International Journal of
Molecular Sciences

MDPI

Article

Benzenesulfonamides Incorporating Hydantoin Moieties Effectively Inhibit Eukaryoticand Human Carbonic Anhydrases

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Abstract: A series of novel 1-(4-benzenesulfonamide)-3-alkyl/benzyl-hydantoin derivatives were synthesized and evaluated for the inhibition of eukaryotic and human carbonic anhydrases (CAs, EC 4.2.1.1). The prepared compounds were screened for their hCA inhibitory activities against three cytosolic isoforms as well as two β-CAs from fungal pathogens. The best inhibition was observed against hCA II and VII as well as *Candida glabrata* enzyme CgNce103. hCA I and *Malassezia globosa* MgCA enzymes were, on the other hand, less effectively inhibited by these compounds. The inhibitory potency of these compounds against CAs was found to be dependent on the electronic and steric effects of substituent groups on the N3-position of the hydantoin ring, which included alkyl, alkenyl and substituted benzyl moieties. The interesting results against CgNce103 make the compounds of interest for investigations in vivo as potential antifungals.

Keywords: carbonic anhydrase inhibitors; sulfonamides; hydantoin

Citation: Abdoli, M.; De Luca, V.; Capasso, C.; Supuran, C.T.; Žalubovskis, R. Benzenesulfonamides Incorporating Hydantoin Moieties Effectively Inhibit Eukaryoticand Human Carbonic Anhydrases. *Int. J. Mol. Sci.* **2022**, *23*, 14115. https://doi.org/10.3390/ijms232214115

Academic Editor: Alexey Nazarov

Received: 18 October 2022
Accepted: 12 November 2022
Published: 15 November 2022

1. Introduction

Due to the involvement of enzymes in many pathological conditions, their inhibitors are recognized as promising targets for developing novel drugs [1,2]. Interestingly, greater than one-third of current drug discovery pipelines are focused on enzyme drug targets and half of all marketed drugs are enzyme inhibitors [3]. Carbonic anhydrases (CAs, E.C.4.2.1.1) are an important family of metalloenzymes that assist the reversible interconversion of carbon dioxide and water to bicarbonate and proton ($CO_2 + H_2O \leftrightharpoons HCO_3^- + H^+$) and thereby play fundamental roles in many processes such as respiration, electrolyte secretion, pH homeostasis, and bone resorption [4–6]. They are, therefore, a common and valuable drug target for the treatment or prevention of a variety of disorders [7–9]. Two of the fifteen known human (h) CA isoforms, hCA II and VII, are key cytosolic isoforms involved in brain metabolism and neuronal excitation [10]. Consequently, isoform-selective hCA II/VII inhibitors are identified as potential therapeutic targets for neurological diseases and disorders such as epilepsy, seizures, and Alzheimer disease [11,12]. In this context, inhibition of these isozymes was recently proposed as a new approach for the management of neuropathic pain [13–15]. It should be noted that the lack of approved medicines for the treatment of neuropathic pain as well as many other conditions in which CA activity is unbalanced is one of the major challenges in medicine [16–40]. Due to their unique zinc-binding properties as anions, primary sulfonamides ($-SO_2NH_2$) are the main classes of CAs inhibitors (CAIs) [16–26] and, not surprisingly, the majority of reported CA inhibitors (CAIs) contain at least one sulfonamide moiety in their structures [27–29]. Very recently,

our group disclosed that the clinically used antibiotic Furagin (Figure 1a), which contains hydantoin moiety, shows effective inhibitory activity on several hCAs [30]. Along this line, we herein extend this earlier investigation to series of 1-(4-benzenesulfonamide)-3-alkyl/benzyl-hydantoin derivatives, with special emphasize on their inhibitory effects against CA II and VII (Figure 1b). The newly developed compounds were also tested for the inhibition of two β-CAs from fungal pathogens. Indeed, in many pathogenic bacteria [41–47] and fungi [48–52], CAs belonging to several genetic familieshaverelevant physiologic functions and their inhibition may lead to anti-infective effects [53–57].

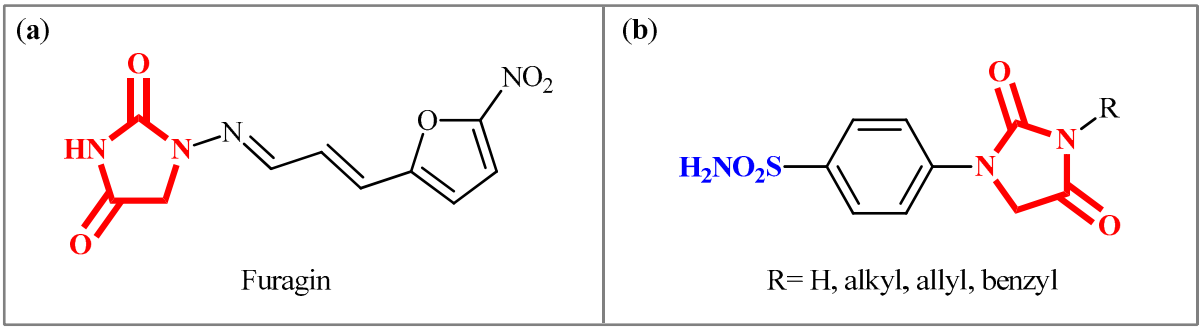


Figure 1. (**a**) Structure of Furagin; (**b**) General structure of 1-(4-benzenesulfonamide)-3-alkyl/benzyl-hydantoins discussed in the paper.

2. Results and Discussion

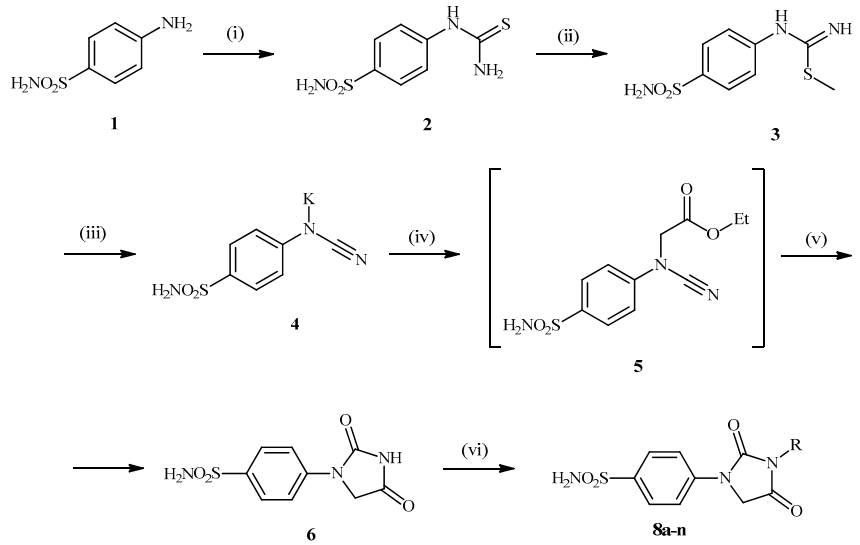
2.1. Compounds Design and Synthesis

Considering the fact that hydantoins already possess CA inhibitory effects [30], the drug design strategy that we propose in this paper is to incorporate in the same molecule both a zinc binder fragment of the benzene-sulfonamide type [4–9,16–18] as well as the tail based on the 3-substituted-hydantoin motif.

The synthesis of the target 1-(4-benzenesulfonamide)-3-alkyl/benzyl-hydantoin derivatives is shown in Scheme 1. The synthesis started from sulfanilamide **1,** which was converted to 4-thioureidobenzenesulfonamide (**2**)via reaction with KSCN in aqueous, acidic medium [33]. The key intermediate, potassium cyano(4-sulfamoylphenyl)amide **4,** was prepared by the selective *S*-methylation of thiourea **2** via treatment with 1 equiv. of MeI, followed by elimination of metheylthiolate from the formed methyl (4-sulfamoylphenyl) carbamimidothioate (**3**) by treatment with K_2CO_3 at elevated temperature. Subsequently, intermediate **4** was treated with ethyl 2-bromoacetate, leading to **5,** which was treated with hydrochloric acid at an elevated temperature, thus affording 4-(2,4-dioxoimidazolidin-1-yl)benzenesulfonamide (**6**). In the final step, the selective *N*-alkylation/benzylation of the NH hydantoin moiety with various alkyl/allyl/benzyl-halides (**7a–n**) provided the desired compounds (**8a–n**) in acceptable to good yield. ^{1}H NMR, ^{13}C NMR, and HRMS techniques were used to confirm the chemical structure of all of the synthesized compounds. All the analyzed compounds were >95% HPLC pure.

