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Review

Carbonic Anhydrase Inhibitors Targeting Metabolism and Tumor Microenvironment

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Abstract: The tumor microenvironment is crucial for the growth of cancer cells, triggering particular biochemical and physiological changes, which frequently influence the outcome of anticancer therapies. The biochemical rationale behind many of these phenomena resides in the activation of transcription factors such as hypoxia-inducible factor 1 and 2 (HIF-1/2). In turn, the HIF pathway activates a number of genes including those involved in glucose metabolism, angiogenesis, and pH regulation. Several carbonic anhydrase (CA, EC 4.2.1.1) isoforms, such as CA IX and XII, actively participate in these processes and were validated as antitumor/antimetastatic drug targets. Here, we review the field of CA inhibitors (CAIs), which selectively inhibit the cancer-associated CA isoforms. Particular focus was on the identification of lead compounds and various inhibitor classes, and the measurement of CA inhibitory on-/off-target effects. In addition, the preclinical data that resulted in the identification of SLC-0111, a sulfonamide in Phase Ib/II clinical trials for the treatment of hypoxic, advanced solid tumors, are detailed.

Keywords: carbonic anhydrase; hypoxia; pH regulation; inhibitor; sulfonamide; SLC-0111

1. Introduction

The microenvironment of tumor cells is different from that of normal cells and plays a crucial role in shaping the behavior of tumors, which, in turn, frequently influences treatment outcomes as well as treatment strategies [1,2]. Many decades ago, Warburg [3] recognized this phenomenon, which later became known as the "Warburg effect" and constitutes a hallmark of many cancers: these cells are hypoxic, more acidic than normal, and possess a dysregulated glucose (and not only glucose) metabolism [3,4]. In the last 20 years, it became obvious that the orchestrators of all these phenomena are transcription factors called hypoxia-inducible factor 1 and 2 (HIF-1/2). The understanding of the intricate biochemical and physiological processes by which HIF-1/2 sense tumor oxygen levels and regulate genes involved in metabolism (such as the glucose transporters), pH regulation (such as monocarboxylate transporters, MCTs; and carbonic anhydrases, CAs), and angiogenesis (vascular endothelial growth factor (VEGF)) led to the award of the 2019 medicine Nobel prize to three scientists who contributed significantly to the field, Kaelin [5], Ratcliffe [6], and Semenza [7,8]. Their crucial discoveries and those from many other laboratories [9–17] established the basis for exploiting the tumor microenvironment abnormalities for developing a new generation of antitumor therapies and drugs, which should specifically target tumor cells without relevant toxicity to normal cells and tissues [18,19].

As a result of HIF-1/2 activation, two of the proteins that were identified to be highly overexpressed in many tumor types were the CA isoforms, CA IX and XII [9–19]. CAs are a superfamily of metalloenzymes present in all kingdoms of life that catalyze the conversion of CO_2 to bicarbonate and protons [20–26]. There are 15 CA isoforms that are known in humans (h); i.e., hCA I - hCA XIV, including two V-type isoforms, hCA VA and hCA VB) [27–30]. The field of CAs and their inhibitors were recently reviewed and will not be discussed in detail here [19–24]. Briefly, the X-ray crystal structures are available for the two tumor-associated isoforms hCA IX and XII [25,26], as well as for many other members of the human CA family [27–30]. Such structures of the enzymes alone and in complex with many types of inhibitors have been highly relevant for the design of compounds with a range of applications, not only in the anticancer field but also in the renal, central nervous system, ophthalmologic, obesity, and other medical fields [31–39]. Furthermore, recently, CA inhibitors were shown to be of potential use in the management of cerebral ischemia, neuropathic pain, and arthritis [40–44], which are conditions for which this class of pharmacologic agents was previously considered inappropriate. However, the attentive search for novel classes of compounds with efficacy and selectivity for the different isoforms involved in these quite diverse conditions resulted in proof of concept studies, which have suggested that all catalytically active hCA isoforms may be considered interesting drug targets.

2. Validation of CA IX/XII as Anticancer Drug Targets

2.1. Sulfonamides and Other Classes of CA inhibitors (CAIs): Selectivity for Tumor-Associated vs. Cytosolic Isoforms

hCA IX was discovered in 1994 by Pastorekova's group [45] and hCA XII in 1998 by Türeci et al. [46]. Both isoforms were shown to be extracellular, multi-domain proteins, with the enzyme active site situated outside the cell [45,46]. Years later, with the demonstration that both enzymes are activated through the HIF-1/2 pathway, a reason for this localization became obvious: both enzymes, together with a range of other proteins that will be not discussed here, are involved in the pH regulation and metabolism of the cancer cell [1,2,47–53]. Both enzymes possess significant catalytic activity for the CO_2 hydration reaction [19–21].

The hypothesis that interfering with the activity of such proteins may have anticancer effects was proposed by Pouysségur in 2006 [18], but no selective inhibitors for cancer-associated CAs or other proteins involved in the regulation of the tumor microenvironment (MCTs, bicarbonate transporters, or Na+/H+-exchangers, etc.) were available at that time. Thus, a program for developing CA IX/XII selective compounds was initiated in some of our (CT Supuran and S Dedhar) laboratories. Acetazolamide **1** (Figure 1), the classical sulfonamide inhibitor, was the starting point [54,55]. Indeed,

primary sulfonamides [56–58] such as acetazolamide were known for decades to potently inhibit CAs, as they bind as anions (RSO_2NH^-) to the metal ion in the enzyme active site, as shown in a classical crystallographic study from Liljas' group [59]. However, acetazolamide is a non-selective CAI [20], which potently inhibits most human CA isoforms and is, thus, not an appropriate compound for designing isoform-selective compounds. The tail approach was thus employed for developing CA IX/XII-selective inhibitors [60]. The idea is very simple: attach moieties that may induce the desired properties (e.g., enhanced hydrosolubility) to scaffolds of simple sulfonamides (e.g., sulfanilamide, metanilamide, and their derivatives; 5-amino-1,3,4-thiadiazole-2-sulfonamide, etc.) that may lead to interactions with the external part of the CA isoforms active site, which is the region at the entrance of the cavity that is more diverse than the active site residues between the 12 catalytically active human isoforms [60,61]. Detailed kinetic and crystallographic studies were performed on a large number of derivatives (more than 10,000 sulfonamides were synthesized and investigated in our laboratories). This research demonstrated that it was possible to design CAIs selective for the various isoforms by tailoring the dimensions (length, bulkiness, etc.) as well as the chemical nature of the various tails [60–69]. For example, the fluorescein-derivatized sulfonamides **2** and **3** were only mildly selective for CA IX vs. CA II [47,48], but they were highly useful for understanding the role of CA IX in tumor pH regulation [47–50]. In contrast, the ureido-substituted sulfonamides **4** and **5** were members of a congeneric series in which many CA IX/XII selective inhibitors were discovered [51,52], and their selectivity for the tumor-associated vs. the cytosolic isoforms was explained by detailed X-ray crystallographic data [70,71]. Another highly interesting approach was reported by Neri's group [72–74], who designed DNA-encoded libraries of sulfonamides and identified highly potent in vitro CA IX inhibitors in a series of very interesting papers [75–77].

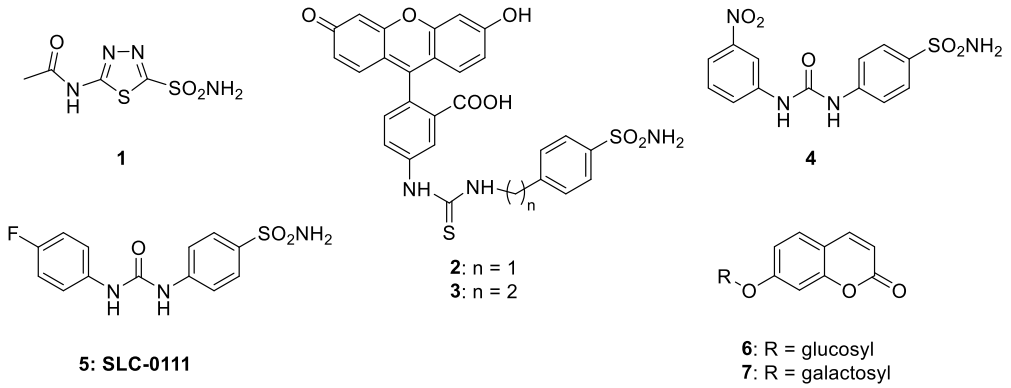


Figure 1. Carbonic anhydrase inhibitors (CAIs) that were crucial for validation of CA IX/XII as antitumor targets: acetazolamide **1** is the classical inhibitor in clinical use for decades; the fluorescein-derivatized sulfonamides **2** and **3** were used in the proof of concept studies to demonstrate the involvement of CA IX in acidification of the external pH of the tumor cell [47–50], the ureido-substituted-sulfonamides **4** and **5** were among the first CAIs to show significant antitumor effects in animal models of hypoxic tumors [51,52], together with coumarin inhibitors **6** and **7** [53].

Thus, by 2009–2010, sulfonamide CAIs targeting CA IX with high specificity were available [78], which fostered renewed interest in targeting not only primary tumors but also metastases [79–81]. However, in parallel with these studies mentioned above, the last decade also brought important developments in the discovery of non-sulfonamide CAIs. The first highly relevant new class of CAIs is represented by the coumarins [82–85]. The coumarins were shown to act as "prodrug inhibitors": they undergo CA-mediated hydrolysis of the lactone ring (as in the compounds **6** and **7** of Figure 1), which generates the de facto inhibitor, belonging to the 2-hydroxycinnamic acid class of compounds. Extensive kinetic, crystallographic, and synthetic efforts led to a thorough understanding of this innovative inhibition mechanism [82–85]. In fact, the 2-hydroxycinnamic acids formed by coumarin hydrolysis do not interact with the catalytic metal ion but instead bind at the entrance of the active site

cavity, in a highly variable region of the different CA isoforms, which explains the very isoform-selective inhibitory effects of many representatives of this class of CAIs [82–85]. Furthermore, this new CA inhibitory class inspired the discovery of many other chemotypes, such as the sulfocoumarins and the homosulfocoumarins by Zalubovskis group [86–88], the homocoumarins [89], the thiocoumarins by Ferraroni et al. [90], etc.

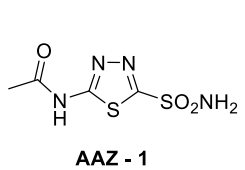
By comparing the sulfur-based, carbon-based [91], and phosphorus-based zinc-binding groups, a range of novel classes of inhibitors that interact with the zinc ion were reported, including phosphonamidates [92], dithiocarbamates [93], monothiocarbamates [94], xanthates and trithiocarbonates [95], selenols [96,97], carboxylates [98] and hydroxamates [99], benzoxaboroles [100–102], as well as carbamates [103]. Additional inhibitors that have been developed exhibit a different inhibition mechanism: they anchor to the zinc-coordinated water molecule [104,105]. Polyamines, such as spermine and its derivatives [104], and the simple phenol [105] and many of its derivatives [106–109] together with polyphenols [110] inhibit CAs in this way. This inhibition mechanism is also shared by the sulfocoumarins, as demonstrated by Zalubovskis group [86] by using kinetic and crystallographic studies. However, probably, one of the strangest CA inhibition mechanism is the one reported by De Simone's and Carradori's group for a benzoic acid derivative that binds outside the active site cavity [111].

2.2. Measurement of Inhibition Efficacy

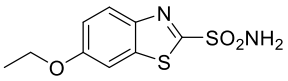
All these inhibitors discussed above and many others that are not mentioned here have been profiled for their inhibition of many CA isoforms by using a stopped-flow CO_2 hydrase assay, which was originally reported in 1971 by Khalifah [112] and validated by others as a rapid method for determining the enzyme kinetics and inhibitory constants of various classes of compounds against different CAs [113–118]. In a recent paper by Jonsson and Liljas [119], some inhibition data from several papers from the Supuran group were considered. Jonsson and Liljas queried the enzyme and substrate concentrations that were used in some experiments. Many of the early stopped-flow assay papers cited above [113–118], which are not from Supuran's group but from several well-established laboratories, provided exactly the same level of information. That is, the range of CO_2 concentrations at which the experiments were performed was reported, without detailing enzyme concentrations. On the contrary, in many of our papers, the enzyme concentrations are reported either for the CO_2 hydrase or esterase assays [60,62,68,69,120–127], and they range between 3.5 and 14 nM. The vast majority of the analyses performed by Jonsson and Liljas [119] are based on supplementary information that was reported in six of our papers (two of the other papers that they considered in the analysis contained CA inhibitory data obtained from a completely different laboratory). The presumed lack of precision results from erroneously typed concentrations for the enzymes in one of the analyzed paper's supplementary information [128]. That is, a concentration of 10^{-7} M was erroneously written, which is, in fact, the stock solution of enzyme and not the enzyme concentration at which the measurements were performed. As mentioned above, in most of our experiments, we work at enzyme concentrations ranging from 3.5 and 14 nM, and sometimes even lower when exceedingly strong inhibitors are analyzed. Jonsson and Liljas [119] also raised a query about the enzyme inhibition curves from the supplementary information of another paper [129]. For these figures, the uncatalyzed reaction was not subtracted in the curves plotted in the supplementary figures, but those values were automatically subtracted when the IC_{50} was calculated due to the use of the algorithm in the computer software that is used for the stopped-flow instrument. Despite these minor errors (now corrected through submission of errata), the inhibitory constants (K_is) determined by the University of Florence stopped-flow kinetic measurements of CA enzyme inhibition have been validated by native mass spectrometry (MS) measurements performed in another laboratory on a number of sulfonamides, which gave results in excellent agreement with the kinetic inhibition data [130,131]. Dissociation constants (K_ds) were obtained from native mass spectrometry measurements of buffered aqueous solutions containing hCA I and hCA II with acetazolamide, ethoxzolamide, brinzolamide, furosemide,

dichlorophenamide, and indapamide, which are well known CAIs [20]. K_d values were obtained by either measuring individual ligand–protein interactions in single experiments or simultaneously in competition experiments (Table 1) [130]. K_d values are equivalent to K_i values for inhibitors that bind at the site of the substrate, which is the case for sulfonamide CAIs. The agreement between the native MS measurements and those for the stopped-flow kinetic assay was excellent. For example, the measured K_d values of all nanomolar inhibitors were within 30% of the K_i values obtained by stopped-flow kinetic experiments. Moreover, K_d values obtained by native MS for the micromolar inhibitors were within 50% of the K_i values. More recently, native MS was used to measure the K_d values of 15 perfluoroalkyl substances with either carboxylate, sulfate, or sulfonamide zinc-binding groups to hCA I and hCA II [131]. The 30 measured K_d values obtained from native MS measurements were also in excellent agreement with the corresponding K_i measurements (data not shown here) [131]. In fact, the K_d values were within 37% of the K_i values. The native MS approach to measuring K_d values has also been validated for ligand–DNA interactions compared to other well-established biophysical methods (isothermal titration calorimetry and differential scanning calorimetry) [132].

