Identification of Novel and Potent Indole-Based Benzenesulfonamides as Selective Human Carbonic Anhydrase II Inhibitors: Design, Synthesis, In Vitro, and In Silico Studies

Ahmed Elkamhawy 1,2, Jiyu Woo 1, Hossam Nada 1,3, Andrea Angeli 4, Tarek M. Bedair 5, Claudia T. Supuran 4,5,* and Kyeong Lee 1,5

Abstract: In recent decades, human carbonic anhydrase inhibitors (hCAIs) have emerged as an important therapeutic class with various applications including antiglaucoma, anticonvulsants, and anticancer agents. Herein, a novel series of indole-based benzenesulfonamides were designed, synthesized, and biologically evaluated as potential hCAIs. A regioisomerism of the sulfonamide moiety was carried out to afford a total of fifteen indole-based benzenesulfonamides possessing different amide linkers that enable the ligands to be flexible and develop potential H-bond interaction(s) with the target protein. The activity of the synthesized compounds was evaluated against four hCA isoforms (I, II, IX, and XII). Compounds 2b, 2c, 2d, 2f, 2h, and 2o exhibited potent and selective profiles over the hCA II isoform with Kᵢ values of 7.3, 9.0, 7.1, 16.0, 8.6 and 7.5 nM, respectively. Among all, compound 2a demonstrated the most potent inhibition against the hCA II isoform with an inhibitory constant (Kᵢ) of 5.9 nM, with 13-, 34-, and 9-fold selectivity for hCA II over I, IX, and XII isoforms, respectively. Structure–activity relationship data attained for various substitutions were rationalized. Furthermore, a molecular docking study gave insights into both inhibitory activity and selectivity of the target compounds. Accordingly, this report presents a successful scaffold hoping approach that reveals compound 2a as a highly potent and selective indole-based hCA II inhibitor worthy of further investigation.

Keywords: indole based-benzenesulfonamide; amide coupling; carbonic anhydrase inhibitors; molecular docking; structure–activity relationship (SAR)

1. Introduction

Carbonic anhydrases (CAs) are metalloenzymes found in all living things that are required for the conversion of CO₂ to bicarbonate and protons [1]. CAs observed in humans belong to the α-class (α-CA), and are classified into sixteen isoforms that vary in molecular characteristics, oligomeric structure, cellular localization, distribution in organs and tissues, expression levels, and kinetic properties [2]. The distribution pattern of the CA isoforms can be described as, cytosolic (I, II, III, VII, and XIII), membrane (IV, IX, XII, and XIV), secretory (VI), and mitochondrial (VA and VB) forms [3,4]. Carbonic anhydrases have a role in a variety of physiological processes, including respiration, pH control, ion transport, bone resorption, and gastric fluid secretion. As a result, inhibitors of these enzymes have become an important therapeutic class in recent decades. Topically acting antiglaucoma,
anticonvulsants, and anticancer agents are some of the novel applications of CA inhibitors that have been developed [2,5]. Among the CA isoforms, CA II is widely associated with various types of cancers, with a recent study finding that it is expressed in the endothelium of neovessels in melanoma, esophageal, renal, and lung cancers [6]. Other studies associate CA II with the key target antigen stimulation of an autoantibody response in melanoma patients [7]. The most common approach in designing selective CA inhibitors has been through the modulation of the ring directly linked to the zinc-binding group (ZBG) or by appending different tails to the aromatic ring [8,9].

One of the most prominent ZBG moieties with carbonic anhydrase inhibitory activity are sulfonamides [10]. Among the investigated sulfonamides, aromatic sulfonamides have been shown to be specific and potent inhibitors of CA isoforms, and they have been considered as potential candidates for the further development and enhancement of more potent inhibitors [11]. Conversely, due to the structural similarity and identical subcellular location of the CA isoforms, carbonic anhydrase inhibitors suffer from a lack of selectivity which often results in unwanted side effects [12]. When designing inhibitors for the metalloenzymes carbonic anhydrases, sulfonamides are regarded as one of the most effective ZBGs among the studied sulfonamides. This is due to their structural features that are ideal for binding to the Zn$^{2+}$ ion in the active site of the receptor [13–15]. The negatively charged nitrogen of sulfonamide coordinates the positively charged metal ion [9,16]. Accordingly, the primary sulfonamide group (SO$_2$NH$_2$) plays an important role in CA inhibition mechanism, with more than 20 compounds in clinical use including acetazolamide (AAZ) I, methazolamide II, and ethoxzolamide III (Figure 1).

Figure 1. Chemical structures of some clinically used sulfonamide-containing drugs.

The indole scaffold has been identified to afford highly potent carbonic anhydrase inhibitors. A recent series of indole-1,2,3-triazole chalcone hybrids was synthesized by click chemistry as CA inhibitors (general structure IV, Figure 2). Although some compounds of this series were able to successfully inhibit the cytosolic isoform hCA I in a low nanomolar range of potency (18.8–50.4 nM), they did not show a similar potency over the tumor-associated hCA II isoform. The tumor associated isoform hCA IX was also weakly inhibited by all the synthesized compounds with $K_i$ values ranging from 69.3 nM to 1.4 µM [17]. Another series of sulfonamidoindole-based hydrazones possessing a 2-(hydrazinocarbonyl)-3-phenyl-1H-indole-5-sulfonamide scaffold (general structure V, Figure 2) was reported with CA inhibitory activity for the tumor-associated carbonic anhydrase isoforms, hCA IX and XII. Despite the high potency of these derivatives against hCA IX and XII, they displayed weak inhibitory activity against both hCA I isoform ($K_i$s range: from 3.13 µM to >10.00 µM) and hCA II isoform ($K_i$s range: from 309 nM to >10.00 µM) [18]. Peerzeeda et al. reported a series of sulfonamide derivatives of pyridyl-indole-based chalcone (general structure VI, Figure 2) as hCA IX inhibitors with antitumor activities against MCF-7 and HepG-2 cell lines [19]. N-aroyl-2-oxindole benzenesulfonamide conjugates (general structure VII, Figure 2) were also reported by George et al. to preferentially inhibit the tumor-associated isoforms IX and XII [20]. The same research group reported another series of 2-oxindole benzenesulfonamide conjugates (general structure VIII, Figure 2), with many compounds showing promising activity and selectivity toward CAI, II and IX compared to AAZ (I) [20]. Novel 4/3-((4-oxo-5-(2-oxoidolin-3-ylidene)thiazolidin-2-ylidene)amino)benzenesulfonamides (general structure IX, Figure 2) were also recently reported as CA inhibitors. Isoforms II and IX (tumor-associated) were found to be more susceptible to inhibition by this series, with $K_i$s in the range of 2.6–598.2 nM for hCA II, and
of 16.1–321 nM for hCA IX. Regarding clinical trials, indisulam (X, Figure 2) is one of these indole-based benzenesulfonamide anticancer agents. It was in phase II clinical studies until recently, and it inhibits several CA isoforms, including the tumor-associated isoform IX [21]. Moreover, another example in clinical trials is SLC-0111 (XI), a benzenesulfonamide compound currently in phase I/II clinical trials for the treatment of advanced metastatic solid tumors [22].