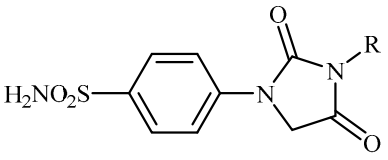
2.2. Carbonic Anhydrase Inhibition

The new compounds designed here were tested as inhibitors of three human enzymes, i.e., isoforms hCA I, II, and VII (all cytosolic ones) [4–9,16–18], as well as two fungal β-CAs from pathogenic organisms: MgCA from *Malassezia globosa*, one of the fungi involved in dandruff formation [58–61]; and CgNce103 from *Candida glabrata*, a species known for its virulence and resistance to many classes of antifungal drugs in clinical use [62–66]. The classical sulfonamide CAI acetazolamide (5-acetamido-1,3,4-thiadiazole-2-sulfonamide, **AAZ**) was used as standard in the measurements reported in Table 1.



Scheme 1. Reagents and conditions: (**i**) KSCN, aq. 3.5 M HCl, reflux, 3 h, 31%; (**ii**) MeI, DMF, 40 °C, 2.5 h, 70%; (**iii**) K_2CO_3, DMF, 100 °C, 1.5 h, 89%; (**iv**) $BrCH_2CO_2Et$, MeOH, 65 °C, 3.5 h; (**v**) MeOH/HCl (8:1), 65 °C, 2.5 h, 92%; (**vi**) R-X (**7a–n**), K_2CO_3, DMF, r.t. 3–5 h. Yields of final products **8a–8n**: **8a**, R = Et; (37%); **8b**, R = $^nC_7H_{15}$; (32%); **8c**, R = allyl; (54%); **8d**, R = benzyl; (61%); **8e**, R = 4-Me-Bn; (51%); **8f**, R = 4-Cl-Bn; (37%); **8g**, R = 4-CN-Bn; (51%); **8h**, R = 4-NO_2-Bn; (49%); **8i**, R = 4-CF_3-Bn; (34%); **8j**, R = 4-OCF_3-Bn; (54%); **8k**, R = 3-Me-Bn; (34%); **8l**, R = 2-F-Bn; (46%); **8m**, R = 3,4-Cl_2-Bn; (48%); **8n**, R = -CH_2-C_6F_5; (54%).

Table 1. Inhibition data of human CA isoforms hCA I, II, and VII and fungal β-CA isoforms MgCA, from *M. globosa*, and CgNce103, from *C. glabrata*, with compounds **6** and **8a–n** in comparison with **AAZ** as standard drug by a stopped flow CO_2hydrase assay [67].



6, 8a–n

Compound	R	K_I (nM) [a]				
		hCA I (α-CA)	hCA II (α-CA)	hCA VII (α-CA)	MgCA (β-CA)	CgNce103 (β-CA)
6	-H	503.9	18.1	61.4	37,170	29.5
8a	-CH_2CH_3	261.7	25.8	30.8	68,090	46.0
8b	-$(CH_2)_6CH_3$	747.3	56.4	187.2	95,700	83.7
8c	-$CH_2CH{=}CH_2$	233.8	32.6	19.5	66,580	54.2
8d	-$CH_2C_6H_5$	837.3	8.7	5.3	64,570	20.9
8e	-CH_2(4-CH_3-C_6H_4)	2926	32.7	3.0	38,930	18.3
8f	-CH_2(4-Cl-C_6H_4)	8789	62.2	15.3	41,460	44.9
8g	-CH_2(4-CN-C_6H_4)	570.5	7.2	12.0	>100,000	38.4
8h	-CH_2(4-NO_2-C_6H_4)	656.6	6.1	30.3	>100,000	6.6
8i	-CH_2(4-CF_3-C_6H_4)	601.1	43.3	14.3	59,580	13.1
8j	-CH_2(4-OCF_3-C_6H_4)	424.9	16.4	22.4	>100,000	5.9
8k	-CH_2(3-CH_3-C_6H_4)	1081	58.3	18.8	>100,000	8.4
8l	-CH_2(2-F-C_6H_4)	446.8	1.2	12.7	81,130	48.8
8m	-CH_2(3,4-diCl-C_6H_3)	687.9	85.6	132.9	>100,000	67.9
8n	-CH_2(C_6F_5)	414.6	91.2	16.9	34,940	35.7
AAZ	-	250	12.5	2.5	74,000	11

[a] Mean from 3 different assays, by a stopped flow technique (errors were in the range of ±5–10% of the reported values).

Data of Table 1 show the following structure-activity relationship (SAR) for the inhibition of these enzymes with hydantoin-substituted benzene-sulfonamides:

(i) hCA I, an abundant cytosolic isoform in many tissues and organs [4–9], was moderately inhibited by compounds **6** and **8** investigated here, with K_I ranging between 233.8 and 8789 nM. Some of the best hCA I inhibitors are as active as **AAZ**, the standard drug (Table 1).

(ii) hCA II; the dominant cytosolic isoform [4–9] was, on the other hand, potently inhibited by most new sulfonamides reported here, with K_I ranging between 1.2 and 91.2 nM. The best inhibitor **8l** incorporates the 2-fluorobenzyl moiety in position 3 of the hydantoin ring, whereas the unsubstituted benzyl derivative **8d** was also a highly effective inhibitor (K_I of 8.7 nM). The alkyl or alkenyl substituted derivatives **8a**–**8c** were slightly less effective (but still potent CAIs), whereas the position and nature of the substituent eventually present on the benzyl fragment in the remaining derivatives seemed to be the factor that strongly influenced the inhibition potency. Indeed, 4-CN, 4-nitro and 2-fluorobenzyl fragments were those associated with the best inhibitory action, whereas 3-methyl, pentafluoro, 4-CF_3 and 4-Cl led to less effective inhibitors.

(iii) The SAR is rather different for the inhibition of CA VII. The unsubstituted hydantoin **6** and the alky-substituted ones, **8a** and **8b**, were moderately active (K_I of 30.8–187.2 nM). The alkeyl and benzylsubstituted hydantoins (except **8m**) were, on the other hand, effective hCA VII inhibitors, with K_I ranging between 3.0–19.5 nM. The best hCA VII inhibitors were the unsubstituted benzyl and the 4-Me-benzyl derivatives **8d** and **8e**, with K_I of 3.0–5.3 nM, in the same range as AAZ.

(iv) MgCA was poorly inhibited by these sulfonamides, which had some activity in the high micromolar range, similarly to AAZ (Table 1).

(v) CgNce103 was, on the other hand, effectively inhibited by hydantoin-substituted benzene-sulfonamides, with K_I ranging between 5.9 and 83.7 nM. The SAR is again diverse from what observed for other isoforms/enzymes. The unsubstituted hydantoin **6** and the alkyl-substituted derivatives **8a**–**8c** showed K_I of 29.5–83.7 nM, whereas most benzyl-substituted derivatives (except **8l** and **8m**) were active in the low nanomolar range.

3. Materials and Methods

3.1. Chemistry

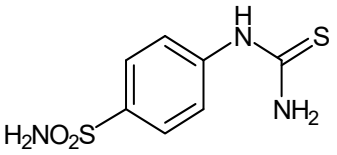
Reagents, starting materials and solvents were obtained from commercial sources and used as received. Thin-layer chromatography was performed on silica gel, spots were visualized with UV light (254 and 365 nm). NMR spectra were recorded on Bruker 300 spectrometer with chemical shifts values (δ) in ppm relative to TMS using the residual DMSO-d_6 signal (^{1}H 2.50; ^{13}C 39.52) see also Supplementary Materials. High-resolution mass spectra (HRMS) were recorded on a mass spectrometer with a Q-TOF micro mass analyzer using the ESI technique.

3.2. Synthesis

3.2.1. 4-Thioureidobenzenesulfonamide (**2**)

4-Aminobenzensulfonamide (**1**) (30 g, 174.3 mmol) was dissolved in aqueous HCl (3.5 M, 180 mL) at 70 °C. After cooling to room temperature, KSCN (16.94 g, 174.3 mmol) was added, and the mixture was refluxed for 3 h. After cooling to room temperature, the reaction mixture was poured onto ice/cold water, and the formed precipitate was collected by filtration, washed with water, and air dried to afford **2** (12.49 g, 31%) as a white powder.

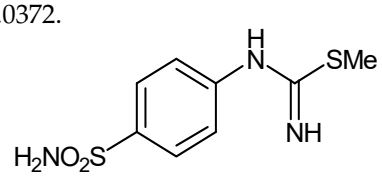
^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.32 (s, 2H), 7.69 (d, 2H, J = 8.6 Hz), 7.77 (d, 2H, J = 8.6 Hz), 10.02 (s, 1H) ppm ^{13}C NMR (75 MHz, DMSO-d_6) δ = 122.8, 127.3, 139.8, 143.9, 182.8 ppm MS (ESI) $[M + H]^+$: m/z 232.0.