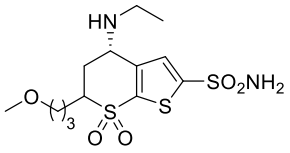
Table 1. Measured dissociation constants (μM) of six sulfonamides to human (h)CA I and hCA II using native mass spectrometry with nanoscale ion emitter tips in individual ligand–protein binding experiments (Single) and simultaneously in a competition experiment (Competition). Data adapted from Reference [130].



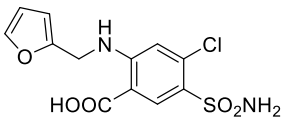
AAZ - 1



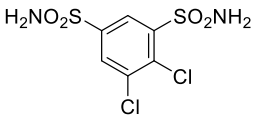
EZA



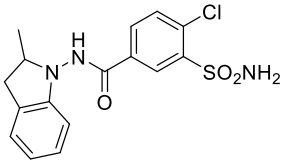
BRZ



FUR



DCP



IND

	hCA I			hCA II		
Inhibitor	**Single**	**Competition**	**Literature**	**Single**	**Competition**	**Literature**
Acetazolamide (AAZ)	0.24 ± 0.02	0.29 ± 0.03	0.25 [a] [133]	0.015 ± 0.001	0.022 ± 0.002	0.012 [a] [133]
Ethoxzolamide (EZA)	0.014 ± 0.002	0.016 ± 0.004	0.025 [a] [133], 0.009 [b] [134]	0.010 ± 0.001	0.014 ± 0.002	0.008 [a] [133]
Brinzolamide (BRZ)	1.06 ± 0.05	1.12 ± 0.06	0.73 ± 0.04[a] [135]	0.005 ± 0.001	0.007 ± 0.001	0.003 [a] [135]
Furosemide (FUR)	0.055 ± 0.005	0.048 ± 0.006	0.062 [a] [130]	0.098 ± 0.009	0.110 ± 0.010	0.065 [a] [135]
Dichlorophenamide (DCP)	1.3 ± 0.1	1.4 ± 0.1	1.2 [a] [136]	0.027 ± 0.002	0.035 ± 0.002	0.038 [a] [136]
Indapamide (IND)	9.2 ± 0.3	9.5 ± 1.0	51.9 [a] [137]	3.22 ± 0.20	3.32 ± 0.31	2.52 [a] [137]

[a] U. Florence stopped-flow kinetic inhibition constant measurements (μM) [68,69,82,120–127]. [b] U. Florida buffer indicator kinetic method measurements [134].

Overall, these native MS data strongly support that K_i values can be obtained using the stopped-flow kinetic method for a range of different types of CAIs with sufficient accuracy for drug discovery and development applications.

2.3. Drug Design Studies Using SLC-0111 as Lead Compound

Compound **5**, SLC-0111, possesses a variable affinity for the diverse hCA isoforms and selective inhibitory action toward tumor expressed CA isoforms IX and XII over the off-target ubiquitous hCA I and hCA II [51,52,70,81]. Compound **5** is characterized by the presence of the ureido

functionality as a linker between the benzenesulfonamide fragment and the tail of the inhibitor. By means of X-ray crystallography on SLC-0111, it was demonstrated that the reason for the isoform selectivity is the ureido linker, which allows great flexibility to the CAI "tails" that may adopt a range of conformations and participate in different interactions within the enzyme active site [52,70]. Such different favorable/unfavorable contacts between the inhibitor tail and the enzyme active site lead to different inhibition profiles for the entire class of sulfonamides to which SLC-0111 belongs [52,70]. The different tail orientations allow the specific interactions between the inhibitor tail and amino acid residues at the entrance of the active site cavity, which is the most variable region in the various α-CA isoforms with medicinal chemistry applications, such as, for example, CA I, II, IX, and XII [19–21,138–142]. SLC-0111 binds selectively to hCA IX coordinating through the SO_2NH^- moiety (the deprotonated form of sulfonamide group) to the positively charged Zn(II) ion in the CA active site. In addition, a second strong contact with the Zn(II) ion involves oxygen of the sulfonamide. In contrast, these interactions are either weak or absent in the case of the hCA II isoform. The large difference in the electrostatic interactions accounts for the selectivity of the ligand to hCA IX. It was suggested that the potency of SLC-0111 against isoform IX is due to the hydrophobic contacts, whereas the selectivity is due to the electrostatic interactions, by means of computational approaches [143].

As a front-runner selective inhibitor for the tumor-associated isoform CA IX, and being currently in Phase Ib/II clinical trials, SLC-0111 has been utilized as a lead CAI for the development of novel promising small molecules with selective inhibitory activity toward CA IX, and with good druggability and lead-likeness characters. Several drug design approaches have been utilized to develop a range of new SLC-0111 analogs. The following subsection will present an overview of most of these drug design approaches.

2.3.1. Modification of the SLC-0111 Benzenesulfonamide Moiety

In 2012, Gieling et al. developed new sulfamate-based SLC-0111 analogs via replacement of the sulfamoyl zinc-binding group (ZBG) in SLC-0111 with the sulfamate functionality (Compound **8**, Figure 2). The reported sulfamate derivatives in this study displayed selective CA IX/XII inhibition, as well as inhibited migration and spreading of breast cancer MDA-MB-231 cells. One of these sulfamate-based CAIs efficiently inhibited the development of MDA-MB-231 metastases in the lung without signs of toxicity [144].

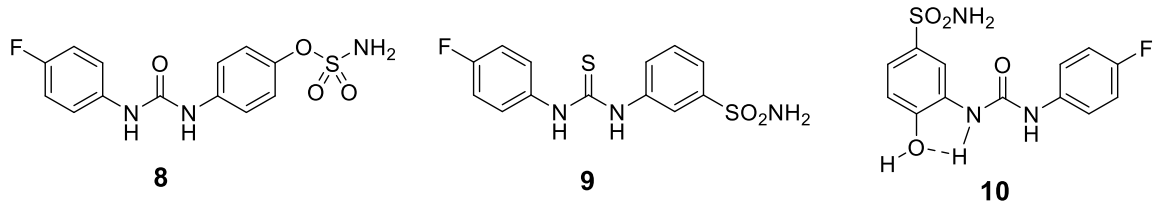


Figure 2. Chemical structures for SLC-0111 analogs (**8**–**10**) developed through modification of the SLC-0111 benzenesulfonamide moiety.

In another study, Carta et al. reported new SLC-0111 regioisomers (Compound **9**, Figure 2). The obtained results revealed that shifting of the sulfamoyl ZBG in SLC-0111 from the para- to meta-position elicited a decrease in the effectiveness toward hCA IX with significant improvement of the inhibitory action against hCA II, which was detrimental to the selectivity profile for this regioisomer [145].

On the other hand, Bozdag et al. reported the design and synthesis of new SLC-0111 congeners incorporating a 2-aminophenol-4-sulfonamide moiety to control the tail flexibility, (Compound **10**, Figure 2). The phenolic OH was able to establish an intra-molecular five-membered ring with the ureido NH group, which may provoke a C2-N′rotational restriction leading to a 20-fold enhanced hCA II/hCA IX selectivity ratio in comparison to SLC-0111 [146].

2.3.2. Modification of the SLC-0111 Ureido Linker

Akocak and co-workers adopted a bioisosteric replacement approach in order to replace the SLC-0111 ureido linker with a cyanoguanidine (Compound **11**, Figure 3) or 1,3-triazene (Compound **12**, Figure 3) linker. The developed *N*,*N*′-diaryl cyanoguanidines and 1,3-diaryltriazenes in both studies emerged as selective hCA II inhibitors [147–149].

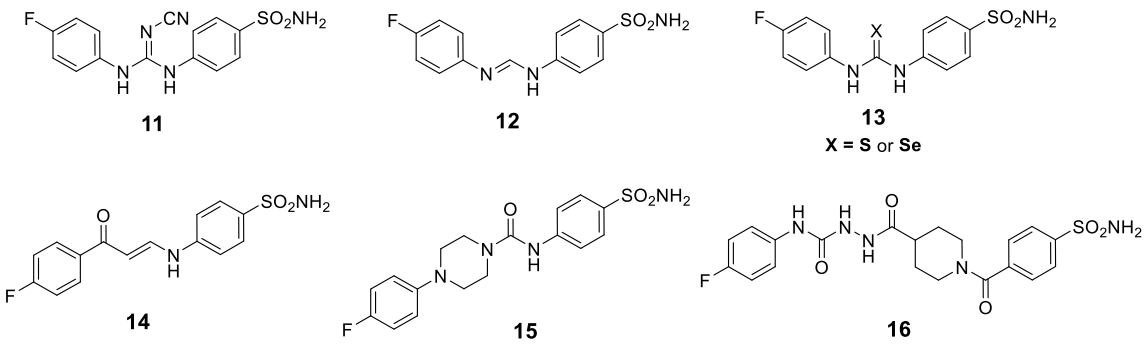


Figure 3. Chemical structures for SLC-0111 analogs (**11**–**16**) developed through modification of the SLC-0111 ureido linker.

The discovery of other SLC-0111 analogs was continued through two new studies that described the synthesis of novel sets of thioureido and selenoureido CAIs. In these studies, the SLC-0111 ureido oxygen was replaced with a sulfur or selenium atom (Compound **13**, Figure 3) [150,151]. The inhibition profile for both thioureido and selenoureido SLC-0111 congeners showed a loss of selectivity for the inhibition of the cancer-associated cytosolic *h*CA isoforms [150,151]. Moreover, Eldehna et al., in 2019, developed a series of 3/4-(3-aryl-3-oxopropenyl)aminobenzenesulfonamide derivatives as novel SLC-0111 enaminone analogs (Compound **14**, Figure 3). All the reported enaminones exhibited good selectivity toward hCA IX over hCA I and II. The structure-activity relationship (SAR) outcomes highlighted the significance of the incorporation of a bulkier aryl tail such as the 2-naphthyl ring [151].

In order to manipulate the flexibility of the SLC-0111 ureido linker, the urea linker outer nitrogen atom was incorporated into a piperazine ring to produce rigidified SLC-0111 analogs (Compound **15**, Figure 3). The rigid congeners displayed a reduction of CA IX/CA II selectivity, although some nanomolar CA IX inhibitors were obtained [152].

Furthermore, to better understand the importance of a rigid heterocyclic scaffold, the piperazine ring was substituted with piperidine (Compound **16**, Figure 3). In this compound series, a hydrazinocarbonyl-ureido moiety was introduced for the tail of the inhibitors. The NH group of the hydrazide moiety may provide a supplementary H-bond donator, which can better interact with the aminoacidic residues in the hydrophilic region of the active site. Depending on the substitution pattern at the piperidino ring, several hydrazidoureidobenzensulfonamides inhibited CA IX at low nanomolar concentrations [153].