![Figure 2. Design strategy of the synthesized hybrids 2a–o.](image)

Inspired by all these efforts related to the development of indole-based hCA inhibitors, we designed a novel series of indole-based benzenesulfonamides (Figure 2) through a hybridization process of these two scaffolds (indole and benzenesulfonamide). Thereafter, a regioisomerism of the ZBG moiety was carried out to afford fifteen indole-based benzenesulfonamides linked together via an amide linker, giving the synthesized compounds flexibility as well as the ability to form hydrogen bond interactions with the target protein. To the best of our knowledge, this hybridization approach of the indole moiety with the most effective ZBG moiety (the benzenesulfonamide group) via a flexible amide linker on different positions on the indole core to develop novel small molecules with a promising potential to inhibit carbonic anhydrase isoforms has not been reported so far.
2. Results and Discussion

2.1. Chemical Synthesis

As shown in Scheme 1, a series of fifteen benzenesulfonamide analogues (2a–o) were synthesized via a coupling reaction of amide synthesis. The reaction was carried out utilizing a range of commercially available appropriate indole-based starting materials; 3-(1H-indol-3-yl)propanoic acid (1a), 1H-indole-5-carboxylic acid (1b), 1H-indole-6-carboxylic acid (1c), or 1H-indol-5-amine (1d). Using coupling catalysts such as EDCI, HOBr, or T3P, several benzenesulfonamide reagents were allowed to react overnight at room temperature with the starting materials 1a–d (as described in Table 1) in a suitable solvent such as MeCN, DMF/DCM (5/1), or THF. As a result, a wide range of indole-based benzenesulfonamide analogues (2a–o) with diverse substituents were afforded in acceptable yields (Table 1).

![Scheme 1. Reagent: appropriate benzenesulfonamide reagent (see Table 1). Conditions: (i) EDCI, HOBr, DIPEA, acetonitrile or DMF/DCM (5/1), rt, 12 h, or (ii) T3P, DIPEA, THF, rt, 12 h.](image)

| Cpd | Starting | Benzenesulfonamide Reagent | Chemical Structure |
|-----|----------|-----------------------------|-------------------|
| 2a  | 1a       |                             |                   |
| 2b  | 1a       |                             |                   |
| 2c  | 1a       |                             |                   |
| 2d  | 1a       |                             |                   |
| 2e  | 1b       |                             |                   |
| 2f  | 1b       |                             |                   |
| 2g  | 1b       |                             |                   |
| 2h  | 1b       |                             |                   |
| 2i  | 1b       |                             |                   |
| 2j  | 1c       |                             |                   |
Table 1. Cont.

| Cpd  | Starting | Benzenesulfonamide Reagent | Chemical Structure |
|------|----------|----------------------------|-------------------|
| 2k   | 1c       | \(\text{C-like} \dots \text{S}\text{H}_2\) | ![Chemical Structure](image) |
| 2l   | 1c       | \(\text{C-like} \dots \text{S}\text{H}_2\) | ![Chemical Structure](image) |
| 2m   | 1c       | \(\text{C-like} \dots \text{S}\text{H}_2\) | ![Chemical Structure](image) |
| 2n   | 1c       | \(\text{C-like} \dots \text{S}\text{H}_2\) | ![Chemical Structure](image) |
| 2o   | 1d       | \(\text{C-like} \dots \text{S}\text{H}_2\) | ![Chemical Structure](image) |

Different techniques were utilized to elucidate the chemical structures and the purity of the synthesized target compounds. The \(^1\text{H}\) NMR spectra of compounds 2a–o were characterized by the appearance of two peaks at 7.20–7.50 ppm attributable to the sulfonamide NH\(_2\) and another peak at 10.20–10.80 ppm attributable to the NH of the amide group confirming the formation of compounds 2a–o. Compound 2a synthesis was further confirmed by the appearance of two triplet peaks in the aliphatic region at 3.05–3.01 and 2.73–2.69 ppm attributable to the two CH\(_2\) groups of the aliphatic linker moiety. Similarly, compound 2b synthesis was easily confirmed by the appearance of the two triplet peaks attributable to the two CH\(_2\) groups, as well as a singlet peak at 10.77 ppm attributable to the OH substituent on the ZBG moiety. Compound 2c was characterized by the presence of four triplet peaks at 3.29–3.26, 2.90–2.88, 2.76–2.74 and 2.43–2.39 attributable to the four CH\(_2\) groups of the aliphatic linker moiety. In the same manner, the presence of triplet peaks in the aliphatic range of the \(^1\text{H}\) NMR spectrum associated with the presence of CH\(_2\) groups in the linker moiety was used to confirm the synthesis of compounds 2h and 2m.

2.2. Carbonic Anhydrase Inhibition

The CA inhibitory activities of the hybrid compounds 2a–o and AAZ, as a standard inhibitor, were measured against four isoforms of hCA by a stopped flow CO\(_2\) hydrase assay (Table 2). The tested CA panel was comprised of the two ubiquitously expressed cytosolic hCA I and II isoforms, along with the two tumor-related transmembrane hCA IX and XII isoforms. The activity of the synthesized hybrids was reported as \(K_i\) (inhibition constant) to assess their binding affinity. Among the synthesized hybrids, compounds 2a, 2d and 2o exhibited potent inhibitory activities against the hCA I isoform with inhibition constants (\(K_i\)) of 79.8, 97.6 and 56.6 nM, respectively, compared to AAZ (250 nM). Compound 2a demonstrated the most potent inhibition among all compounds against the hCA II isoform with an inhibition constant of 5.9 nM. This result indicated that compound 2a possessed 13-fold selectivity for hCA II over the hCA I isoform and 34- and 9-fold selectivity for hCA II over the hCA IX and XII isoforms, respectively. Similarly, compounds 2b, 2c, 2d, 2f, 2h and 2o exhibited potent and selective activity over the hCA II isoform with inhibition constant values of 7.3, 9.0, 7.1, 16.0, 8.6 and 7.5 nM, respectively. On the contrary, the synthesized hybrids only displayed moderate inhibitory activity over the hCA IX isoform with inhibition constant ranging from 169.6–766.3 nM. All the synthesized hybrids displayed potent activity against the tumor-related hCA XII isoform with the most potent hybrids being compounds 2c and 2n, which exhibited an inhibition constant of 36.9 nM. Except for compounds 2f and 2h which possessed moderate hCA XII inhibition constant of 257.0 and 235.6, respectively, the remaining synthesized hybrids displayed double-digit...
nanomolar activity ranging from 40.5–90.9 nM. The inhibitory activity of the synthesized hybrids over the hCA isoforms I, II, IX and XII is demonstrated in Table 2.

Table 2. Inhibition of hCA isoforms I, II, IX and XII by compounds 2a–o; acetazolamide (AAZ) was used as a standard inhibitor.

| Cpd | hCA I (nM) | hCA II (nM) | hCA IX (nM) | hCA XII (nM) |
|-----|------------|-------------|-------------|--------------|
| 2a  | 79.8       | 5.9         | 205.8       | 54.2         |
| 2b  | 2796       | 7.3         | 286.5       | 85.1         |
| 2c  | 217.3      | 9.0         | 221.6       | 36.9         |
| 2d  | 97.6       | 7.1         | 165.5       | 42.2         |
| 2e  | 5575       | 45.5        | 766.3       | 90.9         |
| 2f  | 3105       | 16.0        | 165.1       | 257.0        |
| 2g  | 4772       | 679.9       | 279.6       | 85.3         |
| 2h  | 553.7      | 8.6         | 239.6       | 235.6        |
| 2i  | 7453       | 71.9        | 635.0       | 89.4         |
| 2j  | 5397       | 94.8        | 654.0       | 40.5         |
| 2k  | 4655       | 833.4       | 427.6       | 83.8         |
| 2l  | 4275       | 681.6       | 423.0       | 73.7         |
| 2m  | 252.9      | 38.8        | 277.9       | 85.1         |
| 2n  | 7511       | 670.1       | 487.3       | 36.9         |
| 2o  | 56.6       | 7.5         | 169.6       | 66.9         |
| AAZ | 250.0      | 12.1        | 25.7        | 5.7          |

Generally, the sulfonamides substituted at the para- and meta-positions generally exhibited higher inhibitory activity when compared to the ortho-substituted sulfonamide-indole hybrids. Hydroxy substitutions in compounds 2g and 2l led to a loss of the potent inhibitory activity. However, the hydroxy substituted compound 2b displayed a potent inhibitory activity on hCA II (K\textsubscript{i} = 7.3 nM) and XII (K\textsubscript{i} = 85.1 nM), which could be attributed to the presence of a longer amide linker. Similarly, in all the synthesized compounds, the longer amide linker usually led to higher activity when compared to a shorter one. Moreover, 3-substituted indoles (2a, 2b, 2c and 2d) exhibited the highest activity when compared to 5 or 6-substituted indoles. Interestingly, the reverse amide 2o showed potent inhibitory activities against all hCA isoforms making it a potential lead for a new series of CA inhibitors. All the synthesized compounds showed potent activity against the tumor-related hCA XII with inhibitory activity ranging from 36.9–257.0 nM. A structure activity relationship (SAR) diagram of the synthesized hybrids 2a–o is illustrated in Figure 3.