3.2.2. Methyl (4-Sulfamoylphenyl)carbamimidothioate (**3**)

To a solution of 4-thioureidobenzenesulfonamide (**2**) (300 mg, 1.3 mmol) in DMF (4 mL),MeI (0.08 mL, 1.3 mmol) was added, and the mixture was heated at 40 °C for 2.5 h. After cooling to room temperature, the reaction mixture was extracted with EtOAc (3 × 20 mL). Organic layer was washed with aq. sat. $NaHCO_3$ (2 × 20 mL) and then aq. sat. NH_4Cl (1 × 20 mL), and dried over Na_2SO_4. Solvent removal in vacuum resulted in **3** (223 mg, 70%) as a white powder.

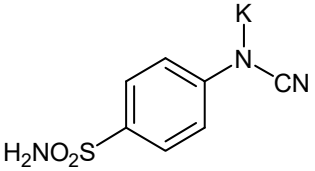
^{1}H NMR (300 MHz, DMSO-d_6) δ = 2.37 (s, 3H), 6.63 (s, 2H), 6.94 (s, 2H), 7.22 (s, 2H), 7.71 (d, 2H, J = 8.4 Hz) ppm ^{13}C NMR (75 MHz, DMSO-d_6) δ = 14.2, 122.8, 127.7, 138.0, 153.9, 157.0 ppm HRMS (ESI) $[M + H]^+$: m/z calcd for ($C_8H_{12}N_3O_2S_2$) 246.0371. Found 246.0372.



3.2.3. Potassium Cyano(4-sulfamoylphenyl)amide (**4**)

To a solution of methyl (4-sulfamoylphenyl) carbamimidothioate (**3**) (500 mg, 2.04 mmol) in DMF (8 mL), K_2CO_3 (564 mg, 4.08 mmol) was added, and the mixture was stirred at 100 °C for 1.5 h. The mixture was cooled to room temperature and precipitate was removed by filtration. To the filtrate, EtOAc (80 mL) was added and precipitate formed was collected by filtration, washed with EtOAc (20 mL), and air dried to afford **4** (427 mg, 89%) as a white powder.

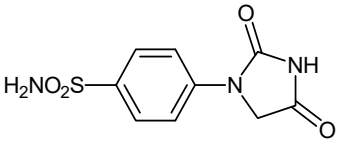
^{1}H NMR (300 MHz, DMSO-d_6) δ = 6.60 (d, 2H, J = 8.6 Hz), 6.85 (s, 2H), 7.29 (s, 1H), 7.38 (d, 2H, J = 8.6 Hz) ppm ^{13}C NMR (75 MHz, DMSO-d_6) δ = 118.0, 125.7, 127.9, 129.0, 160.9 ppm HRMS (ESI) $[M - K]^-$: m/z calcd for ($C_7H_6N_3O_2S$) 196.0181. Found 196.0188.



3.2.4. 4-(2,4-Dioxoimidazolidin-1-yl)benzenesulfonamide (**6**)

To a suspension of potassium cyano(4-sulfamoylphenyl)amide(**4**)(4.0 g, 17 mmol) in MeOH (90 mL), ethyl 2-bromoacetate (1.76 mL, 17 mmol) was added dropwise. The mixture was heated at 65 °C for 3.5 h. After cooling to room temperature conc. HCl (11.25 mL) was dropwise added, and the mixture was stirred for 2.5 h at 65 °C. The solvent was evaporated under reduced pressure and the residue was washed with *i*PrOH (50 mL) and dried in vacuum to afford **6** (3.98 g, 92%) as a white powder.

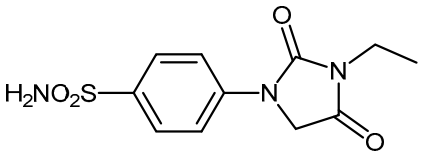
^{1}H NMR (300 MHz, DMSO-d_6) δ = 4.51 (s, 2H), 7.34 (s, 2H), 7.78–7.85 (m, 4H), 11.40 (s, 1H) ppm ^{13}C NMR (75 MHz, DMSO-d_6) δ = 51.9, 118.4, 127.6, 139.2, 141.9, 155.9, 171.1 ppm HRMS (ESI) $[M - 1]^-$: m/z calcd for ($C_9H_8N_3O_4S$) 254.0236. Found 254.0239.



3.2.5. 4-(3-Ethyl-2,4-dioxoimidazolidin-1-yl)benzenesulfonamide (**8a**)

To a stirred solution of 4-(2,4-dioxoimidazolidin-1-yl)benzenesulfonamide (**6**) (250 mg, 0.98 mmol) and ethyl iodide (0.079 mL, 0.98 mmol) in DMF (5 mL) K_2CO_3 (270 mg, 1.96 mmol) was added at room temperature and the mixture was stirred at this temperature for 5 h. It was extracted with DCM (3 × 20 mL), the organic phase was dried over Na_2SO_4, and volatiles were removed in vacuum to afford **8a** (103 mg, 37%) as a white solid.

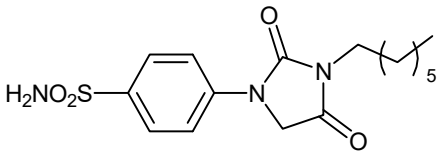
^{1}H NMR (300 MHz, DMSO-d_6) δ = 1.16 (t, 3H, J = 7.1 Hz), 3.51 (q, 2H, J = 7.1), 4.50 (s, 2H), 7.35 (s, 2H), 7.78–7.86 (m, 4H) ppm ^{13}C NMR (125 MHz, DMSO-d_6) δ = 14.3, 34.7, 51.0, 119.0, 128.2, 139.5, 142.1, 155.6, 170.1 ppm HRMS (ESI) $[M - 1]^-$: m/z calcd for ($C_{11}H_{12}N_3O_4S$) 282.0549. Found 282.0557.



3.2.6. 4-(3-Heptyl-2,4-dioxoimidazolidin-1-yl)benzenesulfonamide (**8b**)

To a stirred solution of 4-(2,4-dioxoimidazolidin-1-yl)benzenesulfonamide (**6**) (250 mg, 0.98 mmol) and 1-iodoheptane (0.160 mL, 0.98 mmol) in DMF (5 mL), K_2CO_3 (270 mg, 1.96 mmol) was added at room temperature and the mixture was stirred at this temperature for 5 h. Water was added to the reaction mixture and the precipitate former was collected by filtration, washed with water, and air dried to afford **8b** (109 mg, 32%) as a white solid.

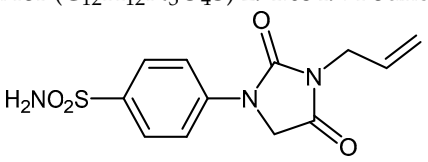
^{1}H NMR (300 MHz, DMSO-d_6) δ = 0.90 (t, 3H, J = 6.6 Hz), 1.31 (br. s, 8H), 1.53–1.62 (m, 2H), 3.47 (t, 2H, J = 6.6 Hz), 4.53 (s, 2H), 7.35 (s, 2H), 7.78–7.86 (m, 4H) ppm ^{13}C NMR (75 MHz, DMSO-d_6) δ = 14.8, 22.9, 27.0, 28.3, 29.1, 32.0, 39.1, 50.6, 118.4, 127.7, 139.4, 141.7, 155.3, 169.7 ppm HRMS (ESI) $[M - 1]^-$: m/z calcd for ($C_{16}H_{22}N_3O_4S$) 352.1331. Found 352.1341.



3.2.7. 4-(3-Allyl-2,4-dioxoimidazolidin-1-yl)benzenesulfonamide (**8c**)

To a stirred solution of 4-(2,4-dioxoimidazolidin-1-yl)benzenesulfonamide (**6**) (250 mg, 0.98 mmol) and allyl bromide (0.085 mL, 0.98 mmol) in DMF (5 mL), K_2CO_3 (270 mg, 1.96 mmol) was added at room temperature, and the mixture was stirred at this temperature for 3 h. Water was added to the reaction mixture and it was extracted with DCM (3 × 20 mL), the organic phase was dried over Na_2SO_4, and the solvent was evaporated in vacuum to give **8c** (156 mg, 54%) as a white solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ = 4.12 (d, 2H, J = 3.4 Hz), 4.61 (s, 2H), 5.17–5.25 (m, 2H), 5.82–5.92 (m, 1H), 7.35 (s, 2H), 7.82–7.89 (m, 4H) ppm ^{13}C NMR (75 MHz, DMSO-d_6) δ = 50.7, 117.7, 118.5, 127.7, 132.7, 139.5, 141.7, 154.9, 169.4 ppm HRMS (ESI) $[M - 1]^-$: m/z calcd for ($C_{12}H_{12}N_3O_4S$) 294.0549. Found 294.0551.