2.3.3. Modification of the SLC-0111 Tail

The replacement of the 4-fluorophenyl tail with a 3-nitrophenyl group afforded low nanomolar CA IX/XII inhibitor with good selectivity for the transmembrane over the cytosolic isoforms (Compound **4**, Figure 1). The same SLC-0111 analog significantly inhibited the formation of metastases by the highly aggressive 4T1 mammary tumor cells [52]. Furthermore, novel SLC-0111 congeners were synthesized either via grafting different substituents within the phenyl tail, rather than *p*-fluoro (Compound **17**, Figure 4) or via replacement of the 4-fluorophenyl moiety with polycyclic tails (Compound **18**, Figure 4) [52]. On the other hand, replacement of the 4-fluorophenyl tail of SLC-0111 with 4-arylthiazole (Compound **19**, Figure 4) and 5-arylthiadiazole (Compound **20**, Figure 4) successfully improved the inhibitory activity toward hCA IX. Unfortunately, the most active thiadiazole analogs

showed a decrease of the hCA IX/II selectivity as compared to SLC-0111 [154]. Another recent study has utilized the bioisosteric replacement approach to design and synthesize new SLC-0111 analogs featuring 3-methylthiazolo[3,2-*a*]benzimidazole moiety as a tail that connected to the zinc-anchoring benzenesulfonamide moiety via a ureido linker (Compound **21**, Figure 4). Thereafter, the ureido linker was either elongated (Compound **22**, Figure 4) or replaced by an enaminone linker (Compound **23**, Figure 4) [155]. The results obtained from the stopped-flow CO_2 hydrase assay elucidated that three compounds possessed single-digit nanomolar CA IX inhibitory action with good selectivity profile toward hCA IX over hCA I and II. Moreover, these compounds exerted effective anti-proliferative and pro-apoptotic activities toward breast cancer MCF-7 and MDA-MB-231 cell lines. It is worth noting that the molecular docking analysis disclosed that thiazolo[3,2-*a*]benzimidazole moiety is a good bioisoster for the SLC-0111 phenyl tail due to its ability to establish many hydrophobic interactions within the hCA IX and XII active sites, as well as involving the sp^2 nitrogen of the tricyclic ring in hydrogen bonding. The fluorine atom from SLC-0111 was also replaced by metal-complexing polycyclic amines, which coordinate positron-emitting (PET) metal ions (e.g., ^{111}In and ^{90}Y) for PET imaging [156].

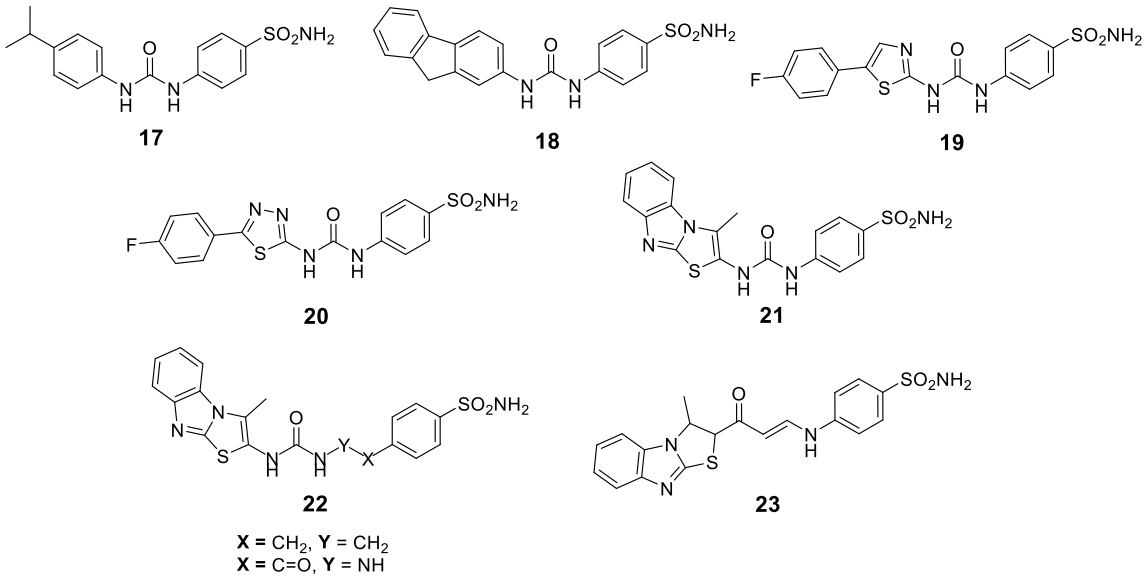


Figure 4. Chemical structures for SLC-0111 analogs (**17**–**23**) developed through modification of the SLC-0111 tail.

2.3.4. Development of SLC-0111 Hybrids

In 2016, Eldehna et al. has utilized a hybrid pharmacophore approach to merge the pharmacophoric elements of the isatin, a privileged scaffold in cancer drug discovery, and SLC-0111 in a single chemical framework to develop a new series of novel isatins-SLC-0111 hybrids (Compound **24**, Figure 5) [157]. Whilst most of the prepared hybrids exerted excellent inhibition for hCA XII in the sub- to low-nanomolar range, they weakly inhibited hCA IX. Thereafter, a structural extension approach has been exploited through *N*-alkylation and *N*-benzylation of the isatin moiety in order to enhance the hydrophobic interactions of the tail within the CA IX binding site, with the prime goal of improving the activity and selectivity toward the CA IX isoform (Compound **25**, Figure 5). As planned, an improvement of hCA IX inhibitory activity for the hybrids, in comparison to *N*-unsubstituted counterparts (Compound **24**), was achieved. Furthermore, one of the developed *N*-substituted isatin-SLC-0111 hybrids showed potent VEGFR-2 inhibitory activity and good anti-proliferative action toward breast cancer MDA-MB-231 and MCF-7 cell lines under hypoxic conditions. Furthermore, it disrupted the MDA-MB-231 cell cycle via alteration of the Sub-G_1 phase and arrest of G_2-M stage, as well as, it resulted in a significant increase in the percent of Annexin V positive apoptotic cells [158].

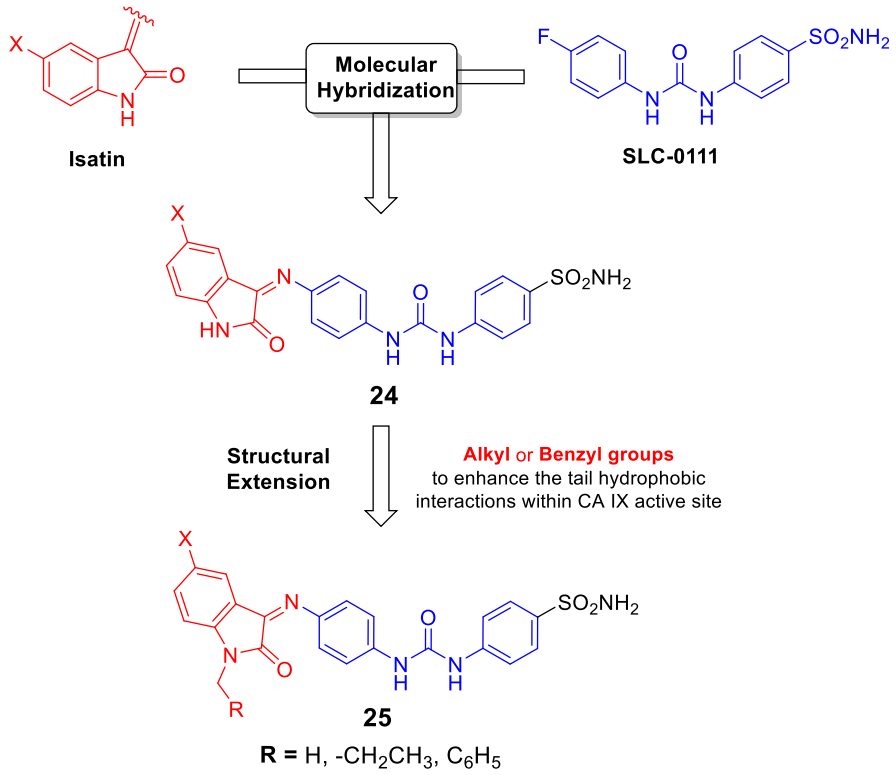


Figure 5. Design of the *N*-(un)substituted isatins-SLC-0111 hybrids **24** and **25** [157,158].

3. In Vivo Studies, Preclinical and Clinical Trials of CA IX/XII Inhibitors

Overall, the methodology used to determine the inhibitory constants for the various compounds determined in the papers discussed above, and validated by independent technologies (see above), represent bona fide, accurate data, which in no way compromise the development of CA IX and CA XII-specific inhibitors for validation in preclinical cancer models and in clinical trials, based on the vast biological literature demonstrating CA IX/CA XII as promising cancer therapeutic targets in hypoxic solid tumors.

The compounds from which SLC-0111, the lead clinical CA IX/CA XII inhibitor, was derived (**5** in Figure 1), were identified by the Supuran group [51,52]. These ureidobenzene sulfonamide compounds were then assessed for "druggability" criteria, including ADME (Absorption, Distribution, Metabolism, Excretion) analysis, from which SLC-0111 was selected for further in vitro and in vivo analysis in appropriate cancer models. The evaluation of CA IX/CA XII inhibitors as anticancer compounds takes a different path from the development of other cytotoxic anticancer drugs. This is because the targets, CA IX/CA XII are only expressed within the hypoxic niches of solid tumors and may represent a minor portion of the total tumor cell population. However, these hypoxic cells have the properties for self-renewal [159–161], migration/invasion [162–164], and survival in an acidic tumor microenvironment [163–168] and significantly contribute to resistance to chemo-, radiation, and immunotherapies. Thus, CA IX/CA XII inhibitors are not likely to have a major effect on tumor growth and metastasis by themselves as mono-therapeutics but need to be used in combination with chemo-, radiation-, and immunotherapies to eliminate resistant populations and for maximum durable suppression of tumor growth and metastasis.

Indeed, the extensive preclinical models carried out by several independent groups with the lead CA IX/XII inhibitors, including SLC-0111, have demonstrated that the use of such inhibitors in combination with chemotherapy agents [51,169–171], immunotherapy [172–174], and radiotherapy [173–176] is highly important and desirable for sustained therapeutic response. The extensive studies reported in these and other papers, utilizing multiple in-depth in vivo models,

provided solid positive preclinical data to warrant the initiation of Phase 1 clinical trials in 2014, of which a Phase 1 safety trial with SLC-0111 (as a monotherapeutic agent), has been completed [177] and a Phase 1b trial is currently underway to evaluate SLC-0111 in combination with gemcitabine in metastatic pancreatic cancer patients whose tumors are CA IX positive (ClinicalTrials.gov Identifier: NCT03450018).

A multitude of other combination therapy studies has been performed with CA IX inhibitors, including SLC-0111, in combination with proton pump inhibitors [178], antimetabolites [179], cisplatin [180], APE1-Ref-1 inhibitors [181], and histone deacetylase (HDAC) inhibitors [182]. All these studies showed a synergistic effect between the CAI and the second antitumor agent, as well as the lack of endothelial toxicity [183].

Other groups also used SLC-0111 in various biomedical studies in which selective inhibition of some CA isoforms was needed. These include the effects on prostate cancer cells of SLC-0111 alone or in combination with daunorubicin [184], radiobiological effects of CA IX inhibition in human breast cancer cells [185], microvascular endothelial cell pH regulation [186], glycolysis and migration suppression in pulmonary microvascular endothelial cells [187], and the involvement of CA isoforms in mitochondrial biogenesis and in the control of lactate production in human Sertoli cells [188]. All these studies confirm the usefulness of this clinical candidate in tumors and other biomedical conditions.

4. Conclusions

The tumor microenvironment is critical in cancer cell growth and can substantially influence the outcome of anticancer interventions. Transcription factors, such as HIF-1/2, can activate a number of key genes including those involved in tumor pH regulation. The human carbonic anhydrase isoforms CA IX and XII have active roles in regulating the extracellular pH in cancers including in advanced solid metastatic tumors. As a result of careful preclinical studies involving X-ray crystallography [189] and the screening of tens of thousands of potential inhibitors [190–193] by use of a functional kinetic enzyme inhibition assay based on stopped-flow kinetic measurements, key lead compounds for CA IX and XII, including the sulfonamide CA inhibitor SLC-0111, have been discovered, developed, and validated. The results from the stopped-flow kinetic enzyme inhibition assay are in excellent agreement with: (i) orthogonal native mass spectrometry measurements that can be used to directly measure protein–ligand interactions and (ii) kinetic inhibition measurements that do not involve a stopped-flow apparatus. Thus, the stopped-flow kinetic method has been used to screen tens of thousands of potential CA inhibitors with sufficiently high accuracy for drug discovery and development applications. Based on the vast literature demonstrating the development and validation of specific CA IX and CA XII inhibitors, including in preclinical cancer models and in clinical trials, these two enzymes are promising cancer therapeutic targets in advanced, hypoxic solid tumors. We anticipate that the use of selective CA IX/XII inhibitors, such as SLC-0111, will be beneficial in combination with chemo-, radiation-, and immunotherapies to eliminate resistant cancer cell populations and for maximum durable suppression of tumor growth and metastasis.