Figure 3. Structure activity relationship (SAR) diagram of the synthesized hybrids 2a–o.

2.3. Molecular Docking Study

The prediction of the binding modes between a ligand and its intended target, as well as the correlation of the obtained scores with prospective activity, are all different applications of molecular docking in computer-aided drug design research [23]. The ability to estimate the impact of specific amino acid mutations on the ligand’s activity profile is another utility of molecular docking studies [24]. Additionally, visualizing the docking study’s resulting interactions aids future ligand modification, resulting in molecules with
better affinity properties [25]. Accordingly, a molecular docking study for synthesized hybrid compounds was performed for hCA isozymes I, II and IX to correlate the structural characteristics with the inhibitory activity and investigate the binding modes of the synthesized hybrids. Docking of the synthesized hybrids revealed that the benzensulfonamide ring fits deeply inside the shallow CA active site, anchoring the zinc ion via a metal bond with Zn$^{2+}$, which is typical for sulfonamide CAIs. Compound 2a was selected as a representative ligand to be docked over the different isoforms of CA due to its inhibitory profile, where it displayed a remarkable inhibitory profile over the tested isoforms with inhibition constant values of 79.8, 5.9, 205.8 and 54.2 nM over hCA I, II, IX and XII, respectively. For hCA I, compound 2a adhered to the common pattern of benzensulfonamides, where the benzensulfonamide ring fit deeply inside the surface CA active site through coordination with the zinc moiety in the distinctive manner of sulfonamide CAIs via NH$^-\cdot$Zn$^{2+}$ bond (Figure 4A). Additionally, compound 2a established two hydrogen bonds between the oxygens of the sulfonamide moiety with THR199 and HIS200 amino acid residues, which stabilized the binding orientation of compound 2a inside the active site cavity (Figure 4B). The presence of the hydrogen bonds and metal coordination bonds, as well as the weak hydrophobic interactions, contributed to the potent inhibition profile exhibited by compound 2a, as well as indicating a strong affinity to the active site of hCAI.

![Figure 4. The interaction patterns of compound 2a in the active sites of hCAI. (A) 3D model of compound 2a surface CA active site, with the surface viewed in the mesh format. (B) 2D interaction pattern of compound 2a with hCA I active site.](image)

Similarly, docking of compound 2a into the active site of hCA II exhibited the typical metal interaction of the sulfonamide group with the Zn metal (Figure 5B). Compound 2a established two additional hydrogen bonds with THR199 amino acid residue of hCA II which increased the stability of the interaction. Furthermore, compound 2a occupied most of the available space of the shallow active site (Figure 5A) indicating a strong binding affinity between the compound and the active site residues. Docking of compound 2a into the active site of hCA IX (Figure 5C,D) established two metal interactions between the sulfonamide group and Zn$^{2+}$ in the active site. It also exhibited a similar binding pattern when docked over the different isoforms of hCA, with the flexible amide linker allowing the ligand to fit deeply inside the active site surface to form the essential metal interaction with the Zn$^{2+}$.
Figure 5. The interaction patterns of compound 2a in the active sites of hCA II and IX. (A) 3D model of compound 2a surface hCA II active site, with the surface viewed in the mesh format. (B) 2D interaction pattern of compound 2a with hCA II active site. (C) 3D model of compound 2a surface hCA IX active site, with the surface viewed in the mesh format. (D) 2D interaction pattern of compound 2a with hCA IX active site.

3. Materials and Methods

3.1. Chemistry

The commercial chemicals utilized herein were of reagent grade and were used without further purification. Thermo Scientific 9200 apparatus was employed to measure the melting point of the synthesized compounds. Proton nuclear magnetic resonance (1H NMR) spectra were established using a Varian (400 MHz) spectrometer (Varian Medical Systems, Inc., Palo Alto, CA, USA). The 1H NMR spectra multiplicity was shown as in the subsequent abbreviations: singlet (s), doublet (d), doublet of doublet (dd), triplet (t), quartet (q), multiplet (m), and broad (b). Chemical shift values are expressed in δ values (ppm), while the coupling...
constant \((J)\) values are reported in hertz. \(^{13}\)C NMR spectra were obtained employing a Varian (100 MHz) spectrometer and the chemical shifts values are reported in parts per million (ppm) downfield from tetramethylsilane (internal standard) with coupling constants in hertz, as previously reported [26,27]. The G2 QTOF mass spectrometer (Waters Corporation, Milford, MA, USA) was employed to produce the mass spectra, high-resolution mass spectrometry (HRMS, ESI−MS), as previously used [28]. Infrared (IR) spectra were documented as KBr disks utilizing a Shimadzu FT-IR 8400S infrared spectrophotometer. The purification of the products was carried out employing column or flash column chromatography (Biotage, Uppsala, Sweden) with silica gel 60 (230–400 mesh Kieselgel 60), as previously carried out [29]. Moreover, monitoring of the reaction was achieved by employing thin-layer chromatography on 0.25 mm silica plates (E. Merck, silica gel 60 F254). High-performance liquid chromatography (HPLC) analysis was used to assess the optical purity of the synthesized compounds: Chiralpak IG-3 (4.6 × 150, 3), hexane/EtOH/MeOH = 85:10:5, 1.5 mL/min, and \(\lambda = 280 \text{ nm.}\)

**3-(1H-indol-3-yl)-N-(3-sulfamoylphenyl)propanamidine (2a).** In a round-bottom flask, the starting material \(1a\) (3-(1H-indol-3-yl)propanoic acid, 0.10 g, 0.50 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI, 0.14 g, 0.75 mmol) and hydroxybenzotriazole (HOBT, 0.10 g, 0.75 mmol) were dissolved in 8 mL of acetonitrile (MeCN) and stirred at room temperature for 30 min, followed by addition of the 3-amino-benzenesulfonamide (0.09 g, 0.75 mmol). The reaction mixture was stirred at room temperature overnight. After completion of the reaction, excess solvent was evaporated. The obtained residue was diluted with EA, and the organic layer was washed with water and brine. The organic layer was then separated and dried over magnesium sulfate and filtered. The residue was purified by column chromatography on silica gel (0–20% EA:Hex) to obtain the product. White solid. Yield: 35%, mp: 202–204 °C, HPLC purity: 9 min, 99.79%, \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 10.78 (s, 1H, indole-NH), 10.24 (s, 1H, amide-NH), 8.18 (s, 1H, Ph-H2′), 7.76–7.74 (m, 1H, Ph-H6′), 7.58–7.56 (d, \(J = 8.00\) Hz, 1H, indolyl-H4), 7.49–7.47 (d, \(J = 8.00\) Hz, 2H, Ph-H4′ and 5′), 7.35 (s, 2H, NH2), 7.34–7.32 (d, 1H, indolyl-H7), 7.13 (s, 1H, indolyl-H2), 7.08–7.04 (t, \(J = 8.00\) Hz, 1H, indolyl-H6), 7.00–6.96 (t, \(J = 8.00\) Hz, 1H, indolyl-H5), 3.05–3.01 (t, \(J = 8.00\) Hz, 2H, CH2), 2.73–2.69 (t, \(J = 8.00\) Hz, 2H, CH2). \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 171.81 (C=O amide), 144.98 (Ph-C3′), 140.03 (Ph-C1′), 136.65 (iodine-C7a), 129.84 (Ph-C5′), 127.40 (iodine-C3a), 122.63 (iodine-C2), 122.25 (Ph-C6′), 121.38 (iodine-C6), 120.49 (Ph-C4′), 118.78 (Ph-C2′), 118.62 (iodine-C5), 116.42 (iodine-C4), 113.95 (iodine-C3), 111.78 (iodine-C7), 37.70 (CH2-C=O), 21.17 (indole-CH2). HRMS (ESI) \(m/z\) calculated for \(C_{17}H_{17}N_3O_4S [M + H]^+\): 344.1069. Found: 344.1087.