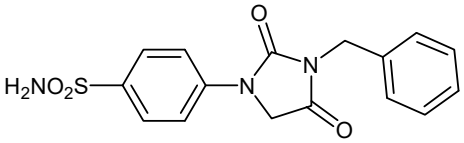


3.2.8. 4-(3-Benzyl-2,4-dioxoimidazolidin-1-yl)benzenesulfonamide (**8d**)

To a stirred solution of 4-(2,4-dioxoimidazolidin-1-yl)benzenesulfonamide (**6**) (250 mg, 0.98 mmol) and benzyl bromide (0.116 mL, 0.98 mmol) in DMF (5 mL), K_2CO_3 (270 mg, 1.96 mmol) was added at room temperature, and the mixture was stirred at this temperature for 5 h. Water was added to the reaction mixture and the precipitate formed was collected

by filtration, washed with water and Et_2O, and air dried to afford **8d** (205 mg, 61%) as a white solid.

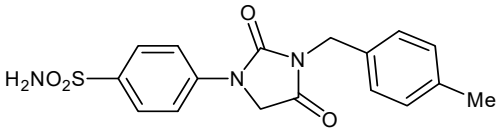
^{1}H NMR (300 MHz, DMSO-d_6) δ = 4.66 (s, 2H), 4.70 (s, 2H), 7.32–7.40 (m, 7H), 7.81–7.90 (m, 4H) ppm ^{13}C NMR (75 MHz, DMSO-d_6) δ = 42.6, 50.9, 118.6, 127.7, 128.5, 128.5, 129.4, 137.0, 139.5, 141.6, 155.1, 169.7 ppm HRMS (ESI) $[M-1]^-$: m/z calcd for ($C_{16}H_{14}N_3O_4S$) 344.0705. Found 344.0708.



3.2.9. 4-(3-(4-Methylbenzyl)-2,4-dioxoimidazolidin-1-yl)benzenesulfonamide (**8e**)

To a stirred solution of 4-(2,4-dioxoimidazolidin-1-yl)benzenesulfonamide (**6**) (250 mg, 0.98 mmol) and 4-methylbenzyl bromide (181 mg, 0.98 mmol) in DMF (5 mL) K_2CO_3 (270 mg, 1.96 mmol) was added at room temperature and the mixture was stirred at this temperature for 5 h. Water was added to the reaction mixture and precipitate formed was collected by filtration, washed with water and Et_2O and air dried to afford **8e** (179 mg, 51%) as a white solid.

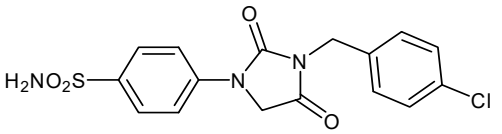
^{1}H NMR (300 MHz, DMSO-d_6) δ = 2.31 (s, 3H), 4.55–4.64 (m, 4H), 7.18 (d, 2H, J = 12.3 Hz), 7.28 (d, 2H, J = 12.3 Hz), 7.36 (s, 2H), 7.76–7.84 (m, 4H) ppm ^{13}C NMR (75 MHz, DMSO-d_6) δ = 21.6, 42.4, 50.9, 118.6, 127.8, 128.6, 130.0, 134.1, 137.7, 139.6, 141.6, 155.1, 169.6 ppm HRMS (ESI) $[M-1]^-$: m/z calcd for ($C_{17}H_{16}N_3O_4S$) 358.0862. Found 358.0869.



3.2.10. 4-(3-(4-Chlorobenzyl)-2,4-dioxoimidazolidin-1-yl)benzenesulfonamide (**8f**)

To a stirred solution of 4-(2,4-dioxoimidazolidin-1-yl)benzenesulfonamide (**6**) (250 mg, 0.98 mmol) and 4-chlorobenzyl bromide (201 mg, 0.98 mmol) in DMF (5 mL), K_2CO_3 (270 mg, 1.96 mmol) was added at room temperature, and the mixture was stirred at this temperature for 3.5 h. Water was added to the reaction mixture and precipitate formed was collected by filtration, washed with water and DCM, and air dried to afford **8f** (137 mg, 37%) as a white solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ = 4.64 (s, 2H), 4.69 (s, 2H), 7.37 (s, 2H), 7.40–7.47 (m, 4H), 7.82–7.89 (m, 4H) ppm ^{13}C NMR (75 MHz, DMSO-d_6) δ = 41.9, 50.9, 118.5, 127.7, 129.4, 130.5, 133.1, 136.0, 139.6, 141.6, 155.0, 169.6 ppm HRMS (ESI) $[M–1]^-$: m/z calcd for ($C_{16}H_{13}N_3O_4SCl$) 378.0315. Found 378.0320.

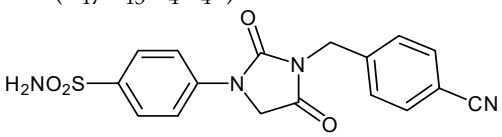


3.2.11. 4-(3-(4-Cyanobenzyl)-2,4-dioxoimidazolidin-1-yl)benzenesulfonamide (**8g**)

To a stirred solution of 4-(2,4-dioxoimidazolidin-1-yl)benzenesulfonamide (**6**) (250 mg, 0.98 mmol) and 4-cyanobenzyl bromide (192 mg, 0.98 mmol) in DMF (5 mL), K_2CO_3 (270 mg, 1.96 mmol) was added at room temperature, and the mixture was stirred at this temperature for 3 h. Water was added to the reaction mixture and precipitate formed was collected by filtration, washed with water and Et_2O, and air dried to afford **8g** (184 mg, 51%) as a white solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ = 4.66 (s, 2H), 4.80 (s, 2H), 7.37 (s, 2H), 7.60 (d, 2H, J = 7.9 Hz), 7.82–7.91 (m, 6H) ppm ^{13}C NMR (75 MHz, DMSO-d_6) δ = 42.3, 51.0, 111.2, 118.5,

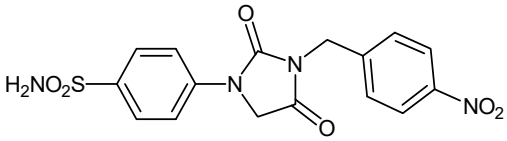
119.6, 127.7, 129.2, 133.4, 139.6, 141.6, 142.6, 155.0, 169.7 ppm HRMS (ESI) $[M-1]^-$: m/z calcd for ($C_{17}H_{13}N_4O_4S$) 369.0658. Found 369.0663.



3.2.12. 4-(3-(4-Nitrobenzyl)-2,4-dioxoimidazolidin-1-yl)benzenesulfonamide (**8h**)

To a stirred solution of 4-(2,4-dioxoimidazolidin-1-yl)benzenesulfonamide (**6**) (250 mg, 0.98 mmol) and 4-nitrobenzyl bromide (211 mg, 0.98 mmol) in DMF (5 mL), K_2CO_3 (270 mg, 1.96 mmol) was added at room temperature, and the mixture was stirred at this temperature for 5 h. Water was added to the reaction mixture and precipitate formed was collected by filtration, washed with water and Et_2O, and air dried to afford **8h** (188 mg, 49%) as a white solid.

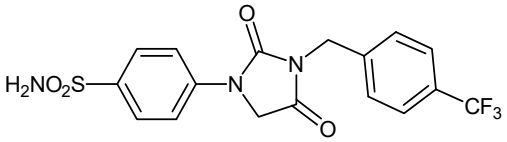
^{1}H NMR (300 MHz, DMSO-d_6) δ = 4.67 (s, 2H), 4.85 (s, 2H), 7.37 (s, 2H), 7.68 (d, 2H, J = 7.2 Hz), 7.83–7.91 (m, 4H), 8.25 (d, 2H, J = 7.2 Hz) ppm ^{13}C NMR (75 MHz, DMSO-d_6) δ = 42.1, 51.0, 118.5, 124.5, 127.7, 129.6, 139.6, 141.6, 144.7, 147.8, 155.0, 169.7 ppm HRMS (ESI) $[M-1]^-$: m/z calcd for ($C_{16}H_{13}N_4O_6S$) 389.0556. Found 389.0556.



3.2.13. 4-(2,4-Dioxo-3-(4-(trifluoromethyl)benzyl)imidazolidin-1-yl)benzenesulfonamide (**8i**)

To a stirred solution of 4-(2,4-dioxoimidazolidin-1-yl)benzenesulfonamide (**6**) (250 mg, 0.98 mmol) and 4-(trifluoromethyl)benzyl bromide (234 mg, 0.98 mmol) in DMF (5 mL), K_2CO_3 (270 mg, 1.96 mmol) was added at room temperature and the mixture was stirred at this temperature for 3 h. Water was added to the reaction mixture and precipitate formed was collected by filtration, washed with water and Et_2O, and air dried to afford **8i** (138 mg, 34%) as a white solid.