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References

1. Chafe, S.C.; McDonald, P.C.; Dedhar, S. pH regulators of the tumor microenvironment. A general overview. In *pH-Interfering Agents as Chemosensitizers in Cancer Therapy*; Supuran, C.T., Carradori, S., Eds.; Elsevier: London, UK, 2020; pp. 13–33.
2. Chiarugi, P.; Ippolito, L. Tumors and their microenvironment. In *pH-Interfering Agents as Chemosensitizers in Cancer Therapy*; Supuran, C.T., Carradori, S., Eds.; Elsevier: London, UK, 2020; pp. 3–11.
3. Warburg, O. On the origin of cancer cells. *Science* **1956**, *123*, 309–314. [CrossRef] [PubMed]
4. Schwartz, L.; Supuran, C.T.; Alfarouk, K.O. The Warburg effect and the hallmarks of cancer. *Anti-Cancer Agents Med. Chem.* **2017**, *17*, 164–170. [CrossRef] [PubMed]
5. Kaelin, W.G., Jr. The VHL Tumor Suppressor Gene: Insights into Oxygen Sensing and Cancer. *Trans. Am. Clin. Climatol. Assoc.* **2017**, *128*, 298–307. [PubMed]
6. Pugh, C.W.; Ratcliffe, P.J. New horizons in hypoxia signaling pathways. *Exp. Cell Res.* **2017**, *356*, 116–121. [CrossRef] [PubMed]
7. Semenza, G.L. Pharmacologic Targeting of Hypoxia-Inducible Factors. *Annu. Rev. Pharmacol. Toxicol.* **2019**, *59*, 379–403. [CrossRef] [PubMed]
8. Schito, L.; Semenza, G.L. Hypoxia-Inducible Factors: Master Regulators of Cancer Progression. *Trends Cancer* **2016**, *2*, 758–770. [CrossRef]
9. Wykoff, C.C.; Beasley, N.J.; Watson, P.H.; Turner, K.J.; Pastorek, J.; Sibtain, A.; Wilson, G.D.; Turley, H.; Talks, K.L.; Maxwell, P.H.; et al. Hypoxia-inducible expression of tumor-associated carbonic anhydrases. *Cancer Res.* **2000**, *60*, 7075–7083.
10. Wykoff, C.C.; Beasley, N.; Watson, P.H.; Campo, L.; Chia, S.K.; English, R.; Pastorek, J.; Sly, W.S.; Ratcliffe, P.; Harris, A.L. Expression of the hypoxia-inducible and tumor-associated carbonic anhydrases in ductal carcinoma in situ of the breast. *Am. J. Pathol.* **2001**, *158*, 1011–1019. [CrossRef]
11. Beasley, N.J.; Wykoff, C.C.; Watson, P.H.; Leek, R.; Turley, H.; Gatter, K.; Pastorek, J.; Cox, G.J.; Ratcliffe, P.; Harris, A.L. Carbonic anhydrase IX, an endogenous hypoxia marker, expression in head and neck squamous cell carcinoma and its relationship to hypoxia, necrosis, and microvessel density. *Cancer Res.* **2001**, *61*, 5262–5267.
12. Loncaster, J.A.; Harris, A.L.; Davidson, S.E.; Logue, J.P.; Hunter, R.D.; Wycoff, C.C.; Pastorek, J.; Ratcliffe, P.J.; Stratford, I.J.; West, C.M. Carbonic anhydrase (CA IX) expression, a potential new intrinsic marker of hypoxia: Correlations with tumor oxygen measurements and prognosis in locally advanced carcinoma of the cervix. *Cancer Res.* **2001**, *61*, 6394–6399.
13. Giatromanolaki, A.; Koukourakis, M.I.; Sivridis, E.; Pastorek, J.; Wykoff, C.C.; Gatter, K.C.; Harris, A.L. Expression of hypoxia-inducible carbonic anhydrase-9 relates to angiogenic pathways and independently to poor outcome in non-small cell lung cancer. *Cancer Res.* **2001**, *61*, 7992–7998.
14. Turner, K.J.; Crew, J.P.; Wykoff, C.C.; Watson, P.H.; Poulsom, R.; Pastorek, J.; Ratcliffe, P.J.; Cranston, D.; Harris, A.L. The hypoxia-inducible genes VEGF and CA9 are differentially regulated in superficial vs invasive bladder cancer. *Br. J. Cancer* **2002**, *86*, 1276–1282. [CrossRef]
15. Mandriota, S.J.; Turner, K.J.; Davies, D.R.; Murray, P.G.; Morgan, N.V.; Sowter, H.M.; Wykoff, C.C.; Maher, E.R.; Harris, A.L.; Ratcliffe, P.J.; et al. HIF activation identifies early lesions in VHL kidneys: Evidence for site-specific tumor suppressor function in the nephron. *Cancer Cell* **2002**, *1*, 459–468. [CrossRef]
16. Stessels, F.; Van den Eynden, G.; Van der Auwera, I.; Salgado, S.; Van den Heuvel, E.; Harris, A.L.; Jackson, D.G.; Colpaert, C.G.; van Marck, E.A.; Dirix, L.Y.; et al. Breast adenocarcinoma liver metastases, in contrast to colorectal cancer liver metastases, display a non-angiogenic growth pattern that preserves the stroma and lacks hypoxia. *Br. J. Cancer* **2004**, *90*, 1429–1436. [CrossRef] [PubMed]
17. Chia, S.K.; Wykoff, C.C.; Watson, P.H.; Han, C.; Leek, R.D.; Pastorek, J.; Gatter, K.C.; Ratcliffe, P.; Harris, A.L. Prognostic significance of a novel hypoxia-regulated marker, carbonic anhydrase IX, in invasive breast carcinoma. *J. Clin. Oncol.* **2001**, *19*, 3660–3668. [CrossRef] [PubMed]
18. Pouysségur, J.; Dayan, F.; Mazure, N.M. Hypoxia signalling in cancer and approaches to enforce tumour regression. *Nature* **2006**, *441*, 437–443. [CrossRef] [PubMed]
19. Neri, D.; Supuran, C.T. Interfering with pH regulation in tumours as a therapeutic strategy. *Nat. Rev. Drug Discov.* **2011**, *10*, 767–777. [CrossRef] [PubMed]

20. Supuran, C.T. Carbonic anhydrases: Novel therapeutic applications for inhibitors and activators. *Nat. Rev. Drug Discov.* **2008**, *7*, 168–181. [CrossRef]
21. Alterio, V.; Di Fiore, A.; D'Ambrosio, K.; Supuran, C.T.; De Simone, G. Multiple binding modes of inhibitors to carbonic anhydrases: How to design specific drugs targeting 15 different isoforms? *Chem. Rev.* **2012**, *112*, 4421–4468. [CrossRef]
22. Supuran, C.T. Structure and function of carbonic anhydrases. *Biochem. J.* **2016**, *473*, 2023–2032. [CrossRef]
23. Supuran, C.T. Carbonic Anhydrases and Metabolism. *Metabolites* **2018**, *8*, 25. [CrossRef]
24. Supuran, C.T. Carbonic Anhydrase Inhibition and the Management of Hypoxic Tumors. *Metabolites* **2017**, *7*, 48. [CrossRef]
25. Alterio, V.; Hilvo, M.; Di Fiore, A.; Supuran, C.T.; Pan, P.; Parkkila, S.; Scaloni, A.; Pastorek, J.; Pastorekova, S.; Pedone, C.; et al. Crystal structure of the catalytic domain of the tumor-associated human carbonic anhydrase IX. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 16233–16238. [CrossRef] [PubMed]
26. Whittington, D.A.; Waheed, A.; Ulmasov, B.; Shah, G.N.; Grubb, J.H.; Sly, W.S.; Christianson, D.W. Crystal structure of the dimeric extracellular domain of human carbonic anhydrase XII, a bitopic membrane protein overexpressed in certain cancer tumor cells. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 9545–9550. [CrossRef]
27. Di Fiore, A.; Truppo, E.; Supuran, C.T.; Alterio, V.; Dathan, N.; Bootorabi, F.; Parkkila, S.; Monti, S.M.; De Simone, G. Crystal structure of the C183S/C217S mutant of human CA VII in complex with acetazolamide. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5023–5026. [CrossRef] [PubMed]
28. Di Fiore, A.; Monti, S.M.; Hilvo, M.; Parkkila, S.; Romano, V.; Scaloni, A.; Pedone, C.; Scozzafava, A.; Supuran, C.T.; De Simone, G. Crystal structure of human carbonic anhydrase XIII and its complex with the inhibitor acetazolamide. *Proteins* **2009**, *74*, 164–175. [CrossRef]
29. Alterio, V.; Pan, P.; Parkkila, S.; Buonanno, M.; Supuran, C.T.; Monti, S.M.; De Simone, G. The structural comparison between membrane-associated human carbonic anhydrases provides insights into drug design of selective inhibitors. *Biopolymers* **2014**, *101*, 769–778. [CrossRef]
30. Pilka, E.S.; Kochan, G.; Oppermann, U.; Yue, W.W. Crystal structure of the secretory isozyme of mammalian carbonic anhydrases CA VI: Implications for biological assembly and inhibitor development. *Biochem. Biophys. Res. Commun.* **2012**, *419*, 485–489. [CrossRef]
31. Supuran, C.T. Advances in structure-based drug discovery of carbonic anhydrase inhibitors. *Expert Opin. Drug Discov.* **2017**, *12*, 61–88. [CrossRef]
32. Carta, F.; Supuran, C.T. Diuretics with carbonic anhydrase inhibitory action: A patent and literature review (2005–2013). *Expert Opin. Ther. Pat.* **2013**, *23*, 681–691. [CrossRef]
33. Nocentini, A.; Supuran, C.T. Advances in the structural annotation of human carbonic anhydrases and impact on future drug discovery. *Expert Opin. Drug Discov.* **2019**, *14*, 1175–1197. [CrossRef] [PubMed]
34. Supuran, C.T. Carbonic anhydrase inhibitors and their potential in a range of therapeutic areas. *Expert Opin. Ther. Pat.* **2018**, *28*, 709–712. [CrossRef] [PubMed]
35. Scozzafava, A.; Supuran, C.T.; Carta, F. Antiobesity carbonic anhydrase inhibitors: A literature and patent review. *Expert Opin. Ther. Pat.* **2013**, *23*, 725–735. [CrossRef] [PubMed]
36. Supuran, C.T. Applications of carbonic anhydrases inhibitors in renal and central nervous system diseases. *Expert Opin. Ther. Pat.* **2018**, *28*, 713–721. [CrossRef]
37. Supuran, C.T.; Altamimi, A.S.A.; Carta, F. Carbonic anhydrase inhibition and the management of glaucoma: A literature and patent review 2013–2019. *Expert Opin. Ther. Pat.* **2019**, *29*, 781–792. [CrossRef]
38. Supuran, C.T. The management of glaucoma and macular degeneration. *Expert Opin. Ther. Pat.* **2019**, *29*, 745–747. [CrossRef]
39. Supuran, C.T. How many carbonic anhydrase inhibition mechanisms exist? *J. Enzym. Inhib. Med. Chem.* **2016**, *31*, 345–360. [CrossRef]
40. Di Cesare Mannelli, L.; Micheli, L.; Carta, F.; Cozzi, A.; Ghelardini, C.; Supuran, C.T. Carbonic anhydrase inhibition for the management of cerebral ischemia: In vivo evaluation of sulfonamide and coumarin inhibitors. *J. Enzym. Inhib. Med. Chem.* **2016**, *31*, 894–899. [CrossRef]
41. Carta, F.; Di Cesare Mannelli, L.; Pinard, M.; Ghelardini, C.; Scozzafava, A.; McKenna, R.; Supuran, C.T. A class of sulfonamide carbonic anhydrase inhibitors with neuropathic pain modulating effects. *Bioorg. Med. Chem.* **2015**, *23*, 1828–1840. [CrossRef]
42. Supuran, C.T. Carbonic anhydrase inhibition and the management of neuropathic pain. *Expert Rev. Neurother.* **2016**, *16*, 961–968. [CrossRef]

