Benzenesulfonamides Incorporating Hydantoin Moieties Effectively Inhibit Eukaryotic and 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

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, EC 4.2.1.1) are an important family of metalloenzymes that assist the reversible interconversion of carbon dioxide and water to bicarbonate and proton (CO₂ + H₂O ⇋ HCO₃⁻ + 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 (-SO₂NH₂) 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 families have relevant physiologic functions and their inhibition may lead to anti-infective effects [53–57].

![Figure 1](image_url)  
*Figure 1. (a) Structure of Furagin; (b) General structure of 1-(4-benzenesulphonamide)-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-benzenesulphonamide)-3-alkyl/benzyl-hydantoin derivatives is shown in Scheme 1. The synthesis started from sulfanilamide 1, which was converted to 4-thioureido-benzenesulfonamide (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 methylethiolate from the formed methyl (4-sulfamoylphenyl) carbamimidothioate (3) by treatment with K2CO3 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. 1H NMR, 13C 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.
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 CO₂ hydrase assay [67].

| Compound | R        | K₁ (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       | -CH₂CH₃  | 261.7     | 25.8     | 30.8     | 68,090     | 46.0     |
| 8b       | -(CH₂)₂CH₃| 747.3     | 56.4     | 187.2    | 95,700     | 83.7     |
| 8c       | -CH₂CH=CH₂| 233.8     | 32.6     | 19.5     | 66,580     | 54.2     |
| 8d       | -CH₂C₅H₅ | 837.3     | 8.7      | 5.3      | 64,570     | 20.9     |
| 8e       | -CH₂(4-CH₃-C₆H₄) | 2926 | 32.7 | 3.0 | 38,930 | 18.3 |
| 8f       | -CH₂(4-Cl-C₆H₄) | 8789 | 62.2 | 15.3 | 41,460 | 44.9 |
| 8g       | -CH₂(4-CN-C₆H₄) | 570.5 | 7.2 | 12.0 | >100,000 | 38.4 |
| 8h       | -CH₂(4-NO₂-C₆H₄) | 656.6 | 6.1 | 30.3 | >100,000 | 6.6 |
| 8i       | -CH₂(4-CF₃-C₆H₄) | 601.1 | 43.3 | 14.3 | 59,580 | 13.1 |
| 8j       | -CH₂(4-OCF₃-C₆H₄) | 424.9 | 16.4 | 22.4 | >100,000 | 5.9 |
| 8k       | -CH₂(3-CH₃-C₆H₄) | 1081 | 58.3 | 18.8 | >100,000 | 8.4 |
| 8l       | -CH₂(2-F-C₆H₄) | 446.8 | 1.2 | 12.7 | 81,130 | 48.8 |
| 8m       | -CH₂(3,4-diCl-C₆H₄) | 867.9 | 85.6 | 132.9 | >100,000 | 67.9 |
| 8n       | -CH₂(C₆F₅) | 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 8i 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 alkyl-substituted ones, 8a and 8b, were moderately active ($K_I$ of 30.8–187.2 nM). The alkyl and benzyloxy-substituted 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 ($\delta$) 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$_6$) $\delta =$ 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$_6$) $\delta =$ 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-thioureidobenzesulfonamide (2) (300 mg, 1.3 mmol) in DMF (4 mL), Mel (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. NaHCO3 (2 × 20 mL) and then aq. sat. NH4Cl (1 × 20 mL), and dried over Na2SO4. Solvent removal in vacuum resulted in 3 (223 mg, 70%) as a white powder.

1H NMR (300 MHz, DMSO-d6) 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 13C NMR (75 MHz, DMSO-d6) δ = 14.2, 122.8, 127.7, 138.0, 153.9, 157.0 ppm HRMS (ESI) [M + H]+: m/z calcd for (C7H6N3O2S) 196.0181. Found 196.0188.

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), K2CO3 (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.

1H NMR (300 MHz, DMSO-d6) 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 13C NMR (75 MHz, DMSO-d6) δ = 118.0, 125.7, 127.9, 129.0, 160.9 ppm HRMS (ESI) [M – K]−: m/z calcd for (C7H6N3O2S) 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 iPrOH (50 mL) and dried in vacuum to afford 6 (3.98 g, 92%) as a white powder.

