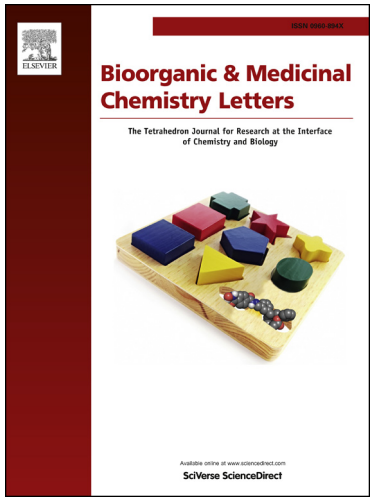

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Pyridazinone substituted benzenesulfonamides as potent carbonic anhydrase inhibitors

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Abstract

A series of sulfonamide derivatives (**2a-l**) incorporating substituted pyridazinone moieties were investigated for the inhibition of two human cytosolic carbonic anhydrase isoforms, hCA I and hCA II. All these compounds, together with the clinically used sulfonamide acetazolamide were investigated as inhibitors of the physiologically relevant isozymes I and II. These sulfonamides showed very strong inhibition against all these isoforms with K_I's in the range of 0.98 to 8.5 nM which makes such molecules possible to be used as leads for discovery of novel effective CA inhibitors targeting other isoforms with medicinal chemistry applications.

Keywords: Human carbonic anhydrase, enzyme inhibitors, sulfonamides.

The involvement of the metalloenzymes family carbonic anhydrases (carbonate hydrolyase, EC 4.2.1.1; CA) in various physiological processes have been recognized for a long period, being shown that the deregulated expression or abnormal performance of the 16 isozymes presently known may have important pathological consequences.[1,2] In fact, there are several human diseases whose pathopysiological characteristics include disbalance in the conversion between these isonzymes substrates (carbon dioxide and bicarbonate), resulting in perturbed ion transport, shift in pH, abnormal fluid secretion, etc.[1,2] Therefore, it seems plausible that modulation of CA activity to normal levels either by inhibition or activation offers interesting therapeutic options.[1] Because of their favorable outcomes, sulfonamides became widely accepted drugs in the treatment of several CA-based diseases, especially as antiglaucoma agents, diuretics, and antiulcer agents among others.[1,2] However, systemic and even topically administered CA inhibitors regularly showed serious side effects.[1-4] It is now understood that these undesired effects are due to the existence of at least 16 different CA isoforms,[1-5] that are indiscriminately inhibited irrespective of whether they play a real role in disease or are just coexpressed in the same tissue and elsewhere in the body. Moreover, certain drugs directed primarily against different CA unrelated targets may also inhibit activity of CAs. This may be exemplified by the antiinflammatory cyclooxygenase-2-selective drugs celecoxib and valdecoxib that show nanomolar affinity to several CA isoforms, but are generally well tolerated and give clinical responses in several disorders.[1,2] Thus, it is critically important to thoroughly characterize the affinity of different isozymes for sulfonamide CAIs, due to the wide range of applications of such drugs, and also to better understand the side effects due to inhibition of isozymes, which do not constitute the main target for a certain disease/application.[1-4]

Our groups recently investigated the interaction of CA I and II isozymes with several types of phenols, pyrrole derivatized sulfonamides, dopaminergic bromophenolic compounds and several of its substituted derivatives, e.g., salicyclates and some of their derivatives.[4] Here we extend these earlier investigations to a series of sulfonamides, some of which are widely used as prodrug or as drugs. Sulfonamides possess many types of biological activities, and representatives of this class of pharmacological agents are widely used in clinic as antibacterial, hypoglycemic, diuretic, anti-hypertensive and antiviral drugs among others.[1,2] Recently, a host of structurally novel sulfonamide derivatives have been reported to show substantial antitumor activity *in vitro* and/or *in vivo*.[3-4]

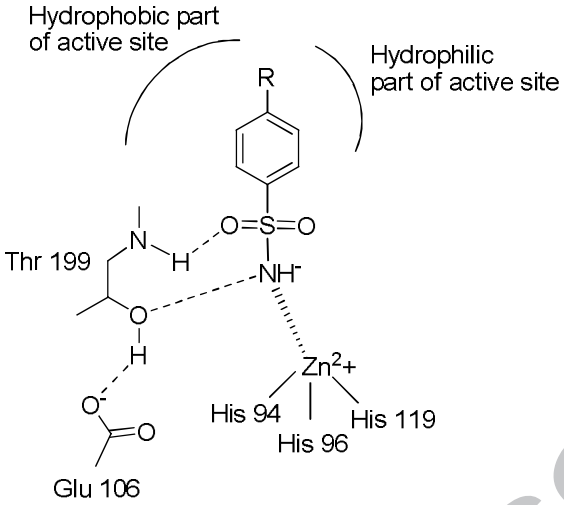


Figure 1. Estimated active site region in the hCA II-benzenesulfonamide complex X-ray structure, showing residues participating in recognition of the inhibitor molecule.