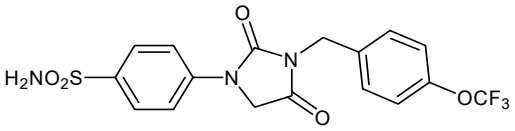
^{1}H NMR (500 MHz, DMSO-d_6) δ = 4.66 (s, 2H), 4.80 (s, 2H, 7.36 (s, 2H), 7.63 (d, 2H, J = 7.2 Hz), 7.76 (d, 2H, J = 7.2 Hz), 7.84–7.89 (m, 4H) ppm ^{13}C NMR (125 MHz, DMSO-d_6) δ = 42.2, 51.0, 118.5, 125.1 (q, J = 271.9 Hz) 126.3, 126.4, 127.7, 129.1 (q, J = 31.4 Hz) 129.3, 139.6, 141.6, 141.7, 155.0, 169.7 ppm ^{19}F NMR (470 MHz) δ = –60.9 ppm HRMS (ESI) $[M-1]^-$: m/z calcd for ($C_{17}H_{13}N_3O_4F_3S$) 412.0579. Found 412.0597.



3.2.14. 4-(2,4-Dioxo-3-(4-(trifluoromethoxy)benzyl)imidazolidin-1-yl)benzenesulfonamide (**8j**)

To a stirred solution of 4-(2,4-dioxoimidazolidin-1-yl)benzenesulfonamide (**6**) (250 mg, 0.98 mmol) and 4-(trifluoromethoxy)benzyl bromide (0.157 mL, 0.98 mmol) in DMF (5 mL), K_2CO_3 (270 mg, 1.96 mmol) was added at room temperature, and the mixture was stirred at this temperature for 3 h. Water was added to the reaction mixture and precipitate formed was collected by filtration, washed with water and Et_2O, and air dried to afford **8j** (226 mg, 54%) as a white solid.

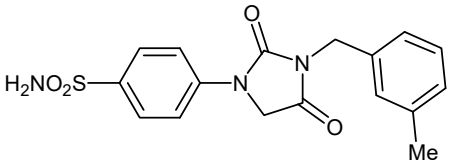
^{1}H NMR (500 MHz, DMSO-d_6) δ = 4.65 (s, 2H), 4.73 (s, 2H), 7.36 (s, 2H), 7.39 (d, 2H, J = 7.2 Hz), 7.54 (d, 2H, J = 7.2 Hz), 7.83–7.89 (4H, m) ppm ^{13}C NMR (125 MHz, DMSO-d_6) δ = 41.9, 50.9, 118.5, 122.0, 121.0 (q, J = 256.0 Hz), 127.7, 130.6, 136.5, 139.6, 141.6, 148.6, 155.0, 169.6 ppm ^{19}F NMR (470 MHz) δ = –56.8 ppm HRMS (ESI) $[M-1]^-$: m/z calcd for ($C_{17}H_{13}N_3O_5SF_3$) 428.0528. Found 428.0533.



3.2.15. 4-(3-(3-Methylbenzyl)-2,4-dioxoimidazolidin-1-yl)benzenesulfonamide (**8k**)

To a stirred solution of 4-(2,4-dioxoimidazolidin-1-yl)benzenesulfonamide (**6**) (250 mg, 0.98 mmol) and 3-methylbenzyl bromide (0.133 mL, 0.98 mmol) in DMF (5 mL), K_2CO_3 (270 mg, 1.96 mmol) was added at room temperature, and the mixture was stirred at this temperature for 5 h. Water was added to the reaction mixture and precipitate formed was collected by filtration, washed with water and Et_2O, and air dried to afford **8k** (120 mg, 34%) as a white solid.

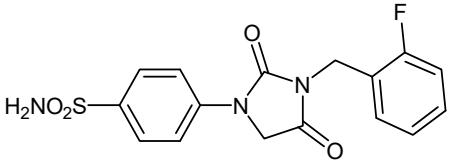
^{1}H NMR (300 MHz, DMSO-d_6) δ = 2.32 (s, 3H), 4.65 (s, 2H), 4.66 (s, 2H), 7.15–7.29 (m, 4H), 7.36 (s, 2H), 7.80–7.89 (m, 4H) ppm ^{13}C NMR (75 MHz, DMSO-d_6) δ = 21.4, 42.0, 50.3, 118.0, 125.2, 127.2, 128.6, 128.8, 136.5, 138.1, 139.0, 141.1, 154.6, 169.1 ppm HRMS (ESI) $[M-1]^-$: m/z calcd for ($C_{17}H_{16}N_3O_4S$) 358.0862. Found 358.0869.



3.2.16. 4-(3-(2-Fluorobenzyl)-2,4-dioxoimidazolidin-1-yl)benzenesulfonamide (**8l**)

To a stirred solution of 4-(2,4-dioxoimidazolidin-1-yl)benzenesulfonamide (**6**) (250 mg, 0.98 mmol) and 2-fluorobenzyl bromide (0.118 mL, 0.98 mmol) in DMF (5 mL), K_2CO_3 (270 mg, 1.96 mmol) was added at room temperature, and the mixture was stirred at this temperature for 5 h. Water was added to the reaction mixture and precipitate formed was collected by filtration, washed with water and Et_2O, and air dried to afford **8l** (164 mg, 46%) as a white solid.

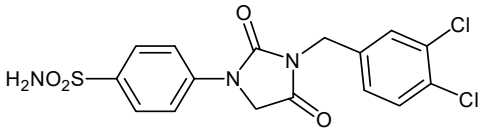
^{1}H NMR (500 MHz, DMSO-d_6) δ = 4.66 (s, 2H), 4.75 (s, 2H), 7.20–7.27 (m, 2H), 7.36 (s, 2H), 7.37–7.47 (m, 2H), 7.83–7.88 (m, 4H) ppm ^{13}C NMR (125 MHz, DMSO-d_6) δ = 36.5 (d, J = 4.6 Hz), 50.9, 116.2 (d, J = 20.9 Hz), 118.5, 123.6 (d, J = 14.2 Hz), 125.3 (d, J = 3.4 Hz), 127.7, 130.6 (d, J = 8.1 Hz), 130.7 (d, J = 3.6 Hz), 139.6, 141.6, 154.9, 160.8 (d, J = 245.9 Hz), 169.5 ppm ^{19}F NMR (470 MHz) –118.0 ppm HRMS (ESI) $[M-1]^-$: m/z calcd for ($C_{16}H_{13}N_3O_4FS$) 362.0611. Found 362.0619.



3.2.17. 4-(3-(3,4-Dichlorobenzyl)-2,4-dioxoimidazolidin-1-yl)benzenesulfonamide (**8m**)

To a stirred solution of 4-(2,4-dioxoimidazolidin-1-yl)benzenesulfonamide (**6**) (250 mg, 0.98 mmol) and 3,4-dichlorobenzyl bromide (0.142 mL, 0.98 mmol) in DMF (5 mL), K_2CO_3 (270 mg, 1.96 mmol) was added at room temperature, and the mixture was stirred at this temperature for 2.5 h. Water was added to the reaction mixture and precipitate formed was collected by filtration, washed with water and Et_2O, and air dried to afford **8m** (194 mg, 48%) as a white solid.

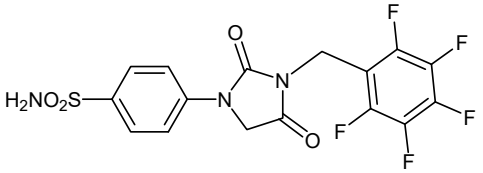
^{1}H NMR (500 MHz, DMSO-d_6) δ = 4.64 (s, 2H), 4.71 (s, 2H), 7.36 (s, 2H), 7.40 (d, 1H, J = 8.6 Hz), 7.66 (d, 2H, J = 8.6 Hz), 7.81–7.90 (m, 4H) ppm ^{13}C NMR (75 MHz, DMSO-d_6) δ = 41.5, 51.0, 118.5, 127.7, 128.9, 130.5, 131.1, 131.5, 132.0, 138.1, 139.6, 141.6, 155.0, 169.7 ppm HRMS (ESI) $[M-1]^-$: m/z calcd for ($C_{16}H_{12}N_3O_4SCl_2$) 411.9926. Found 411.9933.



3.2.18. 4-(2,4-Dioxo-3-((perfluorophenyl)methyl)imidazolidin-1-yl)benzenesulfonamide (**8n**)

To a stirred solution of 4-(2,4-dioxoimidazolidin-1-yl)benzenesulfonamide (**6**) (250 mg, 0.98 mmol) and 2,3,4,5,6-pentafluorobenzyl bromide (0.148 mL, 0.98 mmol) in DMF (5 mL), K_2CO_3 (270 mg, 1.96 mmol) was added at room temperature and the mixture was stirred at this temperature for 5 h. Water was added to the reaction mixture and precipitate formed was collected by filtration, washed with water and Et_2O, and air dried to afford **8n** (229 mg, 54%) as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ = 4.59 (s, 2H), 4.81 (s, 2H), 7.35 (s, 2H), 7.81–7.87 (m, 4H) ppm ^{13}C NMR (125 MHz, DMSO-d_6) δ = 36.5 (d, J = 4.6 Hz), 50.9, 116.2 (d, J = 20.9 Hz), 118.5, 123.6 (d, J = 14.2 Hz), 125.3 (d, J = 3.4 Hz), 127.7, 130.6 (d, J = 8.1 Hz), 130.7 (d, J = 3.6 Hz), 139.6, 141.6, 154.9, 160.8 (d, J = 245.9 Hz), 169.5 ppm ^{19}F NMR (470 MHz) δ = −140.8 (dd, 2F, J = 16.5, 6.3 Hz), −155.1 (t, 1F, J = 21.9 Hz), −163.2–−163.3 (2F, m) ppm HRMS (ESI) $[M-1]^-$: m/z calcd for ($C_{16}H_9N_3O_4F_5S$) 434.0234. Found 434.0246.