**N-(2-hydroxy-5-sulfamoylphenyl)-3-(1H-indol-3-yl)propanamidine (2b).** In a dry round-bottom flask, compound \(1a\) (0.20 g, 1.06 mmol), propylphosphonic anhydride (T3P, 1.20 mL, 2.10 mmol) and DIPEA (0.55 mL, 3.17 mmol) were dissolved in 8 mL of tetrahydrofuran (THF) and stirred at room temperature overnight for 30 min, followed by the addition of the 3-amino-4-hydroxybenzenesulfonamide (0.24 g, 1.27 mmol). The reaction mixture was stirred at room temperature overnight. After completion of the reaction, excess solvent was evaporated. The obtained residue was diluted with EA, and the organic layer was washed with water and brine. The organic layer was then separated and dried over magnesium sulfate and filtered. The residue was purified by column chromatography on silica gel (0–5% EA:Hex) to obtain the product. White solid. Yield: 8.5%, mp: 203–205 °C, HPLC purity: 8 min, 97.00%, \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 10.77 (s, 1H, indole-NH), 9.37 (s, 1H, amide-NH), 8.42 (s, 1H, Ph-H6′), 7.59–7.57 (d, \(J = 7.66\) Hz, 1H, indolyl-H4), 7.41–7.38 (dd, \(J = 8.4, 2.3\) Hz, 1H, Ph-H5′), 7.34–7.32 (d, \(J = 7.86\) Hz, 1H, indolyl-H7), 7.14 (s, 1H, indolyl-H2), 7.13 (s, 2H, NH2), 7.08–7.05 (t, 1H, indolyl-H6), 7.00–6.94 (dd, \(J = 14.5, 8.0\) Hz, 2H, Ph-H3′ and indolyl-H5′), 3.04–2.98 (t, 2H, CH2), 2.87–2.74 (t, 2H, CH2). \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 174.25 (amidine-C=O), 153.10 (Ph-C2′), 138.83 (Ph-C5′), 137.01 (iodine-C7a), 129.63 (Ph-C1′), 128.92 (iodine-C3a), 124.94 (Ph-C4′), 124.83 (iodine-C2), 123.51 (iodine-C6), 122.33 (Ph-C3′), 121.01 (iodine-C5), 120.76 (iodine-C4), 117.50 (Ph-C6′), 116.22 (iodine-C3), 113.91 (iodine-C7), 39.31 (CH2-C=O), 23.46 (indole-CH2). HRMS (ESI) \(m/z\) calculated for \(C_{17}H_{17}N_3O_4S [M + H]^+\): 360.1018. Found: 360.1010.
3-(1H-indol-3-yl)-N-(4-sulfamoylphenethyl)propanamide (2c). Following the synthetic procedure and work-up conditions of compound 2a, 4-(2-aminoethyl)benzenesulfonamide was reacted with the starting material 1a. The obtained residue was purified by column chromatography on silica gel (0–15% EA:Hex). White solid. Yield: 25%, mp: 236–237 °C. HPLC purity: 13 min, 99.00%. 1H NMR (400 MHz, DMSO-d6) δ 10.75 (s, 1H, indole-NH), 7.95 (t, 1H, amide-NH), 7.72–7.70 (d, J = 8.00 Hz, 2H, Ph-H3' and H5'), 7.53–7.51 (d, J = 8.20 Hz, 1H, indolyl-H4), 7.33 (s, 2H, NH2), 7.31–7.29 (d, J = 8.20 Hz, 3H, indolyl-H7, and Ph-H2' and H6'), 7.08 (s, 1H, indolyl-H2), 7.06–7.04 (d, J = 8.20 Hz, 1H, indolyl-H6), 6.99–7.95 (t, J = 7.40 Hz, 1H, indolyl-H5), 3.29–3.26 (q, J = 6.50 Hz, 2H, CH2-NH), 2.90–2.88 (t, J = 7.4 Hz, 2H, CH2), 2.76–2.74 (t, J = 7.00 Hz, 2H, CH2), 2.43–2.39 (t, J = 7.60 Hz, 2H, CH2). HRMS (ESI) m/z calculated for C19H21N2O3S [M + H]+: 372.1382. Found: 372.1371.

3-(1H-indol-3-yl)-N-(4-sulfamoylphenyl)propanamide (2d). Following the synthetic procedure and work-up conditions of compound 2b, 4-aminobenzensulfonamide was reacted with the starting material 1a. The obtained residue was purified by column chromatography on silica gel (0–25% EA:Hex). White solid. Yield: 30%, mp: 198–199 °C. HPLC purity: 9 min, 99.98%. 1H NMR (400 MHz, DMSO-d6) δ 10.78 (s, 1H, indole-NH), 10.27 (s, 1H, amide-NH), 7.75 (s, 4H, Ph-H2', 3', 5', and 6'), 7.58–7.56 (d, J = 8.00 Hz, 1H, indolyl-H4), 7.34–7.32 (d, J = 8.00 Hz, 1H, indolyl-H6), 7.24 (s, 2H, NH2), 7.13 (s, 1H, indolyl-H2), 7.08–7.05 (t, J = 8.00 Hz, 1H, indolyl-H6), 7.00–6.96 (t, J = 8.00 Hz, 1H, indolyl-H5), 3.05–3.01 (t, J = 8.00 Hz, 2H, CH2), 2.71 (t, J = 8.00 Hz, 2H, CH2).

13C NMR (100 MHz, DMSO-d6) δ 171.98 (amide-C=O), 142.60 (Ph-C1'), 138.48 (indole-C7a), 136.65 (Ph-C4'), 127.40 (indole-C3a), 120.66 (Ph-C2' and C6'), 122.53 (indole-C2), 121.30 (indole-C6), 118.78 (indole-C5), 118.54 (indole-C4), 112.46 (indole-C3), 111.71 (indole-C7), 36.75 (CH2-NH), 35.28 (CH2-C=O), 31.13 (CH2-Ph), 21.42 (indole-C2). HMRs (ESI) m/z calculated for C19H21N2O3S [M + H]+: 344.1065. Found: 344.1069.