1H NMR (300 MHz, DMSO-d6) δ = 4.51 (s, 2H), 7.34 (s, 2H), 7.78–7.85 (m, 4H), 11.40 (s, 1H) ppm 13C NMR (75 MHz, DMSO-d6) δ = 51.9, 118.4, 127.6, 139.2, 141.9, 155.9, 171.1 ppm HRMS (ESI) [M – I]−: m/z calcd for (C10H8N3O3S) 254.0236. Found 254.0239.
3.2.5. 4-(3-Ethyl-2,4-dioxiimidazolidin-1-yl)benzenesulfonamide (8a)

To a stirred solution of 4-(2,4-dioxiimidazolidin-1-yl)benzenesulfonamide (6) (250 mg, 0.98 mmol) and ethyl iodide (0.079 mL, 0.98 mmol) in DMF (5 mL) K₂CO₃ (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 Na₂SO₄, and volatiles were removed in vacuum to afford 8a (103 mg, 37%) as a white solid.

³¹H NMR (300 MHz, DMSO-d₆) 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 ¹³C NMR (125 MHz, DMSO-d₆) 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 (C₁₆H₁₄N₃O₄S) 352.1331. Found 352.1341.

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

To a stirred solution of 4-(2,4-dioxiimidazolidin-1-yl)benzenesulfonamide (6) (250 mg, 0.98 mmol) and 1-iodoheptane (0.160 mL, 0.98 mmol) in DMF (5 mL), K₂CO₃ (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 air dried to afford 8b (109 mg, 32%) as a white solid.

³¹H NMR (300 MHz, DMSO-d₆) 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 ¹³C NMR (75 MHz, DMSO-d₆) 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 (C₁₇H₂₂N₃O₄S) 358.0862. Found 358.0869.

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

To a stirred solution of 4-(2,4-dioxiimidazolidin-1-yl)benzenesulfonamide (6) (250 mg, 0.98 mmol) and allyl bromide (0.085 mL, 0.98 mmol) in DMF (5 mL), K₂CO₃ (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 the precipitate formed was collected by filtration, washed with water, and air dried to afford 8c (156 mg, 54%) as a white powder.

³¹H NMR (300 MHz, DMSO-d₆) 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 ¹³C NMR (75 MHz, DMSO-d₆) δ = 14.3, 34.7, 51.0, 119.0, 128.2, 132.7, 139.5, 141.7, 154.8, 169.4 ppm HRMS (ESI) [M − 1]⁻: m/z calcd for (C₁₂H₁₂N₃O₄S) 294.0549. Found 294.0551.

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

To a stirred solution of 4-(2,4-dioxiimidazolidin-1-yl)benzenesulfonamide (6) (250 mg, 0.98 mmol) and benzyl bromide (0.116 mL, 0.98 mmol) in DMF (5 mL), K₂CO₃ (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 air dried to afford 8d (270 mg, 51%) as a white solid.
by filtration, washed with water and Et₂O, and air dried to afford 8d (205 mg, 61%) as a white solid.

1H NMR (300 MHz, DMSO-d₆) δ = 4.66 (s, 2H), 4.70 (s, 2H), 7.32–7.40 (m, 7H), 7.81–7.90 (m, 4H) ppm 13C NMR (75 MHz, DMSO-d₆) 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 (C₁₇H₁₃N₄O₄S) 369.0658. Found 369.0663.

3.2.7. 4-(3-Allyl-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), K₂CO₃ (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 Et₂O and air dried to afford 8e (179 mg, 51%) as a white solid.

1H NMR (300 MHz, DMSO-d₆) 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 13C NMR (75 MHz, DMSO-d₆) δ = 21.6, 42.4, 50.9, 118.5, 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 (C₁₇H₁₆N₄O₄S) 389.0556. Found 389.0556.

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), K₂CO₃ (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.

1H NMR (300 MHz, DMSO-d₆) 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 13C NMR (75 MHz, DMSO-d₆) 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 (C₁₆H₁₅N₃O₄SCl) 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), K₂CO₃ (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 Et₂O, and air dried to afford 8g (184 mg, 51%) as a white solid.