43. Margheri, F.; Ceruso, M.; Carta, F.; Laurenzana, A.; Maggi, L.; Lazzeri, S.; Simonini, G.; Annunziato, F.; Del Rosso, M.; Supuran, C.T.; et al. Overexpression of the transmembrane carbonic anhydrase isoforms IX and XII in the inflamed synovium. *J. Enzym. Inhib. Med. Chem.* **2016**, *31*, 60–63. [CrossRef] [PubMed]
44. Bua, S.; Di Cesare Mannelli, L.; Vullo, D.; Ghelardini, C.; Bartolucci, G.; Scozzafava, A.; Supuran, C.T.; Carta, F. Design and Synthesis of Novel Nonsteroidal Anti-Inflammatory Drugs and Carbonic Anhydrase Inhibitors Hybrids (NSAIDs-CAIs) for the Treatment of Rheumatoid Arthritis. *J. Med. Chem.* **2017**, *60*, 1159–1170. [CrossRef] [PubMed]
45. Pastorek, J.; Pastoreková, S.; Callebaut, I.; Mornon, J.P.; Zelník, V.; Opavský, R.; Zatovicová, M.; Liao, S.; Portetelle, D.; Stanbridge, E.J. Cloning and characterization of MN, a human tumor-associated protein with a domain homologous to carbonic anhydrase and a putative helix-loop-helix DNA binding segment. *Oncogene* **1994**, *9*, 2877–2888.
46. Türeci, O.; Sahin, U.; Vollmar, E.; Siemer, S.; Göttert, E.; Seitz, G.; Parkkila, A.K.; Shah, G.N.; Grubb, J.H.; Pfreundschuh, M.; et al. Human carbonic anhydrase XII: cDNA cloning, expression, and chromosomal localization of a carbonic anhydrase gene that is overexpressed in some renal cell cancers. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 7608–7613. [CrossRef]
47. Svastová, E.; Hulíková, A.; Rafajová, M.; Zatovicová, M.; Gibadulinová, A.; Casini, A.; Cecchi, A.; Scozzafava, A.; Supuran, C.T.; Pastorek, J.; et al. Hypoxia activates the capacity of tumor-associated carbonic anhydrase IX to acidify extracellular pH. *FEBS Lett.* **2004**, *577*, 439–445. [CrossRef]
48. Cecchi, A.; Hulikova, A.; Pastorek, J.; Pastoreková, S.; Scozzafava, A.; Winum, J.Y.; Montero, J.L.; Supuran, C.T. Carbonic anhydrase inhibitors. Design of fluorescent sulfonamides as probes of tumor-associated carbonic anhydrase IX that inhibit isozyme IX-mediated acidification of hypoxic tumors. *J. Med. Chem.* **2005**, *48*, 4834–4841. [CrossRef] [PubMed]
49. Swietach, P.; Wigfield, S.; Cobden, P.; Supuran, C.T.; Harris, A.L.; Vaughan-Jones, R.D. Tumor-associated carbonic anhydrase 9 spatially coordinates intracellular pH in three-dimensional multicellular growths. *J. Biol. Chem.* **2008**, *283*, 20473–20483. [CrossRef]
50. Swietach, P.; Patiar, S.; Supuran, C.T.; Harris, A.L.; Vaughan-Jones, R.D. The role of carbonic anhydrase 9 in regulating extracellular and intracellular pH in three-dimensional tumor cell growths. *J. Biol. Chem.* **2009**, *284*, 20299–20310. [CrossRef] [PubMed]
51. Lou, Y.; McDonald, P.C.; Oloumi, A.; Chia, S.; Ostlund, C.; Ahmadi, A.; Kyle, A.; Auf dem Keller, U.; Leung, S.; Huntsman, D.; et al. Targeting tumor hypoxia: Suppression of breast tumor growth and metastasis by novel carbonic anhydrase IX inhibitors. *Cancer Res.* **2011**, *71*, 3364–3376. [CrossRef]
52. Pacchiano, F.; Carta, F.; McDonald, P.C.; Lou, Y.; Vullo, D.; Scozzafava, A.; Dedhar, S.; Supuran, C.T. Ureido-substituted benzenesulfonamides potently inhibit carbonic anhydrase IX and show antimetastatic activity in a model of breast cancer metastasis. *J. Med. Chem.* **2011**, *54*, 1896–1902. [CrossRef]
53. Touisni, N.; Maresca, A.; McDonald, P.C.; Lou, Y.; Scozzafava, A.; Dedhar, S.; Winum, J.Y.; Supuran, C.T. Glycosyl coumarin carbonic anhydrase IX and XII inhibitors strongly attenuate the growth of primary breast tumors. *J. Med. Chem.* **2011**, *54*, 8271–8277. [CrossRef] [PubMed]
54. Supuran, C.T. Acetazolamide for the treatment of idiopathic intracranial hypertension. *Expert Rev. Neurother.* **2015**, *15*, 851–856. [CrossRef] [PubMed]
55. Supuran, C.T. Drug interaction considerations in the therapeutic use of carbonic anhydrase inhibitors. *Expert Opin. Drug Metab. Toxicol.* **2016**, *12*, 423–431. [CrossRef] [PubMed]
56. Carta, F.; Scozzafava, A.; Supuran, C.T. Sulfonamides (RSO2NH2): A patent review 2008–2012. *Expert Opin. Ther. Pat.* **2012**, *22*, 747–758. [CrossRef] [PubMed]
57. Scozzafava, A.; Carta, F.; Supuran, C.T. Secondary and tertiary sulfonamides: A patent review (2008–2012). *Expert Opin. Ther. Pat.* **2013**, *23*, 203–213. [CrossRef] [PubMed]
58. Monti, S.M.; Supuran, C.T.; De Simone, G. Anticancer carbonic anhydrase inhibitors: A patent review (2008–2013). *Expert Opin. Ther. Pat.* **2013**, *23*, 737–749. [CrossRef] [PubMed]
59. Eriksson, A.E.; Kylsten, P.M.; Jones, T.A.; Liljas, A. Crystallographic studies of inhibitor binding sites in human carbonic anhydrase II: A pentacoordinated binding of the SCN-ion to the zinc at high pH. *Proteins* **1988**, *4*, 283–293. [CrossRef]

60. Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C.T. Carbonic anhydrase inhibitors. Synthesis of water-soluble, topically effective, intraocular pressure-lowering aromatic/heterocyclic sulfonamides containing cationic or anionic moieties: Is the tail more important than the ring? *J. Med. Chem.* **1999**, *42*, 2641–2650. [CrossRef]
61. Supuran, C.T. Exploring the multiple binding modes of inhibitors to carbonic anhydrases for novel drug discovery. *Expert Opin. Drug Discov.* **2020**, *15*, 671–686. [CrossRef]
62. D'Ascenzio, M.; Secci, D.; Carradori, S.; Zara, S.; Guglielmi, P.; Cirilli, R.; Pierini, M.; Poli, G.; Tuccinardi, T.; Angeli, A.; et al. 1,3-Dipolar cycloaddition, HPLC enantioseparation, and docking studies of saccharin/isoxazole and saccharin/isoxazoline derivatives as selective carbonic anhydrase IX and XII inhibitors. *J. Med. Chem.* **2020**, *63*, 2470–2488. [CrossRef]
63. Güzel, O.; Temperini, C.; Innocenti, A.; Scozzafava, A.; Salman, A.; Supuran, C.T. Carbonic anhydrase inhibitors. Interaction of 2-(hydrazinocarbonyl)-3-phenyl-1H-indole-5-sulfonamide with 12 mammalian isoforms: Kinetic and X-ray crystallographic studies. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 152–158. [CrossRef] [PubMed]
64. Biswas, S.; Aggarwal, M.; Güzel, Ö.; Scozzafava, A.; McKenna, R.; Supuran, C.T. Conformational variability of different sulfonamide inhibitors with thienyl-acetamido moieties attributes to differential binding in the active site of cytosolic human carbonic anhydrase isoforms. *Bioorg. Med. Chem.* **2011**, *19*, 3732–3738. [CrossRef] [PubMed]
65. Wagner, J.; Avvaru, B.S.; Robbins, A.H.; Scozzafava, A.; Supuran, C.T.; McKenna, R. Coumarinyl-substituted sulfonamides strongly inhibit several human carbonic anhydrase isoforms: Solution and crystallographic investigations. *Bioorg. Med. Chem.* **2010**, *18*, 4873–4878. [CrossRef] [PubMed]
66. Avvaru, B.S.; Wagner, J.M.; Maresca, A.; Scozzafava, A.; Robbins, A.H.; Supuran, C.T.; McKenna, R. Carbonic anhydrase inhibitors. The X-ray crystal structure of human isoform II in adduct with an adamantyl analogue of acetazolamide resides in a less utilized binding pocket than most hydrophobic inhibitors. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4376–4381. [CrossRef] [PubMed]
67. Carta, F.; Garaj, V.; Maresca, A.; Wagner, J.; Avvaru, B.S.; Robbins, A.H.; Scozzafava, A.; McKenna, R.; Supuran, C.T. Sulfonamides incorporating 1,3,5-triazine moieties selectively and potently inhibit carbonic anhydrase transmembrane isoforms IX, XII and XIV over cytosolic isoforms I and II: Solution and X-ray crystallographic studies. *Bioorg. Med. Chem.* **2011**, *19*, 3105–3119. [CrossRef] [PubMed]
68. Carta, F.; Birkmann, A.; Pfaff, T.; Buschmann, H.; Schwab, W.; Zimmermann, H.; Maresca, A.; Supuran, C.T. Lead Development of Thiazolylsulfonamides with Carbonic Anhydrase Inhibitory Action. *J. Med. Chem.* **2017**, *60*, 3154–3164. [CrossRef] [PubMed]
69. Alterio, V.; Vitale, R.M.; Monti, S.M.; Pedone, C.; Scozzafava, A.; Cecchi, A.; De Simone, G.; Supuran, C.T. Carbonic anhydrase inhibitors: X-ray and molecular modeling study for the interaction of a fluorescent antitumor sulfonamide with isozyme II and IX. *J. Am. Chem. Soc.* **2006**, *128*, 8329–8335. [CrossRef]
70. Pacchiano, F.; Aggarwal, M.; Avvaru, B.S.; Robbins, A.H.; Scozzafava, A.; McKenna, R.; Supuran, C.T. Selective hydrophobic pocket binding observed within the carbonic anhydrase II active site accommodate different 4-substituted-ureido-benzenesulfonamides and correlate to inhibitor potency. *Chem. Commun.* **2010**, *46*, 8371–8373. [CrossRef]
71. Mboge, M.Y.; Mahon, B.P.; Lamas, N.; Socorro, L.; Carta, F.; Supuran, C.T.; Frost, S.C.; McKenna, R. Structure activity study of carbonic anhydrase IX: Selective inhibition with ureido-substituted benzenesulfonamides. *Eur. J. Med. Chem.* **2017**, *132*, 184–191. [CrossRef]
72. Ahlskog, J.K.; Dumelin, C.E.; Trüssel, S.; Mårlind, J.; Neri, D. In vivo targeting of tumor-associated carbonic anhydrases using acetazolamide derivatives. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4851–4856. [CrossRef]
73. Buller, F.; Steiner, M.; Frey, K.; Mircsof, D.; Scheuermann, J.; Kalisch, M.; Buhlmann, P.; Supuran, C.T.; Neri, D. Selection of Carbonic Anhydrase IX Inhibitors from One Million DNA-Encoded Compounds. *ACS Chem. Biol.* **2011**, *6*, 336–344. [CrossRef] [PubMed]
74. Krall, N.; Pretto, F.; Mattarella, M.; Müller, C.; Neri, D. A 99mTc-Labeled Ligand of Carbonic Anhydrase IX Selectively Targets Renal Cell Carcinoma in vivo. *J. Nucl. Med.* **2016**, *57*, 943–949. [CrossRef] [PubMed]
75. Krall, N.; Pretto, F.; Decurtins, W.; Bernardes, G.J.L.; Supuran, C.T.; Neri, D. A small-molecule drug conjugate for the treatment of carbonic anhydrase IX expressing tumors. *Angew. Chem. Int. Ed.* **2014**, *53*, 4231–4235. [CrossRef] [PubMed]