N-(3-sulfamoylphenyl)-1H-indole-5-carboxamide (2e). Following the synthetic procedure and work-up conditions of compound 2a, 3-aminobenzensulfonamide was reacted with the starting material 1b. The obtained residue was purified by column chromatography on silica gel (0–20% EA:Hex) to obtain the product. White solid. Yield: 60%, mp: 182–182 °C. HPLC purity: 15 min, 96.78%. 1H NMR (400 MHz, DMSO-d6) δ 11.83 (s, 1H, indole-NH), 8.66 (s, 1H, indolyl-H4), 8.20–8.18 (d, J = 8.70 Hz, 1H, indolyl-H6), 7.98–7.96 (d, J = 8.60 Hz, 1H, Ph-H6'), 7.00–7.88 (d, J = 8.30 Hz, 1H, indolyl-H7), 7.69–7.67 (d, J = 8.60 Hz, 2H, Ph-H4' and H5'), 7.62 (s, 1H, Ph-H2'), 7.57–7.53 (t, J = 7.60 Hz, 1H, indolyl-H2'), 6.75 (s, 1H, indolyl-H3). 13C NMR (100 MHz, DMSO-d6) δ 164.22 (amide-C=O), 143.26 (Ph-C3'), 140.24 (Ph-C1'), 129.65 (Ph-C5'), 128.99 (indole-C7a), 128.81 (indole-C5), 128.11 (Ph-C6'), 125.72 (indole-C3a), 125.45 (indole-C2), 123.26 (Ph-C4'), 120.31 (Ph-C2'), 114.36 (indole-C4), 112.86 (indole-C7), 109.76 (indole-C6), 103.73 (indole-C3). HMRs (ESI) m/z calculated for C13H15N3O3S [M + H]+: 316.0756. Found: 316.0782, 230.0630, 144.0434, 120.0550, and 111.0198 (see Supplementary Materials).

N-(4-sulfamoylphenyl)-1H-indole-5-carboxamide (2f). Following the synthetic procedure and work-up conditions of compound 2a, 4-aminobenzensulfonamide was reacted with the starting material 1b. The obtained residue was diluted with EA and the organic layer was washed with water and brine. The obtained residue was purified by column chromatography on silica gel (0–20% EA:Hex). White solid. Yield: 17%, mp: 257–259 °C. HPLC purity: 15 min, 100%. 1H NMR (400 MHz, DMSO-d6) δ 11.82 (s, 1H, indole-NH), 8.66 (s, 1H, indolyl-H4), 8.20–8.18 (d, J = 8.30 Hz, 1H, indolyl-H6), 7.98–7.95 (d, J = 8.7 Hz, 1H, indolyl-H7), 7.90–7.88 (d, J = 8.6 Hz, 1H, indolyl-H2), 7.69–7.55 (m, 4H, Ph-H2', H3', H5', and H6'), 6.75 (s, 1H, indolyl-H3). 13C NMR (100 MHz, DMSO-d6) δ 164.22 (amide-C=O), 143.26 (Ph-C1'), 140.24 (Ph-C4'), 129.65 (Ph-C3' and 5'), 128.81 (indole-C7a), 128.11 (indole-C5), 125.45 (indole-C3a), 123.26 (indole-C2), 120.31 (Ph-C2' and 6'), 114.36 (indole-C4), 112.86 (indole-C7), 109.76 (indole-C6), 103.73 (indole-C3). HMRs (ESI) m/z calculated for C13H15N3O3S [M + H]+: 316.0756. Found: 316.0782, 230.0630, 144.0434, 120.0550, and 111.0198 (see Supplementary Materials).
C₁₅H₁₇N₃O₅S [M + H]+: 316.0756. Found: 279.0888, 144.0443, 120.0555, and 111.0207 (see Supplementary Materials).

\( N \)-(2-hydroxy-5-sulfamoylphenyl)-1H-indole-5-carboxamide (2g). Following the synthetic procedure in dimethylformamide/dichloromethane (DMF/DCM = 5/1) and work-up conditions of compound 2a, 3-amino-4-hydroxybenzenesulfonamide was reacted with the starting material 1b. The obtained residue was purified by column chromatography on silica gel (0–10% EA:Hex). White solid. Yield: 0.63%, mp: 189–190 °C, HPLC purity: 15 min, 99.62%, \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 11.82 (s, 1H, indole-NH), 8.66 (s, 1H, indolyl-H4), 8.20–8.18 (d, \(J = 8.50\) Hz, 1H, indolyl-H6), 7.91–7.89 (m, 1H, Ph-H4'), 7.90–7.88 (d, \(J = 8.00\) Hz, 1H, indolyl-H7), 7.69–7.66 (m, 2H, Ph-H2' and H6'), 7.63–7.61 (m, 1H, indolyl-H2), 6.75 (s, 1H, indolyl-H3). 13C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 168.84 (amide-C=O), 138.74 (indole-C7a), 128.22 (Ph-C5'), 127.76 (indole-C3a), 127.59 (Ph-C1'), 124.93 (indole-C2), 123.22 (Ph-C4'), 121.80 (indole-C4), 119.57 (Ph-C6'), 111.52 (indole-C7), 110.02 (indole-C6), 102.90 (indole-C3). HRMS (ESI) m/z calculated for C\(_{15}\)H\(_{17}\)N\(_3\)O\(_5\)S [M + H]+: 332.0705. Found: 332.0701.

\( N \)-(4-sulfamoylphenethyl)-1H-indole-5-carboxamide (2h). Following the synthetic procedure and work-up conditions of compound 2b, 4-(2-aminoethyl)benzenesulfonamide was reacted with the starting material 1b. The obtained residue was purified by column chromatography on silica gel (0–6% DCM:MeOH). Orange solid. Yield: 35%, mp: 209–211 °C, HPLC purity: 6 min, 97.68%, \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 11.31 (s, 1H, indole-NH), 8.43–8.41 (t, 1H, amide-NH), 8.08 (s, 1H, indolyl-H4), 7.75–7.73 (d, \(J = 8.00\) Hz, 2H, Ph-H3' and H5'), 7.61–7.59 (d, \(J = 8.00\) Hz, 1H, indolyl-H6), 7.45–7.39 (m, 4H, indolyl-H2 and H7 & Ph-H2' and H6'), 7.28 (s, 2H, NH2), 6.52 (s, 1H, indolyl-H3), 3.55–3.50 (m, 2H, CH2), 2.96–2.92 (t, \(J = 8.00\) Hz, 2H, CH2-Ph). 13C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 167.71 (amide-C=O), 144.42 (Ph-C1'), 142.39 (Ph-C4'), 137.75 (indole-C7a), 129.56 (Ph-C3' and C5'), 127.38 (indole-C3a), 127.05 (indole-C5), 126.10 (Ph-C2' and C6'), 125.95 (indole-C2), 120.86 (indole-C4), 120.17 (indole-C7), 111.26 (indole-C6), 102.47 (indole-C3), 35.40 (CH2-NH), 31.12 (CH2-Ph). HRMS (ESI) m/z calculated for C\(_{17}\)H\(_{17}\)N\(_3\)O\(_5\)S [M + H]+: 344.1069. Found: 344.1060.

\( N \)-(2-sulfamoylphenyl)-1H-indole-6-carboxamide (2i). Following the synthetic procedure and work-up conditions of compound 2a, 2-aminothiazole-4-carboxamide was reacted with the starting material 1b. The obtained residue was purified by column chromatography on silica gel (0–15% EA:Hex). White solid. Yield: 23%, mp: 192–193 °C, HPLC purity: 15 min, 98.02%, \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 12.40 (s, 1H, amide-NH), 11.43 (s, 1H, indole-NH), 8.23 (s, 1H, indolyl-H4), 8.00–7.98 (d, \(J = 8.10\) Hz, 1H, indolyl-H6), 7.74–7.70 (t, \(J = 8.00\) Hz, 2H, Ph-H4' and H5'), 7.57–7.53 (t, 1H, indolyl-H2), 7.45–7.40 (m, 3H, indolyl-H7, and Ph-H3' and H6'), 6.57 (s, 1H, indolyl-H3). 13C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 164.21 (amide-C=O), 143.26 (Ph-C2'), 140.24 (Ph-C1'), 129.63 (Ph-C5'), 128.99 (indole-C7a), 128.80 (indole-C5), 128.11 (Ph-C6'), 125.71 (indole-C3a), 125.44 (indole-C2), 123.25 (Ph-C4'), 120.31 (Ph-C2'), 114.36 (indole-C4), 112.85 (indole-C7), 109.75 (indole-C6), 103.72 (indole-C3).