3.3. CA Inhibition Assay

An applied photophysics stopped-flow instrument was used for assaying the CA catalysed CO_2 hydration activity [67]. Phenol red (at a concentration of 0.2 mM) was used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5) as buffer for α-CAs or 20 mM TRIS (pH 8.4) as buffer for β-CAs, and 20 mM Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalysed CO_2 hydration reaction for a period of 10–100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor, at least six traces of the initial 5–10% of the reaction were used for determining the initial velocity. The uncatalysed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled–deionised water, and dilutions up to 0.01 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 6 h at room temperature prior to assay in order to allow for the formation of the E–I complex. The inhibition constants were obtained by nonlinear least-squares methods using PRISM 3 and the Cheng–Prusoff equation, as reported earlier [68–74], and represent the mean from at least three different determinations. All CA isoforms were recombinant ones obtained in-house as reported earlier [25,58–61,66,75], and their concentrations in the assay system ranged between 9–12 nM.

4. Conclusions

Starting from commercially available inexpensive 4-aminobenzenesulfonamide, a library of novel hydantoin-based benzenesulfonamides were synthesized, and the structures of all derivatives were confirmed by ^{1}H NMR, ^{13}C NMR, and HRMS spectral techniques. The prepared compounds were screened for their hCA inhibitory activities against three cytosolic isoforms as well as two β-CAs from fungal pathogens. The best inhibition was observed against hCA II and VII, as well as *Candida glabrata* enzyme CgNce103. hCA I and MgCA were, on the other hand, less effectively inhibited by these compounds. The interesting results against CgNce103 make the compounds of interest for investigations in vivo as potential antifungals.

Supplementary Materials: The supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms232214115/s1.

Author Contributions: Conceptualization, M.A., R.Ž. and C.T.S.; Syntheses, M.A.; Determination of inhibitory activity against Cas, M.A., V.D.L., C.C. and C.T.S.; Writing—original draft preparation, M.A., R.Ž. and C.T.S.; Writing–review and editing, M.A., V.D.L., C.C., R.Ž. and C.T.S. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the European Regional Development Fund (ERDF, project no. 1.1.1.2/VIAA/3/19/398).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

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

References

1. Copeland, R.A.; Harpel, M.R.; Tummino, P.J. Targeting enzyme inhibitors in drug discovery. *Expert Opin. Ther. Targets* **2007**, *11*, 967–978. [CrossRef] [PubMed]
2. Geronikaki, A. Recent trends in enzyme inhibition and activation in drug design. *Molecules* **2021**, *26*, 17. [CrossRef]
3. Holdgate, G.A.; Meek, T.D.; Grimley, R.L. Mechanistic enzymology in drug discovery: A fresh perspective. *Nat. Rev. Drug Discov.* **2018**, *17*, 115–132. [CrossRef] [PubMed]
4. Supuran, C.T. Carbonic anhydrases: Novel therapeutic applications for inhibitors and activators. *Nat. Rev. Drug. Discov.* **2008**, *7*, 168–181. [CrossRef] [PubMed]
5. Supuran, C.T. Carbon-versus sulphur-based zinc binding groups for carbonic anhydrase inhibitors? *J. Enzym. Inhib. Med. Chem.* **2018**, *33*, 485–495. [CrossRef] [PubMed]
6. Supuran, C.T. Emerging role of carbonic anhydrase inhibitors. *Clin. Sci.* **2021**, *135*, 1233–1249. [CrossRef]
7. Supuran, C.T.; Scozzafava, A. Carbonic anhydrases as targets for medicinal chemistry. *Bioorg. Med. Chem.* **2007**, *15*, 4336–4350. [CrossRef]
8. Supuran, C.T. Carbonic anhydrase inhibitors and their potential in a range of therapeutic areas. *Expert Opin. Ther. Pat.* **2018**, *28*, 709–712. [CrossRef]
9. Supuran, C.T. Novel carbonic anhydrase inhibitors. *Future Med. Chem.* **2021**, *13*, 1935–1937. [CrossRef]
10. Truppo, E.; Supuran, C.T.; Sandomenico, A.; Vullo, D.; Innocenti, A.; Di Fiore, A.; Alterio, V.; De Simone, G.; Monti, S.M. Carbonic anhydrase VII is S-glutathionylated without loss of catalytic activity and affinity for sulfonamide inhibitors. *Bioorganic. Med. Chem. Lett.* **2012**, *22*, 1560–1564. [CrossRef]
11. Lemon, N.; Canepa, E.; Ilies, M.A.; Fossati, S. Carbonic anhydrases as potential targets against neurovascular unit dysfunction in Alzheimer's disease and stroke. *Front. Aging Neurosci.* **2021**, *13*, 772278. [CrossRef] [PubMed]
12. De Luca, L.; Ferro, S.; Damiano, F.M.; Supuran, C.T.; Vullo, D.; Chimirri, A.; Gitto, R. Structure-based screening for the discovery of new carbonic anhydrase VII inhibitors. *Eur. J. Med. Chem.* **2014**, *71*, 105–111. [CrossRef]
13. Supuran, C.T. Carbonic anhydrase inhibition and the management of neuropathic pain. *Expert Rev. Neurother.* **2016**, *16*, 961–968. [CrossRef] [PubMed]
14. Supuran, C.T. Anti-obesity carbonic anhydrase inhibitors: Challenges and opportunities. *J. Enzym. Inhib. Med. Chem.* **2022**, *37*, 2478–2488. [CrossRef] [PubMed]
15. McDonald, P.C.; Chafe, S.C.; Supuran, C.T.; Dedhar, S. Cancer Therapeutic Targeting of Hypoxia Induced Carbonic Anhydrase IX: From Bench to Bedside. *Cancers* **2022**, *14*, 3297. [CrossRef]
16. Carta, F.; Scozzafava, A.; Supuran, C.T. Sulfonamides: A patent review (2008–2012). *Expert Opin. Ther. Pat.* **2012**, *22*, 747–758. [CrossRef]
17. 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]
18. Liguori, F.; Carradori, S.; Ronca, R.; Rezzola, S.; Filiberti, S.; Carta, F.; Turati, M.; Supuran, C.T. Benzenesulfonamides with different rigidity-conferring linkers as carbonic anhydrase inhibitors: An insight into the antiproliferative effect on glioblastoma, pancreatic, and breast cancer cells. *J. Enzym. Inhib. Med. Chem.* **2022**, *37*, 1857–1869. [CrossRef]
19. Tars, K.; Vullo, D.; Kazaks, A.; Leitans, J.; Lends, A.; Grandane, A.; Žalubovskis, 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]
20. Leitans, J.; Kazaks, A.; Balode, A.; Ivanova, J.; Žalubovskis, R.; Supuran, C.T.; Tars, K. Efficient expression and crystallization system of cancer-associated carbonic anhydrase isoform IX. *J. Med. Chem.* **2015**, *58*, 9004–9009. [CrossRef]