76. Favalli, N.; Biendl, S.; Hartmann, M.; Piazzi, J.; Sladojevich, F.; Gräslund, S.; Brown, P.J.; Näreoja, K.; Schüler, H.; Scheuermann, J.; et al. A DNA-encoded library of chemical compounds based on common scaffolding structures reveals the impact of ligand geometry on protein recognition. *ChemMedChem* **2018**, *13*, 1303–1307. [CrossRef]
77. Scheuermann, J.; Neri, D. Dual-pharmacophore DNA-encoded chemical libraries. *Curr. Opin. Chem. Biol.* **2015**, *26*, 99–103. [CrossRef]
78. Ebbesen, P.; Pettersen, E.O.; Gorr, T.A.; Jobst, G.; Williams, K.; Kieninger, J.; Wenger, R.H.; Pastorekova, S.; Dubois, L.; Lambin, P.; et al. Taking advantage of tumor cell adaptations to hypoxia for developing new tumor markers and treatment strategies. *J. Enzym. Inhib. Med. Chem.* **2009**, *24*, 1–39. [CrossRef]
79. Pettersen, E.O.; Ebbesen, P.; Gieling, R.G.; Williams, K.J.; Dubois, L.; Lambin, P.; Ward, C.; Meehan, J.; Kunkler, I.H.; Langdon, S.P.; et al. Targeting tumour hypoxia to prevent cancer metastasis. From biology, biosensing and technology to drug development: The METOXIA consortium. *J. Enzym. Inhib. Med. Chem.* **2015**, *30*, 689–721. [CrossRef]
80. Supuran, C.T.; Alterio, V.; Di Fiore, A.; D'Ambrosio, K.; Carta, F.; Monti, S.M.; De Simone, G. Inhibition of carbonic anhydrase IX targets primary tumors, metastases, and cancer stem cells: Three for the price of one. *Med. Res. Rev.* **2018**, *38*, 1799–1836. [CrossRef]
81. McDonald, P.C.; Winum, J.Y.; Supuran, C.T.; Dedhar, S. Recent developments in targeting carbonic anhydrase IX for cancer therapeutics. *Oncotarget* **2012**, *3*, 84–97. [CrossRef]
82. Maresca, A.; Temperini, C.; Vu, H.; Pham, N.B.; Poulsen, S.A.; Scozzafava, A.; Quinn, R.J.; Supuran, C.T. Non-zinc mediated inhibition of carbonic anhydrases: Coumarins are a new class of suicide inhibitors. *J. Am. Chem. Soc.* **2009**, *131*, 3057–3062. [CrossRef]
83. Maresca, A.; Temperini, C.; Pochet, L.; Masereel, B.; Scozzafava, A.; Supuran, C.T. Deciphering the mechanism of carbonic anhydrase inhibition with coumarins and thiocoumarins. *J. Med. Chem.* **2010**, *53*, 335–344. [CrossRef] [PubMed]
84. Temperini, C.; Innocenti, A.; Scozzafava, A.; Parkkila, S.; Supuran, C.T. The coumarin-binding site in carbonic anhydrase accommodates structurally diverse inhibitors: The antiepileptic lacosamide as an example. *J. Med. Chem.* **2010**, *53*, 850–854. [CrossRef] [PubMed]
85. Supuran, C.T. Coumarin carbonic anhydrase inhibitors from natural sources. *J. Enzym. Inhib. Med. Chem.* **2020**, *35*, 1462–1470. [CrossRef] [PubMed]
86. Tars, K.; Vullo, D.; Kazaks, A.; Leitans, J.; Lends, A.; Grandane, A.; Zalubovskis, R.; Scozzafava, A.; Supuran, C.T. Sulfocoumarins (1,2-benzoxathiine-2,2-dioxides): A class of potent and isoform-selective inhibitors of tumor-associated carbonic anhydrases. *J. Med. Chem.* **2013**, *56*, 293–300. [CrossRef]
87. Grandane, A.; Tanc, M.; Žalubovskis, R.; Supuran, C.T. Synthesis of 6-aryl-substituted sulfocoumarins and investigation of their carbonic anhydrase inhibitory action. *Bioorg. Med. Chem.* **2015**, *23*, 1430–1436. [CrossRef]
88. Pustenko, A.; Stepanovs, D.; Žalubovskis, R.; Vullo, D.; Kazaks, A.; Leitans, J.; Tars, K.; Supuran, C.T. 3H-1,2-benzoxathiepine 2,2-dioxides: A new class of isoform-selective carbonic anhydrase inhibitors. *J. Enzym. Inhib. Med. Chem.* **2017**, *32*, 767–775. [CrossRef]
89. Grandane, A.; Nocentini, A.; Werner, T.; Zalubovskis, R.; Supuran, C.T. Benzoxepinones: A new isoform-selective class of tumor associated carbonic anhydrase inhibitors. *Bioorg. Med. Chem.* **2020**, *28*, 115496. [CrossRef]
90. Ferraroni, M.; Carta, F.; Scozzafava, A.; Supuran, C.T. Thioxocoumarins show an alternative carbonic anhydrase inhibition mechanism compared to coumarins. *J. Med. Chem.* **2016**, *59*, 462–473. [CrossRef]
91. Supuran, C.T. Carbon- versus sulphur-based zinc binding groups for carbonic anhydrase inhibitors? *J. Enzym. Inhib. Med. Chem.* **2018**, *33*, 485–495. [CrossRef]
92. Nocentini, A.; Gratteri, P.; Supuran, C.T. Phosphorus versus sulfur: Discovery of benzenephosphonamidates as versatile sulfonamide-mimic chemotypes acting as carbonic anhydrase inhibitors. *Chemistry* **2019**, *25*, 1188–1192. [CrossRef]
93. Carta, F.; Aggarwal, M.; Maresca, A.; Scozzafava, A.; McKenna, R.; Supuran, C.T. Dithiocarbamates: A new class of carbonic anhydrase inhibitors. Crystallographic and kinetic investigations. *Chem. Commun.* **2012**, *48*, 1868–1870. [CrossRef] [PubMed]
94. Vullo, D.; Durante, M.; Di Leva, F.S.; Cosconati, S.; Masini, E.; Scozzafava, A.; Novellino, E.; Supuran, C.T.; Carta, F. Monothiocarbamates strongly inhibit carbonic anhydrases in vitro and possess intraocular pressure lowering activity in an animal model of glaucoma. *J. Med. Chem.* **2016**, *59*, 5857–5867. [CrossRef] [PubMed]

95. Carta, F.; Akdemir, A.; Scozzafava, A.; Masini, E.; Supuran, C.T. Xanthates and trithiocarbonates strongly inhibit carbonic anhydrases and show antiglaucoma effects in vivo. *J. Med. Chem.* **2013**, *56*, 4691–4700. [CrossRef] [PubMed]
96. Angeli, A.; Tanini, D.; Nocentini, A.; Capperucci, A.; Ferraroni, M.; Gratteri, P.; Supuran, C.T. Selenols: A new class of carbonic anhydrase inhibitors. *Chem. Commun.* **2019**, *55*, 648–651. [CrossRef]
97. Tanini, D.; Capperucci, A.; Ferraroni, M.; Carta, F.; Angeli, A.; Supuran, C.T. Direct and straightforward access to substituted alkyl selenols as novel carbonic anhydrase inhibitors. *Eur. J. Med. Chem.* **2020**, *185*, 111811. [CrossRef]
98. Langella, E.; D'Ambrosio, K.; D'Ascenzio, M.; Carradori, S.; Monti, S.M.; Supuran, C.T.; De Simone, G. A Combined crystallographic and theoretical study explains the capability of carboxylic acids to adopt multiple binding modes in the active site of carbonic anhydrases. *Chemistry* **2016**, *22*, 97–100. [CrossRef]
99. Di Fiore, A.; Maresca, A.; Supuran, C.T.; De Simone, G. Hydroxamate represents a versatile zinc binding group for the development of new carbonic anhydrase inhibitors. *Chem. Commun.* **2012**, *48*, 8838–8840. [CrossRef]
100. Alterio, V.; Cadoni, R.; Esposito, D.; Vullo, D.; Fiore, A.D.; Monti, S.M.; Caporale, A.; Ruvo, M.; Sechi, M.; Dumy, P.; et al. Benzoxaborole as a new chemotype for carbonic anhydrase inhibition. *Chem. Commun.* **2016**, *52*, 11983–11986. [CrossRef]
101. Langella, E.; Alterio, V.; D'Ambrosio, K.; Cadoni, R.; Winum, J.Y.; Supuran, C.T.; Monti, S.M.; De Simone, G.; Di Fiore, A. Exploring benzoxaborole derivatives as carbonic anhydrase inhibitors: A structural and computational analysis reveals their conformational variability as a tool to increase Enzyme selectivity. *J. Enzym. Inhib. Med. Chem.* **2019**, *34*, 1498–1505.
102. Nocentini, A.; Supuran, C.T.; Winum, J.Y. Benzoxaborole compounds for therapeutic uses: A patent review (2010–2018). *Expert Opin. Ther. Pat.* **2018**, *28*, 493–504. [CrossRef]
103. De Simone, G.; Angeli, A.; Bozdag, M.; Supuran, C.T.; Winum, J.Y.; Monti, S.M.; Alterio, V. Inhibition of carbonic anhydrases by a substrate analog: Benzyl carbamate directly coordinates the catalytic zinc ion mimicking bicarbonate binding. *Chem. Commun.* **2018**, *54*, 10312–10315. [CrossRef] [PubMed]
104. Carta, F.; Temperini, C.; Innocenti, A.; Scozzafava, A.; Kaila, K.; Supuran, C.T. Polyamines inhibit carbonic anhydrases by anchoring to the zinc-coordinated water molecule. *J. Med. Chem.* **2010**, *53*, 5511–5522. [CrossRef] [PubMed]
105. Nair, S.K.; Ludwig, P.A.; Christianson, D.W. Two-site binding of phenol in the active site of human carbonic anhydrase II: Structural implications for substrate association. *J. Am. Chem. Soc.* **1994**, *116*, 3659–3660. [CrossRef]
106. Innocenti, A.; Vullo, D.; Scozzafava, A.; Supuran, C.T. Carbonic anhydrase inhibitors. Interactions of phenols with the 12 catalytically active mammalian isoforms (CA I–XIV). *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1583–1587. [CrossRef] [PubMed]
107. Innocenti, A.; Vullo, D.; Scozzafava, A.; Supuran, C.T. Carbonic anhydrase inhibitors. Inhibition of mammalian isoforms I–XIV with a series of substituted phenols including paracetamol and salicylic acid. *Bioorg. Med. Chem.* **2008**, *16*, 7424–7428. [CrossRef] [PubMed]
108. Bayram, E.; Senturk, M.; Kufrevioglu, O.I.; Supuran, C.T. In vitro effects of salicylic acid derivatives on human cytosolic carbonic anhydrase isozymes I and II. *Bioorg. Med. Chem.* **2008**, *16*, 9101–9105. [CrossRef]
109. Nocentini, A.; Bonardi, A.; Gratteri, P.; Cerra, B.; Gioiello, A.; Supuran, C.T. Steroids interfere with human carbonic anhydrase activity by using alternative binding mechanisms. *J. Enzym. Inhib. Med. Chem.* **2018**, *33*, 1453–1459. [CrossRef]
110. Karioti, A.; Carta, F.; Supuran, C.T. Phenols and polyphenols as carbonic anhydrase inhibitors. *Molecules* **2016**, *21*, 1649. [CrossRef]
111. D'Ambrosio, K.; Carradori, S.; Monti, S.M.; Buonanno, M.; Secci, D.; Vullo, D.; Supuran, C.T.; De Simone, G. Out of the active site binding pocket for carbonic anhydrase inhibitors. *Chem. Commun.* **2015**, *51*, 302–305. [CrossRef]
112. Khalifah, R.G. The carbon dioxide hydration activity of carbonic anhydrase. I. Stop-flow kinetic studies on the native human isoenzymes B and C. *J. Biol. Chem.* **1971**, *246*, 2561–2573.
113. Hoffmann, K.M.; Samardzic, D.; van den Heever, K.; Rowlett, R.S. Co(II)-substituted Haemophilus influenzae β-carbonic anhydrase: Spectral evidence for allosteric regulation by pH and bicarbonate ion. *Arch. Biochem. Biophys.* **2011**, *511*, 80–87. [CrossRef] [PubMed]

114. Rowlett, R.S.; Tu, C.; Murray, P.S.; Chamberlin, J.E. Examination of the role of Gln-158 in the mechanism of CO_2 hydration catalyzed by beta-carbonic anhydrase from Arabidopsis thaliana. *Arch. Biochem. Biophys.* **2004**, *425*, 25–32. [CrossRef] [PubMed]
115. Ghannam, A.F.; Tsen, W.; Rowlett, R.S. Activation parameters for the carbonic anhydrase II-catalyzed hydration of CO_2. *J. Biol. Chem.* **1986**, *261*, 1164–1169.
116. Rowlett, R.S.; Gargiulo, N.J.; Santoli, F.A.; Jackson, J.M.; Corbett, A.H. Activation and inhibition of bovine carbonic anhydrase III by dianions. *J. Biol. Chem.* **1991**, *266*, 933–941. [PubMed]
117. Tu, C.; Rowlett, R.S.; Tripp, B.C.; Ferry, J.G.; Silverman, D.N. Chemical rescue of proton transfer in catalysis by carbonic anhydrases in the beta- and gamma-class. *Biochemistry* **2002**, *41*, 15429–15435. [CrossRef] [PubMed]
118. Elder, I.; Han, S.; Tu, C.; Steele, H.; Laipis, P.J.; Viola, R.E.; Silverman, D.N. Activation of carbonic anhydrase II by active-site incorporation of histidine analogs. *Arch. Biochem. Biophys.* **2004**, *421*, 283–289. [CrossRef]
119. Jonsson, B.H.; Liljas, A. Perspectives on the Classical Enzym. Carbonic Anhydrase and the Search for Inhibitors. *Biophys. J.* **2020**, *119*, 1275–1280. [CrossRef]
120. Akocak, S.; Alam, M.R.; Shabana, A.M.; Sanku, R.K.; Vullo, D.; Thompson, H.; Swenson, E.R.; Supuran, C.T.; Ilies, M.A. PEGylated Bis-Sulfonamide Carbonic Anhydrase Inhibitors Can Efficiently Control the Growth of Several Carbonic Anhydrase IX-Expressing Carcinomas. *J. Med. Chem.* **2016**, *59*, 5077–5088. [CrossRef]
121. Supuran, C.T.; Scozzafava, A.; Ilies, M.A.; Iorga, B.; Cristea, T.; Briganti, F.; Chiraleu, F.; Banciu, M.D. Carbonic anhydrase inhibitors—Part 53? Synthesis of substituted-pyridinium derivatives of aromatic sulfonamides: The first non-polymeric membrane-impermeable inhibitors with selectivity for isozyme IV. *Eur. J. Med. Chem.* **1998**, *33*, 577–594. [CrossRef]
122. Loughrey, B.T.; Williams, M.L.; Healy, P.C.; Innocenti, A.; Vullo, D.; Supuran, C.T.; Parsons, P.G.; Poulsen, S.A. Novel organometallic cationic ruthenium(II) pentamethylcyclopentadienyl benzenesulfonamide complexes targeted to inhibit carbonic anhydrase. *J. Biol. Inorg. Chem.* **2009**, *14*, 935–945. [CrossRef]
123. Mujumdar, P.; Teruya, K.; Tonissen, K.F.; Vullo, D.; Supuran, C.T.; Peat, T.S.; Poulsen, S.A. An Unusual Natural Product Primary Sulfonamide: Synthesis, Carbonic Anhydrase Inhibition, and Protein X-ray Structures of Psammaplin C. *J. Med. Chem.* **2016**, *59*, 5462–5470. [CrossRef] [PubMed]
124. Wilkinson, B.L.; Bornaghi, L.F.; Houston, T.A.; Innocenti, A.; Vullo, D.; Supuran, C.T.; Poulsen, S.A. Carbonic anhydrase inhibitors: Inhibition of isozymes I, II, and IX with triazole-linked O-glycosides of benzene sulfonamides. *J. Med. Chem.* **2007**, *50*, 1651–1657. [CrossRef] [PubMed]
125. Lopez, M.; Vu, H.; Wang, C.K.; Wolf, M.G.; Groenhof, G.; Innocenti, A.; Supuran, C.T.; Poulsen, S.A. Promiscuity of carbonic anhydrase II. Unexpected ester hydrolysis of carbohydrate-based sulfamate inhibitors. *J. Am. Chem. Soc.* **2011**, *133*, 18452–18462. [CrossRef] [PubMed]
126. Mahon, B.P.; Lomelino, C.L.; Ladwig, J.; Rankin, G.M.; Driscoll, J.M.; Salguero, A.L.; Pinard, M.A.; Vullo, D.; Supuran, C.T.; Poulsen, S.A.; et al. Mapping Selective Inhibition of the Cancer-Related Carbonic Anhydrase IX Using Structure-Activity Relationships of Glucosyl-Based Sulfamates. *J. Med. Chem.* **2015**, *58*, 6630–6638. [CrossRef] [PubMed]
127. La Regina, G.; Coluccia, A.; Famiglini, V.; Pelliccia, S.; Monti, L.; Vullo, D.; Nuti, E.; Alterio, V.; De Simone, G.; Monti, S.M.; et al. Discovery of 1,1′-Biphenyl-4-sulfonamides as a New Class of Potent and Selective Carbonic Anhydrase XIV Inhibitors. *J. Med. Chem.* **2015**, *58*, 8564–8572. [CrossRef] [PubMed]
128. Moi, D.; Nocentini, A.; Deplano, A.; Balboni, G.; Supuran, C.T.; Onnis, V. Structure-activity relationship with pyrazoline-based aromatic sulfamates as carbonic anhydrase isoforms I, II, IX and XII inhibitors: Synthesis and biological evaluation. *Eur. J. Med. Chem.* **2019**, *182*, 111638. [CrossRef] [PubMed]
129. De Luca, L.; Mancuso, F.; Ferro, S.; Buemi, M.R.; Angeli, A.; Del Prete, S.; Capasso, C.; Supuran, C.T.; Gitto, R. Inhibitory effects and structural insights for a novel series of coumarin-based compounds that selectively target human CA IX and CA XII carbonic anhydrases. *Eur. J. Med. Chem.* **2018**, *143*, 276–282. [CrossRef] [PubMed]
130. Nguyen, G.T.H.; Tran, T.N.; Podgorski, M.N.; Bell, S.G.; Supuran, C.T.; Donald, W.A. Nanoscale ion emitters in native mass spectrometry for measuring ligand-protein binding affinities. *ACS Cent. Sci.* **2019**, *5*, 308–318. [CrossRef]
131. Nguyen, G.T.H.; Nocentini, A.; Angeli, A.; Gratteri, P.; Supuran, C.T.; Donald, W.A. Perfluoroalkyl substances of significant environmental concern can strongly inhibit human carbonic anhydrase isozymes. *Anal. Chem.* **2020**, *92*, 4614–4622. [CrossRef]