\( N \)-(3-sulfamoylphenyl)-1H-indole-6-carboxamide (2j). Following the synthetic procedure and work-up conditions of compound 2a, 3-aminothiazole-4-carboxamide was reacted with the starting material 1c. The obtained residue was recrystallized twice using methanol and DCM. Orange solid. Yield: 32%, mp: 201–211 °C, HPLC purity: 15 min, 99.67%, \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 11.83 (s, 1H, indole-NH), 8.43 (s, 1H, indolyl-H7), 8.20–8.18 (d, \(J = 8.50\) Hz, 1H, indolyl-H4), 7.92–7.80 (m, 4H, indolyl-H5, and Ph-H2', H4', H5'), 7.70–7.66 (m, 1H, Ph-H5'), 7.58–7.54 (m, 1H, indolyl-H2), 6.69 (s, 1H, indolyl-H3). 13C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 164.24 (amide-C=O), 143.26 (Ph-C3'), 135.48 (Ph-C1'), 133.75 (indole-C7a), 131.98 (Ph-C5'), 129.65 (Ph-C6'), 129.00 (indole-C3a), 125.73 (indole-C6), 121.25 (indole-C2), 120.74 (Ph-C4'), 120.31 (Ph-C2'), 115.76 (indole-C4), 115.73 (indole-C5), 109.79 (indole-C7), 102.72 (indole-C3).

\( N \)-(4-sulfamoylphenyl)-1H-indole-6-carboxamide (2k). Following the synthetic procedure and work-up conditions of compound 2a, 4-aminothiazole-4-carboxamide was reacted with the starting material 1c. The obtained residue was purified by column chromatography on silica gel (0–20% EA:Hex). White solid. Yield: 13%, mp: 221–222 °C, found: 279.0888, 144.0443, 120.0555, and 111.0207.
N-(2-hydroxy-5-sulfamoylphenyl)-1H-indole-6-carboxamide (2f). Following the synthetic procedure and work-up conditions of compound 2a, 3-amino-4-hydroxybenzenesulfonamide was reacted with the starting material 1c. The obtained residue was purified by column chromatography on silica gel (0–15% EA:Hex). Orange solid. Yield: 23%, mp: 213–215 °C, HPLC purity: 15 min, 98.03%. 1H NMR (400 MHz, DMSO-d6) δ 11.83 (s, 1H, indole-NH), 8.42 (s, 1H, indolyl-H7), 8.20–8.18 (d, J = 8.5 Hz, 1H, indolyl-H4), 7.92–7.60 (m, 4H, Ph-H2, H3, H5 and H6), 7.70–7.66 (t, 1H, indolyl-H5), 7.58–7.53 (t, 1H, indolyl-H2), 6.69 (s, 1H, indolyl-H3). 13C NMR (100 MHz, DMSO-d6) δ 168.80 (amide-C=O), 135.58 (Ph-C1′), 131.47 (Ph-C4′), 129.32 (indole-C4), 128.23 (indole-C7a), 127.80 (indole-C3a), 124.93 (indole-C6), 123.52 (indole-C2), 120.20 (Ph-C2′ and C6′), 120.01 (Ph-C3′ and C5′), 113.94 (indole-C5), 109.99 (indole-C7), 101.87 (indole-C3).

N-(4-sulfamoylphenethyl)-1H-indole-6-carboxamide (2m). Following the synthetic procedure with solvent (DMF/DCM = 5/1) and work-up conditions of compound 2a, 4-(2-aminoethyl)benzenesulfonamide was reacted with the starting material 1c. The obtained residue was purified by column chromatography on silica gel (0–10% EA:Hex). White solid. Yield: 28%, mp: 250–252 °C, HPLC purity: 1 min, 96.73%. 1H NMR (400 MHz, DMSO-d6) δ 11.37 (s, 1H, indole-NH), 8.49–8.46 (t, J = 8.00 Hz, 1H, amide-NH), 7.92 (s, 1H, indolyl-H7), 7.76–7.74 (d, J = 8.00 Hz, 2H, Ph-H3′ and H5′), 7.57–7.55 (d, J = 8.00 Hz, 1H, indolyl-H5), 7.50–7.48 (m, 2H, indolyl-H2 and H4), 7.45–7.43 (d, J = 8.00 Hz, 2H, Ph-H2′ and H6′), 7.28 (s, 2H, NH2), 6.47 (s, 1H, indolyl-H3), 3.55–3.50 (q, 2H, CH2-NH), 2.95–2.93 (t, J = 8.00 Hz, 2H, CH2-Ph). 13C NMR (100 MHz, DMSO-d6) δ 167.63 (amide-C=O), 144.40 (Ph-C1′), 142.43 (Ph-C4′), 135.65 (indole-C7a), 130.17 (indole-C3a), 129.55 (Ph-C3′ and C5′), 128.31 (indole-C2), 127.82 (indole-C6), 126.11 (Ph-C2′ and C6′), 119.78 (indole-C4), 118.31 (indole-C5), 111.52 (indole-C7), 101.61 (indole-C3), 40.92 (CH2-NH), 35.38 (CH2-Ph).

N-(2-sulfamoylphenyl)-1H-indole-6-carboxamide (2n). Following the synthetic procedure and work-up conditions of compound 2a, 2-aminobenzenesulfonamide was reacted with the starting material 1c. The obtained residue was purified by column chromatography on silica gel (0–12% EA:Hex). White solid. Yield: 10%, mp: 222–223 °C, HPLC purity: 15 min, 98.70%. 1H NMR (400 MHz, DMSO-d6) δ 11.82 (s, 1H, indole-NH), 8.42 (s, 1H, indolyl-H7), 8.20–8.18 (d, J = 8.4 Hz, 1H, indolyl-H4), 7.91–7.89 (d, 2H, Ph-H3′ and H6′), 7.81–7.78 (t, 1H, Ph-H4′), 7.70–7.66 (t, J = 7.3 Hz, 1H, Ph-H5′), 7.57–7.54 (t, 1H, indolyl-H2), 6.69 (s, 1H, indolyl-H3). 13C NMR (100 MHz, DMSO-d6) δ 164.24 (amide-C=O), 143.26 (Ph-C2′), 135.48 (Ph-C1′), 133.74 (indole-C7a), 129.66 (Ph-C3′), 128.99 (indole-C3a), 125.74 (indole-C6), 121.25 (indole-C2), 120.74 (Ph-C5′), 120.32 (Ph-C4′), 120.20 (Ph-C6′), 115.75 (indole-C4), 115.72 (indole-C5), 109.80 (indole-C7), 102.72 (indole-C3).