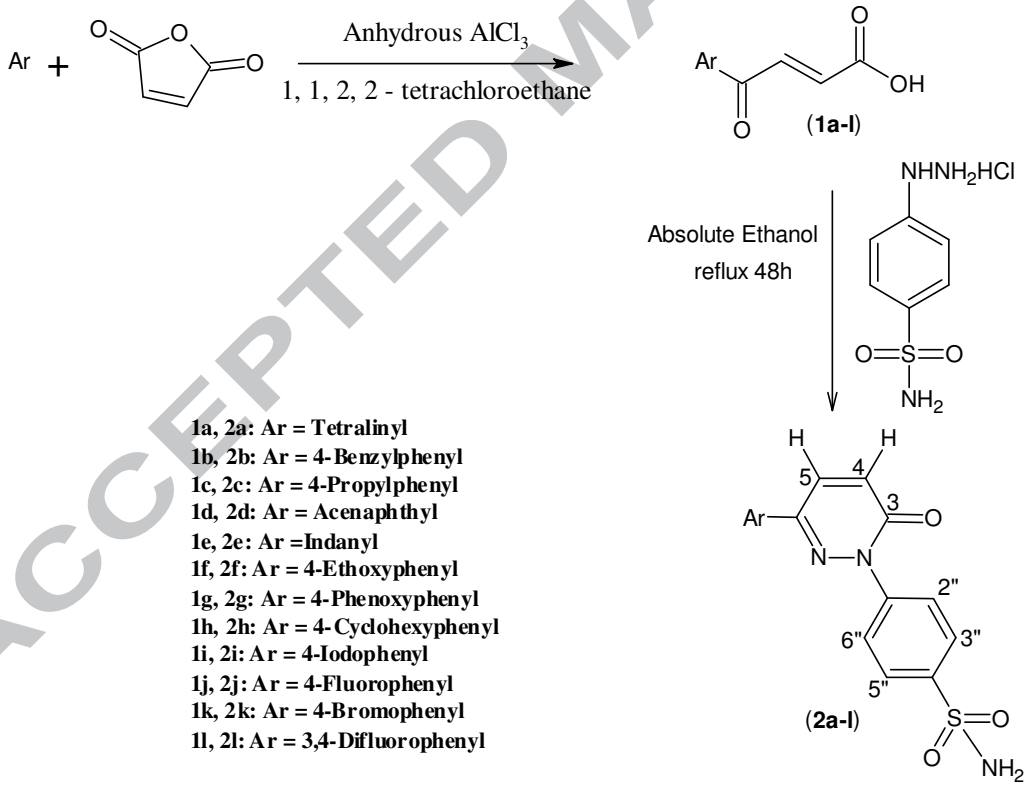
In the present study we have purified human CA I and II isoenzymes and examined the *in vitro* inhibition effects of some sulfonamide compounds (**2a-l**) mentioned above on these enzymes, using the esterase activity.

Sulfonamide type inhibitors binds to CAs, with coordination to the Zn^{2+} ion from the enzyme active site by substituting the fourth, non-protein ligand, a water molecule or hydroxide ion, such as for example acetazolamide (**AZA**), a clinically used compound since 1954.[4,5] The X-ray crystal structure has been extensively used for understanding the inhibition mechanism of CAIs. For example, for the adduct of hCA II with sulfamide,[5] it has been observed that the compound binds to CA by anchoring its SO_2NH^- moiety to the zinc ion of the enzyme active site, through a hydrogen bond as well as through a second hydrogen bond to the NH amide of Thr199, an amino acid conserved in all α-CAs and critically important for the catalytic cycle of these enzymes (Figure 1).[2-5]

The inhibition profile of various CA isozymes with this class of agents is very variable, with inhibition constants ranging from the millimolar to the submicromolar range.[5-7] Thus, it seemed reasonable to us to extend the previous studies,[5-10] including in this investigation a series of Schiff's bases obtained by condensing formylchromone with aminosulfonamides.