132. Nguyen, G.T.H.; Leung, W.Y.; Tran, T.N.; Wang, H.; Murray, V.; Donald, W.A. mechanism for the binding of netropsin to hairpin DNA revealed using nanoscale ion emitters in native mass spectrometry. *Anal. Chem.* **2020**, *92*, 1130–1137. [CrossRef]
133. Garaj, V.; Puccetti, L.; Fasolis, G.; Winum, J.-Y.; Montero, J.-L.; Scozzafava, A.; Vullo, D.; Innocenti, A.; Supuran, C.T. Carbonic anhydrase inhibitors: Novel sulfonamides incorporating 1,3,5-triazine moieties as inhibitors of the cytosolic and tumour-associated carbonic anhydrase isozymes I, II and IX. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3102–3108. [CrossRef] [PubMed]
134. Conroy, C.W.; Maren, T.H. The effect of temperature on the binding of sulfonamides to carbonic anhydrase isoenzymes I, II, and IV. *Mol. Pharmacol.* **1995**, *48*, 486–491. [PubMed]
135. Kohler, K.; Hillebrecht, A.; Wischeler, J.S.; Innocenti, A.; Heine, A.; Supuran, C.T.; Klebe, G. Saccharin inhibits carbonic anhydrases: Possible explanation for its unpleasant metallic aftertaste. *Angew. Chem. Int. Ed.* **2007**, *46*, 7697–7699. [CrossRef] [PubMed]
136. Temperini, C.; Cecchi, A.; Scozzafava, A.; Supuran, C.T. Carbonic anhydrase inhibitors. Comparison of chlorthalidone and indapamide X-ray crystal structures in adducts with isozyme II: When three water molecules and the keto-enol tautomerism make the difference. *J. Med. Chem.* **2009**, *52*, 322–328. [CrossRef] [PubMed]
137. Temperini, C.; Cecchi, A.; Scozzafava, A.; Supuran, C.T. Carbonic anhydrase inhibitors. Interaction of indapamide and related diuretics with 12 mammalian isozymes and X-ray crystallographic studies for the indapamide –isozyme II adduct. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2567–2573. [CrossRef]
138. Aggarwal, M.; Kondeti, B.; McKenna, R. Insights towards sulfonamide drug specificity in α-carbonic anhydrases. *Bioorg. Med. Chem.* **2013**, *21*, 1526–1533. [CrossRef] [PubMed]
139. De Simone, G.; Alterio, V.; Supuran, C.T. Exploiting the hydrophobic and hydrophilic binding sites for designing carbonic anhydrase inhibitors. *Expert Opin. Drug Discov.* **2013**, *8*, 793–810. [CrossRef] [PubMed]
140. Briganti, F.; Pierattelli, R.; Scozzafava, A.; Supuran, C.T. Carbonic anhydrase inhibitors. Part 37. Novel classes of isozyme I and II inhibitors and their mechanism of action. Kinetic and spectroscopic investigations on native and cobalt-substituted enzymes. *Eur. J. Med. Chem.* **1996**, *31*, 1001–1010. [CrossRef]
141. Supuran, C.T. Structure-based drug discovery of carbonic anhydrase inhibitors. *J. Enzym. Inhib. Med. Chem.* **2012**, *27*, 759–772. [CrossRef] [PubMed]
142. Supuran, C.T. Carbonic anhydrases: From biomedical applications of the inhibitors and activators to biotechnological use for CO_2 capture. *J. Enzym. Inhib. Med. Chem.* **2013**, *28*, 229–230. [CrossRef]
143. Chahal, V.; Nirwan, S.; Kakkar, R. A comparative study of the binding modes of SLC-0111 and its analogues in the hCA II and hCA IX active sites using QM/MM, molecular docking, MM-GBSA and MD approaches. *Biophys. Chem.* **2020**, *265*, 106439. [CrossRef] [PubMed]
144. Gieling, R.G.; Babur, M.; Mamnani, L.; Burrows, N.; Telfer, B.A.; Carta, F.; Winum, J.Y.; Scozzafava, A.; Supuran, C.T.; Williams, K.J. Antimetastatic effect of sulfamate carbonic anhydrase IX inhibitors in breast carcinoma xenografts. *J. Med. Chem.* **2012**, *14*, 5591–5600. [CrossRef]
145. Carta, F.; Vullo, D.; Osman, S.M.; AlOthman, Z.; Supuran, C.T. Synthesis and carbonic anhydrase inhibition of a series of SLC-0111 analogs. *Bioorg. Med. Chem.* **2017**, *25*, 2569–2576. [CrossRef] [PubMed]
146. Bozdag, M.; Carta, F.; Ceruso, M.; Ferraroni, F.; McDonald, P.C.; Dedhar, S.; Supuran, C.T. Discovery of 4-hydroxy-3-(3-(phenylureido)benzenesulfonamides as SLC-0111 analogues for the treatment of hypoxic tumors overexpressing carbonic anhydrase IX. *J. Med. Chem.* **2018**, *61*, 6328–6338. [CrossRef]
147. Akocak, S.; Lolak, N.; Bua, S.; Turel, I.; Supuran, C.T. Synthesis and biological evaluation of novel *N*,*N*′-diaryl cyanoguanidines acting as potent and selective carbonic anhydrase II inhibitors. *Bioorg. Chem.* **2018**, *77*, 245–251. [CrossRef] [PubMed]
148. Lolak, N.; Akocak, S.; Bua, S.; Koca, M.; Supuran, C.T. Design and synthesis of novel 1,3-diaryltriazene-substituted sulfonamides as potent and selective carbonic anhydrase II inhibitors. *Bioorg. Chem.* **2018**, *77*, 542–547. [CrossRef] [PubMed]
149. Lomelino, C.L.; Mahon, B.P.; Carta, F.; Supuran, C.T.; McKenna, R. Kinetic and X-ray crystallographic investigations on carbonic anhydrase isoforms I, II, IX and XII of a thioureido analog of SLC-0111. *Bioorg. Med. Chem.* **2016**, *24*, 976–981. [CrossRef] [PubMed]

150. Angeli, A.; Tanini, D.; Peat, T.S.; Di, L.; Mannelli, C.; Bartolucci, G.; Capperucci, A.; Ghelardini, C.; Supuran, C.T.; Carta, F. Discovery of new selenoureido analogues of 4-(4-fluorophenylureido) benzenesulfonamide as carbonic anhydrase inhibitors. *ACS Med. Chem. Lett.* **2017**, *8*, 963–968. [CrossRef] [PubMed]
151. Eldehna, W.M.; Abo-Ashour, M.F.; Berrino, E.; Vullo, D.; Ghabbour, H.A.; Al-Rashood, S.T.; Hassan, G.S.; Alkahtani, H.M.; Almehizia, A.A.; Alharbi, A.; et al. SLC-0111 enaminone analogs, 3/4-(3-aryl-3-oxopropenyl) aminobenzenesulfonamides, as novel selective subnanomolar inhibitors of the tumor-associated carbonic anhydrase isoform IX. *Bioorg. Chem.* **2019**, *83*, 549–558. [CrossRef] [PubMed]
152. Congiu, C.; Onnis, V.; Deplano, A.; Balboni, G.; Dedeoglu, N.; Supuran, C.T. Synthesis of sulfonamides incorporating piperazinyl-ureido moieties and their carbonic anhydrase I, II, IX and XII inhibitory activity. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 3850–3853. [CrossRef] [PubMed]
153. Moi, D.; Nocentini, A.; Deplano, A.; Osman, S.M.; Al Othman, Z.A.; Piras, V.; Balboni, G.; Supuran, C.T.; Onnis, V. Appliance of the piperidinyl-hydrazidoureido linker to benzenesulfonamide compounds: Synthesis, in vitro and in silico evaluation of potent carbonic anhydrase II, IX and XII inhibitors. *Bioorg. Chem.* **2020**, *98*, 103728. [CrossRef] [PubMed]
154. Abo-Ashour, M.F.; Eldehn, W.M.; Nocentini, A.; Ibrahim, H.S.; Bua, S.; Abdel-Aziz, H.A.; Abou, S.M.; Supuran, C.T. Novel synthesized SLC-0111 thiazole and thiadiazole analogues: Determination of their carbonic anhydrase inhibitory activity and molecular modeling studies. *Bioorg. Chem.* **2019**, *87*, 794–802. [CrossRef] [PubMed]
155. Alkhaldi, A.A.M.; Al-Sanea, M.M.; Nocentini, A.; Eldehna, W.M.; Elsayed, Z.M.; Bonardi, A.; Abo-Ashour, M.F.; El-Damasy, A.K.; Abdel-Maksoud, M.S.; Al-Warhi, T.; et al. 3-Methylthiazolo[3,2-a]benzimidazole-benzenesulfonamide conjugates as novel carbonic anhydrase inhibitors endowed with anticancer activity: Design, synthesis, biological and molecular modeling studies. *Eur. J. Med. Chem.* **2020**, *207*, 112745. [CrossRef]
156. Iikuni, S.; Ono, M.; Watanabe, H.; Shimizu, Y.; Sano, K.; Saji, H. Cancer radiotheranostics targeting carbonic anhydrase-IX with ^{111}In- and ^{90}Y-labeled ureidosulfonamide scaffold for SPECT imaging and radionuclide-based therapy. *Theranostics* **2018**, *8*, 2992–3006. [CrossRef]
157. Eldehna, W.M.; Fares, M.; Ceruso, M.; Ghabbour, H.A.; Abou-Seri, S.M.; Abdel- Aziz, H.A.; El Ella, D.A.; Supuran, C.T. Amido/ureidosubstituted benzenesulfonamides-isatin conjugates as low nanomolar/subnanomolar inhibitors of the tumor-associated carbonic anhydrase isoform XII. *Eur. J. Med. Chem.* **2016**, *110*, 259–266. [CrossRef] [PubMed]
158. Eldehna, W.M.; Abo-Ashour, M.F.; Nocentini, A.; El-Haggar, R.S.; Bua, S.; Bonardi, A.; Al-Rashood, S.T.; Hassan, G.S.; Gratteri, P.; Abdel-Aziz, H.A.; et al. Enhancement of the tail hydrophobic interactions within the carbonic anhydrase IX active site via structural extension: Design and synthesis of novel N-substituted isatins-SLC-0111 hybrids as carbonic anhydrase inhibitors and antitumor agents. *Eur. J. Med. Chem.* **2016**, *162*, 147–160. [CrossRef] [PubMed]
159. Lock, F.E.; McDonald, P.C.; Lou, Y.; Serrano, I.; Chafe, S.C.; Ostlund, C.; Aparicio, S.; Winum, J.Y.; Supuran, C.T.; Dedhar, S. Targeting carbonic anhydrase IX depletes breast cancer stem cells within the hypoxic niche. *Oncogene* **2013**, *32*, 5210–5219. [CrossRef] [PubMed]
160. Kreuzer, M.; Banerjee, A.; Birts, C.N.; Darley, M.; Tavassoli, A.; Ivan, M.; Blaydes, J.P. Glycolysis, via NADH-dependent dimerisation of CtBPs, regulates hypoxia-induced expression of CAIX and stem-like breast cancer cell survival. *FEBS Lett.* **2020**. [CrossRef]
161. Gibadulinova, A.; Bullova, P.; Strnad, H.; Pohlodek, K.; Jurkovicova, D.; Takacova, M.; Pastorekova, S.; Svastova, E. 1CAIX-Mediated control of LIN28/let-7 axis contributes to metabolic adaptation of breast cancer cells to hypoxia. *Int. J. Mol. Sci.* **2020**, *21*, 4299. [CrossRef]
162. Swayampakula, M.; McDonald, P.C.; Vallejo, M.; Coyaud, E.; Chafe, S.C.; Westerback, A.; Venkateswaran, G.; Shankar, J.; Gao, G.; Laurent, E.M.N.; et al. The interactome of metabolic Enzyme carbonic anhydrase IX reveals novel roles in tumor cell migration and invadopodia/MMP14-mediated invasion. *Oncogene* **2017**, *36*, 6244–6261. [CrossRef]
163. Lee, S.H.; Griffiths, J.R. How and Why Are Cancers Acidic? Carbonic Anhydrase IX and the Homeostatic Control of Tumour Extracellular pH. *Cancers* **2020**, *12*, 1616. [CrossRef] [PubMed]
164. Ciccone, V.; Filippelli, A.; Angeli, A.; Supuran, C.T.; Morbidelli, L. Pharmacological Inhibition of CA-IX impairs tumor cell proliferation, migration and invasiveness. *Int. J. Mol. Sci.* **2020**, *21*, 2983. [CrossRef] [PubMed]