N-(1H-indol-5-yl)-4-sulfamoylbenzamide (2o). Following the synthetic procedure with solvent (DMF/DCM = 5/1) and work-up conditions of compound 2a, 4-sulfamoylbenzoic acid was reacted with the starting material 1d. The obtained residue was purified by column chromatography on silica gel (0–8% DCM:MeOH). Purple solid. Yield: 25%, mp: 261–262 °C, HPLC purity: 6 min, 97.38%. 1H NMR (400 MHz, DMSO-d6) δ 11.06 (s, 1H, indole-NH), 10.26 (s, 1H, amide-NH), 8.13–8.11 (d, J = 8.00 Hz, 2H, Ph-H2′ and H6′), 8.00 (s, 1H, indolyl-H4′), 7.96–7.94 (d, J = 8.00 Hz, 2H, Ph-H3′ and H5′), 7.52 (s, 2H, NH2), 7.41–7.33 (m, 3H, indolyl-H2, H6, and H7), 6.42 (s, 1H, indolyl-H3). 13C NMR (100 MHz, DMSO-d6) δ 164.44 (amide-C=O), 146.61 (Ph-C4′), 138.79 (Ph-C1′), 133.52 (indole-C7a), 131.07 (indole-C5), 128.64 (Ph-C2′ and C6′), 127.81 (indole-C2), 126.43 (indole-C3a), 126.03 (Ph-C3′ and C5′), 116.38 (indole-C6),
112.64 (indole-C7), 111.53 (indole-C4), 101.63 (indole-C3). HRMS (ESI) m/z calculated for C_{15}H_{13}N_{3}O_{3}S [M + H]^+ 316.0756. Found: 316.0757.

3.2. Carbonic Anhydrase Inhibition

The inhibitor and enzyme solutions were first preincubated together for 15 min at room temperature to enable for the generation of the E–I complex. Photophysics stopped-flow instrument was used to measure the CA-catalyzed CO$_2$ hydration activity. Phenol red (at a concentration of 0.2 mM) was added as an indicator after a period of 10–100 s in the CA-catalyzed CO$_2$ hydration reaction at an absorbance maximum of 557 nm, with 20 mM Hepes (pH = 7.4) and 10 mM NaClO$_4$ (to maintain constant ionic strength) [30]. The kinetic parameters and inhibition constants were calculated at CO$_2$ concentrations ranging from 1.7 to 17 mM [31]. In the initial 5–10% of the reaction, at least six traces were examined to determine the initial velocity for each inhibitor. Similarly, the total measured rates were used to measure the uncatalyzed rates [32]. Distilled–deionized water was used in the preparation of the Stock inhibitor solutions (10 mM), as well as dilutions up to 0.01 nM. The inhibition constants were measured utilizing PRISM 3, whereas the Lineweaver–Burk plots were used to measure the kinetic parameters which comprised the mean of at least three separate measurements [33,34].

3.3. Molecular Docking

hCA I (PDB code: 6Y00, resolution 1.37 Å) [35], hCA II (PDB code: 4BF1, resolution 1.35 Å) and hCA IX (PDB code: 4ZWX, resolution 1.70 Å) [36] crystal structures were retrieved from the Protein Data Bank (www.pdb.org, accessed on 19 February 2022). The downloaded crystal structures were prepared employing the preparation wizard of the Schrodinger 2021 suite package under the default settings with the pH value set at a value of 7.4. All ligands were first sketched via ChemDraw Professional 17.0, then exported as a structure data file format and sent to the Ligprep module. Ligand preparation was achieved utilizing the Schrodinger Ligprep module to optimize the geometry of the studied ligands. Glide’s standard precision module was employed to dock the minimized ligands into the appropriate crystal structure binding site, yielding 10 poses for each docked ligand.

4. Conclusions

A new hybrid series of indole-based benzenesulfonamides were successfully synthesized, characterized, and biologically evaluated against four isoforms of CA (I, II, IX, and XII). The inhibitory activities of the hybrid compounds 2a–o and AAZ, as a standard inhibitor, were measured against the four isoforms of hCA by a stopped flow CO$_2$ hydrase assay. Compared to AAZ, compounds 2a, 2d and 2o showed higher inhibitory activities over hCA I. Among all, compound 2a displayed the most potent activity and selectivity against hCA II. Compounds 2b, 2c, 2d, 2f, 2h and 2o also showed low nanomolar inhibitory activities against hCA II. The selectivity profiles of the synthesized target compounds, as well as their SAR, display the high potential of this series to be further investigated as a promising lead towards more potent and selective hCA II inhibitors.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms23052540/s1. NMR, HPLC, and HRMS representative data for the newly synthesized compounds in this article.

Author Contributions: Conceptualization, A.E. and K.L.; resources, K.L.; project administration, A.E.; synthesis and data analysis, A.E., J.W. and T.M.B.; determination of inhibitory activity against hCA, A.A. and C.T.S.; molecular docking and modeling, H.N.; Structure–activity relationship determination, A.E. and H.N.; writing—original draft preparation, A.E., J.W., H.N., T.M.B.; writing—review and editing, all authors. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2018R1A5A2023127) and the BK21 FOUR program through NRF funded by the Ministry of Education of Korea.
Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Acknowledgments: A.E. extends his appreciation to Korea Institute of Science and Technology (KIST) for supporting this work through “2022 KIST School Partnership project” and in the accomplishment of this project, he would like to thank the Technology Innovation Commercial Office (TICO) at Mansoura University for their highly effective contribution.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Nocentini, A.; Supuran, C.T.; Capasso, C. An overview on the recently discovered iota-carbonic anhydrases. J. Enzym. Inhib. Med. Chem. 2021, 36, 1988–1995. [CrossRef] [PubMed]
2. Supuran, C.T. Carbonic anhydrases: Novel therapeutic applications for inhibitors and activators. Nat. Rev. Drug Discov. 2008, 7, 168–181. [CrossRef] [PubMed]
3. Topal, F.; Aksu, K.; Gulcin, I.; Tümer, F.; Goksu, S. Inhibition Profiles of Some Symmetric Sulfamides Derived from Phenethylamines on Human Carbonic Anhydrase, I, and II Isoenzymes. Chem. Biodivers. 2021, 18, e2100422. [CrossRef] [PubMed]
4. Durdagi, S.; Şentürk, M.; Ekinci, D.; Balaydın, H.T.; Göksu, S.; Küfrevioğlu, Ö.I.; Innocenti, A.; Scozzafava, A.; Supuran, C.T. Kinetic and docking studies of phenol-based inhibitors of carbonic anhydrase isofoms I, II, IX and XII evidence a new binding mode within the enzyme active site. Bioorg. Med. Chem. 2011, 19, 1381–1389. [CrossRef] [PubMed]
5. Supuran, C.T.; Scozzafava, A. Carbonic anhydrase inhibitors and their therapeutic potential. Expert Opin. Ther. Pat. 2000, 10, 575–600. [CrossRef]
6. Mohamed, M.M.; Sloane, B.F. multifunctional enzymes in cancer. Nat. Rev. Cancer 2006, 6, 764–775. [CrossRef]
7. Haapasalo, J.; Nordfors, K.; Järvelä, S.; Bragge, H.; Rantalainen, J.; Parkkila, S.; Laaksonen, H.; Haapasalo, H.; Parkkila, S. Carbonic anhydrase II in the endothelium of glial tumors: A potential target for therapy. Neuro-Oncol. 2007, 9, 308–313. [CrossRef]
8. Cuffaro, D.; Nuti, E.; Rossello, A. An overview of carbohydrate-based carbonic anhydrase inhibitors. J. Enzym. Inhib. Med. Chem. 2020, 35, 1906–1922. [CrossRef]
9. Awadallah, F.M.; Bu, S.; Mahmoud, W.R.; Nada, H.H.; Nocentini, A.; Supuran, C.T. Inhibition studies on a panel of human carbonic anhydrases with N1-substituted secondary sulfonamides incorporating thiazolinone or imidazolone-indole tails. J. Enzym. Inhib. Med. Chem. 2018, 33, 629–638. [CrossRef]
10. Supuran, C.T. Carbon- versus sulphur-based zinc binding groups for carbonic anhydrase inhibitors? J. Enzym. Inhib. Med. Chem. 2018, 33, 485–495. [CrossRef]
11. Sanka, R.K.K. Development and Biological Evaluation of Carbonic Anhydrase Modulators as Potential Nootropics and Anticancer Agents. Ph.D. Thesis, Temple University, Ann Arbor, MI, USA, 2018.
12. Supuran, C.T. Advances in structure-based drug discovery of carbonic anhydrase inhibitors. Expert Opin. Drug Discov. 2017, 12, 61–88. [CrossRef] [PubMed]
13. 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] [PubMed]
14. Aggarwal, M.; Kondeti, B.; McKenna, R. Insights towards sulfonamide drug specificity in α-carbonic anhydrases. Bioorg. Med. Chem. 2013, 21, 1526–1533. [CrossRef] [PubMed]
15. Carta, F.; Supuran, C.T.; Scozzafava, A. Sulfonamides and their isosters as carbonic anhydrase inhibitors. Future Med. Chem. 2014, 6, 1149–1165. [CrossRef] [PubMed]
16. Chiaramonte, N.; Bu, S.; Ferraroni, M.; Nocentini, A.; Bonardi, A.; Bartolucci, G.; Durante, M.; Lucarini, L.; Chiapponi, D.; Dei, S.; et al. 2-Benzylpiperezine: A new scaffold for potent human carbonic anhydrase inhibitors. Synthesis, enzyme inhibition, enantioselectivity, computational and crystallographic studies and in vivo activity for a new class of intraocular pressure lowering agents. Eur. J. Med. Chem. 2018, 151, 363–375. [CrossRef] [PubMed]
17. Singh, P.; Swain, B.; Thacker, P.S.; Sigalapalli, D.K.; Yadav, P.P.; Angeli, A.; Supuran, C.T.; Arifuddin, M. Synthesis and carbonic anhydrase inhibition studies of sulfonamide based indole-1,2,3-triazole chalcone hybrids. Bioorg. Chem. 2020, 99, 103839. [CrossRef] [PubMed]
18. Demir-Yazıcı, K.; Bu, S.; Akgülmuş, N.M.; Akdemir, A.; Supuran, C.T.; Güzel-Akdemir, Ö. Indole-Based Hydrazones Containing A Sulfonamide Moiety as Selective Inhibitors of Tumor-Associated Human Carbonic Anhydrase Isoforms IX and XII. Int. J. Mol. Sci. 2019, 20, 2354. [CrossRef]
19. Peerzada, M.N.; Khan, P.; Ahmad, K.; Hassan, M.I.; Azam, A. Synthesis, characterization and biological evaluation of tertiary sulfonamide derivatives of pyridyl-Indole based heteroaryl chalcone as potential carbonic anhydrase IX inhibitors and anticancer agents. Eur. J. Med. Chem. 2018, 155, 13–23. [CrossRef]
20. George, R.F.; Bu, S.; Supuran, C.T.; Awadallah, F.M. Synthesis of some N-aroyl-2-oxindole benzenesulfonamide conjugates with carbonic anhydrase inhibitory activity. Bioorg. Chem. 2020, 96, 103635. [CrossRef]
21. Assi, R.; Kantarjian, H.M.; Kadia, T.M.; Pemmaraju, N.; Jabbour, E.; Jain, N.; Daver, N.; Estrov, Z.; Uehara, T.; Owa, T.; et al. Final results of a phase 2, open-label study of indisulam, idarubicin, and cytarabine in patients with relapsed or refractory acute myeloid leukemia and high-risk myelodysplastic syndrome. *Cancer* 2018, 124, 2758–2765. [CrossRef] [PubMed]

