov Identifier: NCT03450018).

**Various Other Approaches for Designing CA IX/XII Inhibitors**

The interesting results observed with SLC-0111 and its congeners as antitumor/antimetastatic agents, fostered a large number of research studies in the design of CAIs which used this compound as the lead molecule. For lack of space we will be unable to mention all of them, but some relevant examples will be presented.
As shown in Figure 8, a large number of compounds have been designed and often also investigated in anticancer studies for efficacy, using SLC-0111 as the lead compound. The main approaches for obtaining these compounds were: (i) modification of ZBG which in SLC-0111 is of the primary sulfonamide type, but it has been changed to sulfamate in compound 28; (ii) modification of the primary sulfonamide type, but it has been changed to sulfamate in compound 28;

![Figure 8: Sulfonamides 28–43 designed as anti-cancer derivatives using SLC-0111 as lead molecule.](https://www.dovepress.com/)

107,124–134
position of the sulfamoyl moiety, as in compounds 29 and 30, which have the ZBG in meta, not in para to the ureido functionality;\(^{107}\) (iii) modification of the ureido linker, as in compounds 30–36, which incorporate thioureido, selenoureido, \(N\) cyanoguanidino, triazene, enaminone, piperazino-carboxamide and piperidine-carboxamide functionalities;\(^{124–130}\) (iv) modification of the tail, as in compounds 37–41, in which the 4-fluorophenyl moiety has been changed to various other moieties, similar or much more different to the original compound;\(^{131–133}\) (v) more drastic changes of both the tail and the linker present in the lead, as in derivatives 42 and 43.\(^{134}\) It should be mentioned that polyaminocarboxylate-polycyclic moieties of the DOTA type have also been introduced in the SLC-0111 scaffold (in place of the fluorine atom) for obtaining compounds able to complexate PET emitting isotopes such as \(^{111}\)In- and \(^{90}\)Y-, with such labeled ureidosulfonamides being useful for SPECT imaging.\(^{135}\) For lack of space it is impossible to mention many other highly valuable studies of drug design of various other classes of CAIs.

Essential for these studies were also the X-ray crystallography data obtained by many groups, but in which McKenna’s was undoubtedly the most relevant, with a huge number of compounds belonging to a variety of classes crystallized in complex with hCA II, hCA IX (or one of its mimics) as well as other isozymes.\(^{98,100,136–144}\) This led to a thorough understanding of the favorable interactions between the inhibitor scaffold and the enzyme and paved the way towards more efficient and isoform-selective inhibitors.

A Recent Controversy on Antitumor CAIs

Recently, in a controversial paper, Jonsson and Liljas\(^{145}\) queried the validity of CA IX/XII as antitumor drug targets as well as the inhibition data generated in my laboratory, with a direct attack on the most advanced drug candidate available to date, SLC-0111. They state that “it is doubtful that SLC-0111 has the required Ki to advance to clinical trials” and that the “commercial interests of pharmaceutical companies and patients may be hurt”.\(^{145}\) As shown in this review, and elsewhere, as a reply to this attack,\(^{53,146}\) a multitude of studies form various laboratories all over the world, through an effort of more than 20 years, allowed the validation of the two enzymes as drug targets. Furthermore, very diverse techniques such as stopped-flow kinetics,\(^{53}\) native mass spectrometry measurements,\(^{147–149}\) and X-ray crystallography strongly support the Ki values obtained in our laboratory using the stopped-flow kinetic method for a range of different types of CAIs and demonstrate that this provides sufficient accuracy levels for drug discovery and development applications. It should also be noted that more than 10,000 different CAIs were synthesized and assayed in our laboratory and a number of \(>15,000\) were assayed through collaborations with \(>100\) academic research groups and 15 pharmaceutical companies worldwide during the three decades of activity in CA research. I have not mentioned in detail here, but we have reported more than \(80\%\) of the new chemotypes acting as CAIs and discovered at least three innovative CA inhibition mechanisms, i.e., anchoring to zinc coordinated water, occlusion of the active site entrance and binding out of the active site.\(^{146,150}\) Liljas is a crystallographer who only reported the X-ray crystal structure of just one isofrom (hCA II) in complex with three sulfonamides and five inorganic anions, and never worked in anticancer drug design.\(^{145}\) We thus consider the statements from the above-mentioned paper as totally erroneous and motivated by non-scientific issues.

Conclusions

CA IX/XII are activated through the HIF-1/2 cascade in hypoxic tumors and were validated as antitumor/antimetastatic targets in recent years. CA IX and XII play crucial roles in regulating the extracellular pH in tumor cells, and were shown to be abundantly expressed in many types of advanced solid metastatic tumors present in a variety of organs (kidneys, lung, breast, colon, liver, prostate, melanoma, etc). As a result of careful studies involving chemical synthesis, discovery of new chemotypes and CA inhibition mechanisms, stopped-flow kinetic measurements, X-ray crystallography, mass spectrometry and other techniques, involving the screening of tens of thousands of potential inhibitors, many key lead compounds targeting CA IX and XII emerged, including the sulfonamide CA inhibitor SLC-0111, which is presently in Phase Ib/II clinical trials. A variety of preclinical cancer models were developed, all demonstrating that these two enzymes are promising cancer therapeutic targets in advanced, hypoxic solid tumors, due to the fact that they possess at least three beneficial effects: they reduce the growth of the primary tumor, inhibit the formation of metastases, and deplete the number of cancer stem cells. We anticipate that the use of selective CA
IX/XII inhibitors, such as SLC-0111 or many of the interesting compounds developed in other laboratories, will be beneficial in combination with chemo-, radiation- and immuno- therapies for eliminating resistant cancer cell populations and for a durable suppression of tumor cell growth and metastasis.

Disclosure
Claudiu T Supuran reports Welichem Biotech Inc. is developing the drug that I have discovered, SLC-0111, during the conduct of the study; in addition, Claudiu T Supuran has a patent WO2012/021963 issued to Welichem. The author reports no other potential conflicts of interest for this work.

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