165. Pastorekova, S.; Gillies, R.J. The role of carbonic anhydrase IX in cancer development: Links to hypoxia, acidosis, and beyond. *Cancer Metastasis Rev.* **2019**, *38*, 65–77. [CrossRef] [PubMed]
166. Lee, S.H.; McIntyre, D.; Honess, D.; Hulikova, A.; Pacheco-Torres, J.; Cerdán, S.; Swietach, P.; Harris, A.L.; Griffiths, J.R. Carbonic anhydrase IX is a pH-stat that sets an acidic tumour extracellular pH in vivo. *Br. J. Cancer* **2018**, *119*, 622–630. [CrossRef]
167. McDonald, P.C.; Chafe, S.C.; Brown, W.S.; Saberi, S.; Swayampakula, M.; Venkateswaran, G.; Nemirovsky, O.; Gillespie, J.A.; Karasinska, J.M.; Kalloger, S.E.; et al. Regulation of pH by carbonic anhydrase 9 mediates survival of pancreatic cancer cells with activated KRAS in response to hypoxia. *Gastroenterology* **2019**, *157*, 823–837. [CrossRef] [PubMed]
168. Persi, E.; Duran-Frigola, M.; Damaghi, M.; Roush, W.R.; Aloy, P.; Cleveland, J.L.; Gillies, R.J.; Ruppin, E. Systems analysis of intracellular pH vulnerabilities for cancer therapy. *Nat. Commun.* **2018**, *9*, 2997. [CrossRef] [PubMed]
169. Boyd, N.H.; Walker, K.; Fried, J.; Hackney, J.R.; McDonald, P.C.; Benavides, G.A.; Spina, R.; Audia, A.; Scott, S.E.; Libby, C.J.; et al. Addition of carbonic anhydrase 9 inhibitor SLC-0111 to temozolomide treatment delays glioblastoma growth in vivo. *JCI Insight* **2017**, *2*, e92928. [CrossRef] [PubMed]
170. Iessi, E.; Logozzi, M.; Mizzoni, D.; Di Raimo, R.; Supuran, C.T.; Fais, S. Rethinking the Combination of Proton Exchanger Inhibitors in Cancer Therapy. *Metabolites* **2018**, *8*, 2. [CrossRef]
171. McDonald, P.C.; Swayampakula, M.; Dedhar, S. Coordinated Regulation of Metabolic Transporters and Migration/Invasion by Carbonic Anhydrase IX. *Metabolites* **2018**, *8*, 20. [CrossRef] [PubMed]
172. Chafe, S.C.; McDonald, P.C.; Saberi, S.; Nemirovsky, O.; Venkateswaran, G.; Burugu, S.; Gao, D.; Delaidelli, A.; Kyle, A.H.; Baker, J.H.E.; et al. Targeting hypoxia-induced carbonic anhydrase ix enhances immune-checkpoint blockade locally and systemically. *Cancer Immunol. Res.* **2019**, *7*, 1064–1078. [CrossRef]
173. Damgaci, S.; Ibrahim-Hashim, A.; Enriquez-Navas, P.M.; Pilon-Thomas, S.; Guvenis, A.; Gillies, R.J. Hypoxia and acidosis: Immune suppressors and therapeutic targets. *Immunology* **2018**, *154*, 354–362. [CrossRef]
174. Kuchuk, O.; Tuccitto, A.; Citterio, D.; Huber, V.; Camisaschi, C.; Milione, M.; Vergani, B.; Villa, A.; Alison, M.R.; Carradori, S.; et al. pH regulators to target the tumor immune microenvironment in human hepatocellular carcinoma. *Oncoimmunology* **2018**, *7*, e1445452. [CrossRef] [PubMed]
175. Ward, C.; Meehan, J.; Gray, M.; Kunkler, I.H.; Langdon, S.P.; Argyle, D.J. Carbonic Anhydrase IX (CAIX), Cancer, and Radiation Responsiveness. *Metabolites* **2018**, *8*, 13. [CrossRef] [PubMed]
176. Doyen, J.; Parks, S.K.; Marcié, S.; Pouysségur, J.; Chiche, J. Knock-down of hypoxia-induced carbonic anhydrases IX and XII radiosensitizes tumor cells by increasing intracellular acidosis. *Front. Oncol.* **2013**, *2*, 199. [CrossRef] [PubMed]
177. McDonald, P.C.; Chia, S.; Bedard, P.L.; Chu, Q.; Lyle, M.; Tang, L.; Singh, M.; Zhang, Z.; Supuran, C.T.; Renouf, D.J.; et al. A Phase 1 Study of SLC-0111, a novel inhibitor of carbonic anhydrase ix, in patients with advanced solid tumors. *Am. J. Clin. Oncol.* **2020**, *43*, 484–490. [CrossRef] [PubMed]
178. Federici, C.; Lugini, L.; Marino, M.L.; Carta, F.; Iessi, E.; Azzarito, T.; Supuran, C.T.; Fais, S. Lansoprazole and carbonic anhydrase IX inhibitors sinergize against human melanoma cells. *J. Enzym. Inhib. Med. Chem.* **2016**, *31*, 119–125. [CrossRef]
179. Andreucci, E.; Peppicelli, S.; Carta, F.; Brisotto, G.; Biscontin, E.; Ruzzolini, J.; Bianchini, F.; Biagioni, A.; Supuran, C.T.; Calorini, L. Carbonic anhydrase IX inhibition affects viability of cancer cells adapted to extracellular acidosis. *J. Mol. Med.* **2017**, *95*, 1341–1353. [CrossRef]
180. Bryant, J.L.; Gieling, R.G.; Meredith, S.L.; Allen, T.J.; Walker, L.; Telfer, B.A.; Supuran, C.T.; Williams, K.J.; White, A. Novel carbonic anhydrase IX-targeted therapy enhances the anti-tumour effects of cisplatin in small cell lung cancer. *Int. J. Cancer* **2018**, *142*, 191–201. [CrossRef]
181. Logsdon, D.P.; Grimard, M.; Luo, M.; Shahda, S.; Jiang, Y.; Tong, Y.; Yu, Z.; Zyromski, N.; Schipani, E.; Carta, F.; et al. Regulation of HIF1α under Hypoxia by APE1/Ref-1 Impacts CA9 expression: Dual targeting in patient-derived 3D pancreatic cancer models. *Mol. Cancer Ther.* **2016**, *15*, 2722–2732. [CrossRef]
182. Peppicelli, S.; Andreucci, E.; Ruzzolini, J.; Bianchini, F.; Nediani, C.; Supuran, C.T.; Calorini, L. The Carbonic Anhydrase IX inhibitor SLC-0111 as emerging agent against the mesenchymal stem cell-derived pro-survival effects on melanoma cells. *J. Enzym. Inhib. Med. Chem.* **2020**, *35*, 1185–1193. [CrossRef]
183. Genah, S.; Angeli, A.; Supuran, C.T.; Morbidelli, L. Effect of Carbonic Anhydrase IX inhibitors on human endothelial cell survival. *Pharmacol. Res.* **2020**, *159*, 104964. [CrossRef] [PubMed]

184. Riemann, A.; Güttler, A.; Haupt, V.; Wichmann, H.; Reime, S.; Bache, M.; Vordermark, D.; Thews, O. Inhibition of Carbonic Anhydrase IX by Ureidosulfonamide Inhibitor U104 reduces prostate cancer cell growth, but does not modulate daunorubicin or cisplatin cytotoxicity. *Oncol. Res.* **2018**, *26*, 191–200. [CrossRef] [PubMed]
185. Güttler, A.; Theuerkorn, K.; Riemann, A.; Wichmann, H.; Kessler, J.; Thews, O.; Bache, M.; Vordermark, D. Cellular and radiobiological effects of carbonic anhydrase IX in human breast cancer cells. *Oncol. Rep.* **2019**, *41*, 2585–2594. [PubMed]
186. Lee, J.Y.; Alexeyev, M.; Kozhukhar, N.; Pastukh, V.; White, R.; Stevens, T. Carbonic anhydrase IX is a critical determinant of pulmonary microvascular endothelial cell pH regulation and angiogenesis during acidosis. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **2018**, *315*, L41–L51. [CrossRef] [PubMed]
187. Lee, J.Y.; Onanyan, M.; Garrison, I.; White, R.; Crook, M.; Alexeyev, M.F.; Kozhukhar, N.; Pastukh, V.; Swenson, E.R.; Supuran, C.T.; et al. Extrinsic acidosis suppresses glycolysis and migration while increasing network formation in pulmonary microvascular endothelial cells. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **2019**, *317*, L188–L201. [CrossRef]
188. Bernardino, R.L.; Dias, T.R.; Moreira, B.P.; Cunha, M.; Barros, A.; Oliveira, E.; Sousa, M.; Alves, M.G.; Oliveira, P.F. Carbonic anhydrases are involved in mitochondrial biogenesis and control the production of lactate by human Sertoli cells. *FEBS J.* **2019**, *286*, 1393–1406. [CrossRef]
189. D'Ambrosio, K.; Carradori, S.; Cesa, S.; Angeli, A.; Monti, S.M.; Supuran, C.T.; De Simone, G. Catechols: A new class of carbonic anhydrase inhibitors. *Chem. Commun.* **2020**, in press. [CrossRef]
190. Abdelrahman, M.A.; Ibrahim, H.S.; Nocentini, A.; Eldehna, W.M.; Bonardi, A.; Abdel-Aziz, H.A.; Gratteri, P.; Abou-Seri, S.M.; Supuran, C.T. Novel 3-substituted coumarins as selective human carbonic anhydrase IX and XII inhibitors: Synthesis, biological and molecular dynamics analysis. *Eur. J. Med. Chem.* **2020**, *209*, 112897. [CrossRef]
191. Guglielmi, P.; Rotondi, G.; Secci, D.; Angeli, A.; Chimenti, P.; Nocentini, A.; Bonardi, A.; Gratteri, P.; Carradori, S.; Supuran, C.T. Novel insights on saccharin- and acesulfame-based carbonic anhydrase inhibitors: Design, synthesis, modelling investigations and biological activity evaluation. *J. Enzym. Inhib. Med. Chem.* **2020**, *35*, 1891–1905. [CrossRef]
192. Bouzina, A.; Berredjem, M.; Nocentini, A.; Bua, S.; Bouaziz, Z.; Jose, J.; Le Borgne, M.; Marminon, C.; Gratteri, P.; Supuran, C.T. Ninhydrins inhibit carbonic anhydrases directly binding to the metal ion. *Eur. J. Med. Chem.* **2020**, *209*, 112875. [CrossRef]
193. Mishra, C.B.; Tiwari, M.; Supuran, C.T. Progress in the development of human carbonic anhydrase inhibitors and their pharmacological applications: Where are we today? *Med. Res. Rev.* **2020**, *40*, 2485–2565. [CrossRef] [PubMed]