22. Bozdag, M.; Carta, F.; Ceruso, M.; Ferraroni, M.; 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] [PubMed]

23. Macalino, S.J.Y.; Gosu, V.; Hong, S.; Choi, S. Role of computer-aided drug design in modern drug discovery. *Arch. Pharmacal Res.* 2015, 38, 1686–1701. [CrossRef] [PubMed]

24. Guedes, I.A.; de Magalhães, C.S.; Dardenne, L.E.J.B.R. Receptor–ligand molecular docking. *Biophys. Rev.* 2014, 6, 75–87. [CrossRef] [PubMed]

25. Wang, J.; Li, Y.; Yang, Y.; Du, J.; Zhang, S.; Yang, L. In silico research to assist the investigation of carboxamide derivatives as potent TRPV1 antagonists. *Mol. Biosyst.* 2015, 11, 2885–2899. [CrossRef] [PubMed]

26. Elkamhawy, A.; Kim, N.Y.; Hassan, A.H.E.; Park, J.E.; Yang, J.E.; Oh, K.S.; Lee, B.H.; Lee, M.Y.; Shin, K.J.; Lee, K.T.; et al. Design, synthesis and biological evaluation of novel thiazolidinedione derivatives as irreversible allosteric IKK-β modulators. *Eur. J. Med. Chem.* 2018, 157, 691–704. [CrossRef] [PubMed]

27. Park, J.E.; Elkamhawy, A.; Hassan, A.H.E.; Pae, A.N.; Lee, J.; Paik, S.; Park, B.G.; Roh, E.J. Synthesis and evaluation of new pyridyl/pyrazinyl thiourea derivatives: Neuroprotection against amyloid-β-induced toxicity. *Eur. J. Med. Chem.* 2017, 141, 322–334. [CrossRef] [PubMed]

28. Elkamhawy, A.; Hassan, A.H.E.; Paik, S.; Sup Lee, Y.; Lee, H.H.; Shin, J.S.; Lee, K.T.; Roh, E.J. EGFR inhibitors from cancer to inflammation: Discovery of 4-fluoro-N-(4-(3-(trifluoromethyl)phenoxy)pyrimidin-5-yl)benzamide as a novel anti-inflammatory EGFR inhibitor. *Bioorg. Chem.* 2019, 86, 112–118. [CrossRef] [PubMed]

29. Al-Sanea, M.M.; Elkamhawy, A.; Zakaria, A.; Park, B.S.; Kwon, Y.; Lee, S.H.; Lee, S.W.; Kim, I.T. Synthesis and in vitro screening of phenylbipyridinylpyrazole derivatives as potential antiproliferative agents. *Molecules* 2015, 20, 1031–1045. [CrossRef] [PubMed]

30. Bozdag, M.; Ferraroni, M.; Nuti, E.; Vullo, D.; Rossello, A.; Carta, F.; 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] [PubMed]

31. Alafeefy, A.M.; Elkamhawy, A.; Hassan, A.H.E.; Kwon, Y.; Lee, S.H.; Lee, S.W.; Kim, I.T. Synthesis and in vitro screening of phenylbipyridinylpyrazole derivatives as potential antiproliferative agents. *Molecules* 2015, 20, 1031–1045. [CrossRef] [PubMed]

32. Menchise, V.; De Simone, G.; Alterio, V.; Di Fiore, A.; Pedone, C.; Scozzafava, A.; Supuran, C.T. Metalloenzyme Antitumor Sulfonamide Complexes with Isozyme II. *J. Med. Chem.* 2005, 48, 5721–5727. [CrossRef]

33. 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]

34. Bozdag, M.; Ferraroni, M.; Nuti, E.; Vullo, D.; Rossello, A.; Carta, F.; Scozzafava, A.; Supuran, C.T. Combining the tail and the ring approaches for obtaining potent and isoform-selective carbonic anhydrase inhibitors: Solution and X-ray crystallographic studies. *Bioorg. Med. Chem.* 2014, 22, 334–340. [CrossRef] [PubMed]

35. Ali, M.; Bozdag, M.; Farooq, U.; Angeli, A.; Carta, F.; Berto, P.; Zanotti, G.; Supuran, C.T. Benzylaminoethyureido-Tailed Benzenesulfonamides: Design, Synthesis, Kinetic and X-ray Investigations on Human Carbonic Anhydrases. *Int. J. Mol. Sci.* 2020, 21, 2560. [CrossRef] [PubMed]

36. 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]