21. Grandane, A.; Tanc, M.; Di Cesare Mannelli, L.; Carta, F.; Ghelardini, C.; Žalubovskis, R.; Supuran, C.T. 6-Substituted sulfocoumarins are selective carbonic anhdydrase IX and XII inhibitors with significant cytotoxicity against colorectal cancer cells. *J. Med. Chem.* **2015**, *58*, 3975–3983. [CrossRef] [PubMed]
22. Angeli, A.; Carta, F.; Nocentini, A.; Winum, J.Y.; Žalubovskis, R.; Akdemir, A.; Onnis, V.; Eldehna, W.M.; Capasso, C.; Simone, G.D.; et al. Carbonic anhydrase inhibitors targeting metabolism and tumor microenvironment. *Metabolites* **2020**, *10*, 412. [CrossRef] [PubMed]
23. Grandāne, A.; Nocentini, A.; Domračeva, I.; Žalubovskis, R.; Supuran, C.T. Development of oxathiino [6, 5-b] pyridine 2, 2-dioxide derivatives as selective inhibitors of tumor-related carbonic anhydrases IX and XII. *Eur. J. Med. Chem.* **2020**, *200*, 112300. [CrossRef]
24. Nocentini, A.; Angeli, A.; Carta, F.; Winum, J.Y.; Žalubovskis, R.; Carradori, S.; Capasso, C.; Donald, W.A.; Supuran, C.T. Reconsidering anion inhibitors in the general context of drug design studies of modulators of activity of the classical enzyme carbonic anhydrase. *J. Enzym. Inhib. Med. Chem.* **2021**, *36*, 561–580. [CrossRef] [PubMed]
25. Abdoli, M.; Angeli, A.; Bozdag, M.; Carta, F.; Kakanejadifard, A.; Saeidian, H.; Supuran, C.T. Synthesis and carbonic anhydrase I, II, VII, and IX inhibition studies with a series of benzo [d] thiazole-5-and 6-sulfonamides. *J. Enzym. Inhib. Med. Chem.* **2017**, *32*, 1071–1078. [CrossRef]
26. Abdoli, M.; Bozdag, M.; Angeli, A.; Supuran, C.T. Benzamide-4-sulfonamides are effective human carbonic anhydrase i, ii, vii, and ix inhibitors. *Metabolites* **2018**, *8*, 37. [CrossRef] [PubMed]
27. Bozdag, M.; Poli, G.; Angeli, A.; Lucarini, E.; Tuccinardi, T.; Mannelli, L.D.; Selleri, S.; Ghelardini, C.; Winum, J.Y.; Carta, F.; et al. N-aryl-N′-ureido-O-sulfamates: Potent and selective inhibitors of the human Carbonic Anhydrase VII isoform with neuropathic pain relieving properties. *Bioorg. Chem.* **2019**, *89*, 103033. [CrossRef] [PubMed]
28. Vali, Y.K.; Gundla, R.; Singh, O.V.; Tamboli, Y.; Manelli, L.D.; Ghelardini, C.; Al-Tamimi, A.M.; Carta, F.; Angeli, A.; Supuran, C.T. Spirocyclic sulfonamides with carbonic anhydrase inhibitory and anti-neuropathic pain activity. *Bioorg. Chem.* **2019**, *92*, 103210. [CrossRef]
29. Gitto, R.; Agnello, S.; Ferro, S.; Vullo, D.; Supuran, C.T.; Chimirri, A. Identification of potent and selective human carbonic anhydrase VII (hCA VII) inhibitors. *ChemMedChem* **2010**, *5*, 823–826. [CrossRef]
30. Pustenko, A.; Nocentini, A.; Gratteri, P.; Bonardi, A.; Vozny, I.; Žalubovskis, R.; Supuran, C.T. The antibiotic furagin and its derivatives are isoform-selective human carbonic anhydrase inhibitors. *J. Enzym. Inhib. Med. Chem.* **2020**, *35*, 1011–1020. [CrossRef]
31. 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]
32. Pustenko, A.; Nocentini, A.; Balašova, A.; Krasavin, M.; Žalubovskis, R.; Supuran, C.T. 7-Acylamino-3H-1,2-benzoxathiepine 2,2-dioxides as new isoform-selective carbonic anhydrase IX and XII inhibitors. *J. Enzyme Inhib. Med. Chem.* **2020**, *35*, 650–656. [CrossRef]
33. Abdoli, M.; Giovannuzzi, S.; Supuran, C.T.; Žalubovskis, R. 4-(3-Alkyl/benzyl-guanidino)benzenesulfonamides as selective carbonic anhydrase VII inhibitors. *J. Enzym. Inhib. Med. Chem.* **2022**, *37*, 1568–1576. [CrossRef]
34. Krasavin, M.; Sharonova, T.; Sharoyko, V.; Zhukovsky, D.; Kalinin, S.; Žalubovskis, R.; Tennikova, T.; Supuran, C.T. Combining carbonic anhydrase and thioredoxin reductase inhibitory motifs within a single molecule dramatically increases its cytotoxicity. *J. Enzym. Inhib. Med. Chem.* **2020**, *35*, 665–671. [CrossRef]
35. Krasavin, M.; Žalubovskis, R.; Grandāne, A.; Domračeva, I.; Zhmurov, P.; Supuran, C.T. Sulfocoumarins as dual inhibitors of human carbonic anhydrase isoforms IX/XII and of human thioredoxin reductase. *J. Enzym. Inhib. Med. Chem.* **2020**, *35*, 506–510. [CrossRef] [PubMed]
36. Pustenko, A.; Nocentini, A.; Balašova, A.; Alafeefy, A.; Krasavin, M.; Žalubovskis, R.; Supuran, C.T. Aryl derivatives of 3H-1,2-benzoxathiepine 2,2-dioxide as carbonic anhydrase inhibitors. *J. Enzym. Inhib. Med. Chem.* **2020**, *35*, 245–254. [CrossRef] [PubMed]
37. Podolski-Renić, A.; Dinić, J.; Stanković, T.; Jovanović, M.; Ramović, A.; Pustenko, A.; Žalubovskis, R.; Pešić, M. Sulfocoumarins, specific carbonic anhydrase IX and XII inhibitors, interact with cancer multidrug resistant phenotype through pH regulation and reverse P-glycoprotein mediated resistance. *Eur. J. Pharm. Sci.* **2019**, *138*, 105012. [CrossRef] [PubMed]
38. 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]
39. Ivanova, J.; Balode, A.; Žalubovskis, R.; Leitans, J.; Kazaks, A.; Vullo, D.; Tars, K.; Supuran, C.T. 5-Substituted-benzylsulfanyl-thiophene-2-sulfonamides with effective carbonic anhydrase inhibitory activity: Solution and crystallographic investigations. *Bioorg. Med. Chem.* **2017**, *25*, 857–863. [CrossRef]
40. Alterio, V.; Tanc, M.; Ivanova, J.; Zalubovskis, R.; Vozny, I.; Monti, S.M.; Di Fiore, A.; De Simone, G.; Supuran, C.T. X-ray crystallographic and kinetic investigations of 6-sulfamoyl-saccharin as a carbonic anhydrase inhibitor. *Org. Biomol. Chem.* **2015**, *13*, 4064–4069. [CrossRef]
41. Amedei, A.; Capasso, C.; Nannini, G.; Supuran, C.T. Microbiota, Bacterial Carbonic Anhydrases, and Modulators of Their Activity: Links to Human Diseases? *Mediat. Inflamm.* **2021**, *2021*, 6926082. [CrossRef]