1H NMR (300 MHz, DMSO-d₆) 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 13C NMR (75 MHz, DMSO-d₆) 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 (C₁₇H₁₃N₄O₃S) 369.0658. Found 369.0663.

3.2.10. 4-(2,4-Dioxo-3-(4-(trifluoromethyl)benzyl)imidazolidin-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), K₂CO₃ (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 Et₂O, and air dried to afford 8h (188 mg, 49%) as a white solid.

¹H NMR (300 MHz, DMSO-d₆) 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) ppm ¹³C NMR (75 MHz, DMSO-d₆) 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 (C₁₆H₁₃N₄O₆S) 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), K₂CO₃ (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 Et₂O, and air dried to afford 8i (138 mg, 34%) as a white solid.

¹H NMR (500 MHz, DMSO-d₆) 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 ¹³C NMR (125 MHz, DMSO-d₆) δ = 42.2, 51.0, 118.5, 125.1 (q, J = 271.9 Hz) 126.3, 126.4, 127.7, 129.6, 139.3, 139.6, 141.6, 141.7, 155.0, 169.7 ppm ¹⁹F NMR (470 MHz) δ = –60.9 ppm HRMS (ESI) [M – 1]: m/z calcd for (C₁₇H₁₃N₃O₆F₃) 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), K₂CO₃ (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 Et₂O, and air dried to afford 8j (226 mg, 54%) as a white solid.

¹H NMR (500 MHz, DMSO-d₆) 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 (m, 4H) ppm ¹³C NMR (125 MHz, DMSO-d₆) 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 ¹⁹F NMR (470 MHz) δ = –56.8 ppm HRMS (ESI) [M – 1]: m/z calcd for (C₁₇H₁₃N₃O₅F₃S) 428.0528. Found 428.0533.
3.2.13. 4-(2,4-Dioxo-3-(4-(trifluoromethyl)benzyl)imidazolidin-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), K₂CO₃ (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 Et₂O, and air dried to afford 8k (120 mg, 34%) as a white solid.

¹H NMR (300 MHz, DMSO-d₆) δ = 2.32 (s, 3H), 4.65 (s, 2H), 4.66 (s, 2H), 7.15–7.29 (m, 4H), 7.36 (s, 2H), 7.81–7.89 (m, 4H) ppm ¹³C NMR (75 MHz, DMSO-d₆) 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 (C₁₇H₁₆N₃O₄S) 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), K₂CO₃ (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 Et₂O, and air dried to afford 8l (164 mg, 46%) as a white solid.

¹H NMR (500 MHz, DMSO-d₆) 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 ¹³C NMR (125 MHz, DMSO-d₆) δ = 36.5 (d, J = 21.4 Hz), 50.9, 116.2 (d, J = 21.4 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 ¹⁹F NMR (470 MHz) –118.0 ppm HRMS (ESI) [M − 1]⁻: m/z calcd for (C₁₆H₁₅N₃O₄FS) 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), K₂CO₃ (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 Et₂O, and air dried to afford 8m (194 mg, 48%) as a white solid.

¹H NMR (500 MHz, DMSO-d₆) 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 ¹³C NMR (75 MHz, DMSO-d₆) 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 (C₁₆H₁₂N₃O₄SCl₂) 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), K₂CO₃ (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 Et₂O, and air dried to afford 8n (229 mg, 54%) as a white solid.

1H NMR (500 MHz, DMSO-d₆) δ = 4.59 (s, 2H), 4.81 (s, 2H), 7.35 (s, 2H), 7.81–7.87 (m, 4H) ppm 13C NMR (125 MHz, DMSO-d₆) 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 ¹⁹F 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 (C₁₆H₁₃N₃O₄F₂S) 434.0234. Found 434.0246.

3.3. CA Inhibition Assay

An applied photophysics stopped-flow instrument was used for assaying the CA catalysed CO₂ hydration activity [67]. Phenol red (at a concentration of 0.2 mM) was used as indicator, working at the absorbance maximum of 537 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 Na₂SO₄ (for maintaining constant the ionic strength), following the initial rates of the CA-catalysed CO₂ hydration reaction for a period of 10–100 s. The CO₂ 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 mM 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 ¹H NMR, ¹³C 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.

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