42. 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]
43. Campestre, C.; De Luca, V.; Carradori, S.; Grande, R.; Carginale, V.; Scaloni, A.; Supuran, C.T.; Capasso, C. Carbonic Anhydrases: New Perspectives on Protein Functional Role and Inhibition in *Helicobacter pylori*. *Front. Microbiol.* **2021**, *12*, 629163. [CrossRef]
44. Del Prete, S.; Nocentini, A.; Supuran, C.T.; Capasso, C. Bacterial ι-carbonic anhydrase: A new active class of carbonic anhydrase identified in the genome of the Gram-negative bacterium *Burkholderia territorii*. *J. Enzym. Inhib. Med. Chem.* **2020**, *35*, 1060–1068. [CrossRef]
45. De Luca, V.; Carginale, V.; Supuran, C.T.; Capasso, C. The gram-negative bacterium Escherichia coli as a model for testing the effect of carbonic anhydrase inhibition on bacterial growth. *J. Enzym. Inhib. Med. Chem.* **2022**, *37*, 2092–2098. [CrossRef]
46. Supuran, C.T.; Capasso, C. Antibacterial carbonic anhydrase inhibitors: An update on the recent literature. *Expert Opin. Ther. Pat.* **2020**, *30*, 963–982. [CrossRef]
47. Giovannuzzi, S.; Hewitt, C.S.; Nocentini, A.; Capasso, C.; Costantino, G.; Flaherty, D.P.; Supuran, C.T. Inhibition studies of bacterial α-carbonic anhydrases with phenols. *J. Enzym. Inhib. Med. Chem.* **2022**, *37*, 666–671. [CrossRef]
48. Angeli, A.; Velluzzi, A.; Selleri, S.; Capasso, C.; Spadini, C.; Iannarelli, M.; Cabassi, C.S.; Carta, F.; Supuran, C.T. Seleno Containing Compounds as Potent and Selective Antifungal Agents. *ACS Infect. Dis.* **2022**, *8*, 1905–1919. [CrossRef]
49. D'Agostino, I.; Mathew, G.E.; Angelini, P.; Venanzoni, R.; Angeles Flores, G.; Angeli, A.; Carradori, S.; Marinacci, B.; Menghini, L.; Abdelgawad, M.A.; et al. Biological investigation of N-methyl thiosemicarbazones as antimicrobial agents and bacterial carbonic anhydrases inhibitors. *J. Enzym. Inhib. Med. Chem.* **2022**, *37*, 986–993. [CrossRef]
50. De Luca, V.; Angeli, A.; Mazzone, V.; Adelfio, C.; Carginale, V.; Scaloni, A.; Carta, F.; Selleri, S.; Supuran, C.T.; Capasso, C. Heterologous expression and biochemical characterisation of the recombinant β-carbonic anhydrase (MpaCA) from the warm-blooded vertebrate pathogen malassezia pachydermatis. *J. Enzym. Inhib. Med. Chem.* **2022**, *37*, 62–68. [CrossRef]
51. De Luca, V.; Angeli, A.; Mazzone, V.; Adelfio, C.; Carta, F.; Selleri, S.; Carginale, V.; Scaloni, A.; Supuran, C.T.; Capasso, C. Inhibitory Effects of Sulfonamide Derivatives on the β-Carbonic Anhydrase (MpaCA) from Malassezia pachydermatis, a Commensal, Pathogenic Fungus Present in Domestic Animals. *Int. J. Mol. Sci.* **2021**, *22*, 12601. [CrossRef]
52. Supuran, C.T.; Capasso, C. A Highlight on the Inhibition of Fungal Carbonic Anhydrases as Drug Targets for the Antifungal Armamentarium. *Int. J. Mol. Sci.* **2021**, *22*, 4324. [CrossRef]
53. Supuran, C.T.; Capasso, C. Biomedical applications of prokaryotic carbonic anhydrases. *Expert Opin. Ther. Pat.* **2018**, *28*, 745–754. [CrossRef]
54. Flaherty, D.P.; Seleem, M.N.; Supuran, C.T. Bacterial carbonic anhydrases: Underexploited antibacterial therapeutic targets. *Future Med. Chem.* **2021**, *13*, 1619–1622. [CrossRef]
55. Hewitt, C.S.; Abutaleb, N.S.; Elhassanny, A.E.M.; Nocentini, A.; Cao, X.; Amos, D.P.; Youse, M.S.; Holly, K.J.; Marapaka, A.K.; An, W.; et al. Structure-Activity Relationship Studies of Acetazolamide-Based Carbonic Anhydrase Inhibitors with Activity against Neisseria gonorrhoeae. *ACS Infect. Dis.* **2021**, *7*, 1969–1984.
56. Abutaleb, N.S.; Elhassanny, A.E.M.; Nocentini, A.; Hewitt, C.S.; Elkashif, A.; Cooper, B.R.; Supuran, C.T.; Seleem, M.N.; Flaherty, D.P. Repurposing FDA-approved sulphonamide carbonic anhydrase inhibitors for treatment of Neisseria gonorrhoeae. *J. Enzym. Inhib. Med. Chem.* **2022**, *37*, 51–61. [CrossRef]
57. An, W.; Holly, K.J.; Nocentini, A.; Imhoff, R.D.; Hewitt, C.S.; Abutaleb, N.S.; Cao, X.; Seleem, M.N.; Supuran, C.T.; Flaherty, D.P. Structure-activity relationship studies for inhibitors for vancomycin-resistant *Enterococcus* and human carbonic anhydrases. *J. Enzym. Inhib. Med. Chem.* **2022**, *37*, 1838–1844.
58. Nocentini, A.; Bua, S.; Del Prete, S.; Heravi, Y.E.; Saboury, A.A.; Karioti, A.; Bilia, A.R.; Capasso, C.; Gratteri, P.; Supuran, C.T. Natural Polyphenols Selectively Inhibit β-Carbonic Anhydrase from the Dandruff-Producing Fungus Malassezia globosa: Activity and Modeling Studies. *ChemMedChem* **2018**, *13*, 816–823.
59. Nocentini, A.; Cadoni, R.; Del Prete, S.; Capasso, C.; Dumy, P.; Gratteri, P.; Supuran, C.T.; Winum, J.Y. Benzoxaboroles as Efficient Inhibitors of the β-Carbonic Anhydrases from Pathogenic Fungi: Activity and Modeling Study. *ACS Med. Chem. Lett.* **2017**, *8*, 1194–1198.
60. Supuran, C.T. Bortezomib inhibits bacterial and fungal β-carbonic anhydrases. *Bioorg. Med. Chem.* **2016**, *24*, 4406–4409.
61. Hewitson, K.S.; Vullo, D.; Scozzafava, A.; Mastrolorenzo, A.; Supuran, C.T. Molecular cloning, characterization, and inhibition studies of a β-carbonic anhydrase from Malassezia globosa, a potential antidandruff target. *J. Med. Chem.* **2012**, *55*, 3513–3520. [CrossRef] [PubMed]
62. Bua, S.; Osman, S.M.; AlOthman, Z.; Supuran, C.T.; Nocentini, A. Benzenesulfonamides incorporating nitrogenous bases show effective inhibition of β-carbonic anhydrases from the pathogenic fungi *Cryptococcus neoformans*, *Candida glabrata* and *Malassezia globosa*. *Bioorg. Chem.* **2019**, *86*, 39–43. [CrossRef] [PubMed]
63. Innocenti, A.; Leewattanapasuk, W.; Mühlschlegel, F.A.; Mastrolorenzo, A.; Supuran, C.T. Carbonic anhydrase inhibitors. Inhibition of the beta-class enzyme from the pathogenic yeast Candida glabrata with anions. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4802–4805. [CrossRef] [PubMed]
64. Monti, S.M.; Maresca, A.; Viparelli, F.; Carta, F.; De Simone, G.; Mühlschlegel, F.A.; Scozzafava, A.; Supuran, C.T. Dithiocarbamates are strong inhibitors of the beta-class fungal carbonic anhydrases from *Cryptococcus neoformans*, *Candida albicans* and *Candida glabrata*. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 859–862. [CrossRef]

65. Cottier, F.; Leewattanapasuk, W.; Kemp, L.R.; Murphy, M.; Supuran, C.T.; Kurzai, O.; Mühlschlegel, F.A. Carbonic anhydrase regulation and CO(2) sensing in the fungal pathogen *Candida glabrata* involves a novel Rca1p ortholog. *Bioorg. Med. Chem.* **2013**, *21*, 1549–1554. [CrossRef]
66. Vullo., D.; Leewattanapasuk, W.; Mühlschlegel, F.A.; Mastrolorenzo, A.; Capasso, C.; Supuran, C.T. Carbonic anhydrase inhibitors: Inhibition of the β-class enzyme from the pathogenic yeast *Candida glabrata* with sulfonamides, sulfamates and sulfamides. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 2647–2652. [CrossRef]
67. 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. [CrossRef]
68. Pastorekova, S.; Casini, A.; Scozzafava, A.; Vullo, D.; Pastorek, J.; Supuran, C.T. Carbonic anhydrase inhibitors: The first selective, membrane-impermeant inhibitors targeting the tumor-associated isozyme IX. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 869–873. [CrossRef]
69. Vullo, D.; Voipio, J.; Innocenti, A.; Rivera, C.; Ranki, H.; Scozzafava, A.; Kaila, K.; Supuran, C.T. Carbonic anhydrase inhibitors. Inhibition of the human cytosolic isozyme VII with aromatic and heterocyclic sulfonamides. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 971–976. [CrossRef]
70. 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**, *55*, 5591–5600. [CrossRef]
71. Zimmerman, S.A.; Ferry, J.G.; Supuran, C.T. Inhibition of the archaeal beta-class (Cab) and gamma-class (Cam) carbonic anhydrases. *Curr. Top. Med. Chem.* **2007**, *7*, 901–908. [CrossRef] [PubMed]
72. Supuran, C.T.; Clare, B.W. Carbonic anhydrase inhibitors. Part Quantum chemical QSAR of a group of 1,3,4-thiadiazole and 1,3,4-thiadiazoline disulfonamides with carbonic anhydrase inhibitory properties. *Eur. J. Med. Chem.* **1999**, *34*, 41–50. [CrossRef]
73. Supuran, C.T.; Barboiu, M.; Luca, C.; Pop, E.; Brewster, M.E.; Dinculescu, A. Carbonic anhydrase activators. Part Synthesis of mono- and bis- pyridinium salt derivatives of 2-amino-5-(2-aminoethyl)- and 2-amino-5-(3-aminopropyl)-1,3,4-thiadiazole, and their interaction with isozyme II. *Eur. J. Med. Chem.* **1996**, *31*, 597–606. [CrossRef]
74. Aspatwar, A.; Barker, H.; Aisala, H.; Zueva, K.; Kuuslahti, M.; Tolvanen, M.; Primmer, C.R.; Lumme, J.; Bonardi, A.; Tripathi, A.; et al. Cloning, purification, kinetic and anion inhibition studies of a recombinant β-carbonic anhydrase from the Atlantic salmon parasite platyhelminth Gyrodactylus salaris. *J. Enzym. Inhib. Med. Chem.* **2022**, *37*, 1577–1586. [CrossRef] [PubMed]
75. Del Prete, S.; De Luca, V.; Vullo, D.; Osman, S.M.; AlOthman, Z.; Carginale, V.; Supuran, C.T.; Capasso, C. A new procedure for the cloning, expression and purification of the beta-carbonic anhydrase from the pathogenic yeast Malassezia globosa, an anti-dandruff drug target. *J. Enzym. Inhib. Med. Chem.* **2016**, *31*, 1156–1161. [CrossRef]