The synthetic route used to synthesize title compounds (**2a-l**) is outlined in Scheme 1. The β-aroylacrylic acids (**1a-l**) required for the synthesis of pyridazinoneswere obtained by a Friedel Craft's acylation through reported methods.[11] The cyclization to pyridazinone derivatives

bearing a benzenesulfonamide moiety was afforded by the condensation of appropriate β-aroylacrylic acid and 4-hydrazinobenzenesulfonamide hydrochloride in ethanol in 45-65% yield. The purity of the compounds was checked by TLC (Silica gel G) which was visualized by exposing to iodine vapours. The structures of **2a-l** were determined on the basis of elemental analysis and by various spectroscopic methods such as IR, ^{1}H NMR, ^{13}C NMR and MS. Elemental analysis (C, H, N & S) data were within ±0.4% of the theoretical values. Spectral data IR, ^{1}H NMR, ^{13}C NMR and MS of compounds were found in full agreement with the proposed structure. IR spectra showed prominent bands for NH_2 at 3305-3297 cm^{-1}, and 3140-3120 cm^{-1}, for cyclic carbonyl at 1662-1638 cm^{-1}, for C=N at 1599-1580 cm^{-1} and for SO_2N <at 1328-1322 cm^{-1} and 1156-1133 cm^{-1}. In ^{1}H NMR spectra the aromatic protons were observed at expected ppms. The signal for SO_2NH_2 was observed as two-proton singlet or merged with the signals of aromatic protons in aromatic region.



Scheme 1. Synthesis of substituted-6-oxopyridazin-1(6H)-yl] benzenesulfonamide (**2a-l**).

The purification of the two CA isozymes was performed with a simple one step method by a cellulose-benzyl-sulfanylamide affinity column chromatoghrapy. hCA I was purified, 102.6-fold with a specific activity of 875.12 $EUmg^{-1}$ and overall yield of 52.42 %, hCA II was purified, 867.3-fold with a specific activity of 6970 $EUmg^{-1}$ and overall yield of 63.8 %.[2b,5-10] Inhibitory effects of these sulfonamides **2a-l** on enzyme activities were tested under *in vitro* conditions; K_I values were calculated by using the Cheng-Prusoff equations and are reported in Table 1.

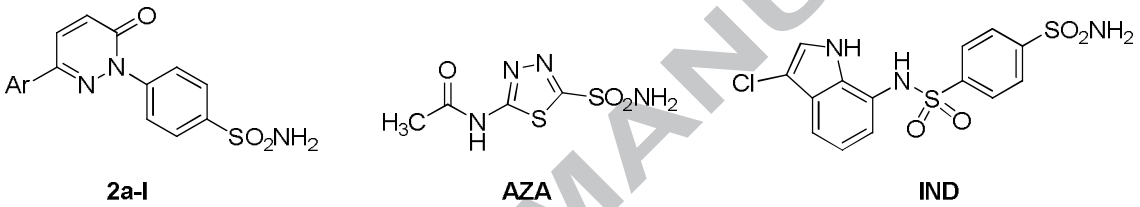
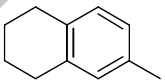
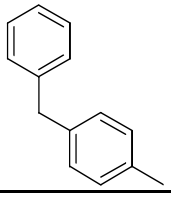
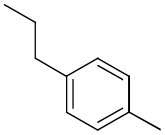
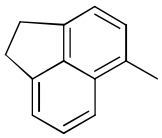
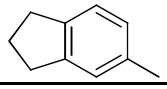
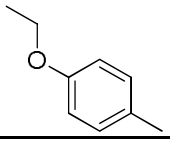
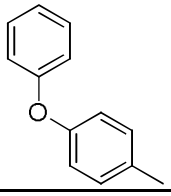
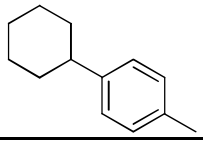
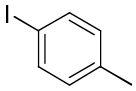
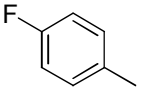
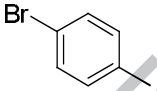
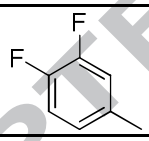


Figure 2. Studied and references molecules.

Table 1. hCA I and II inhibition data with sulfonamides **2a-l** and clinically used inhibitors, and the selectivity ratio hCA I over hCA II.

		K_I (nM)		Selectivity ratio
Compound	**R**	**hCA I**	**hCA II**	**hCA I/hCA II**
2a		5.16	2.16	2.39
2b		8.50	1.91	4.45
2c		1.13	2.90	0.39

2d		2.77	2.89	0.96
2e		3.60	2.15	1.67
2f		2.34	2.85	0.82
2g		1.70	1.15	1.48
2h		4.87	0.98	4.97
2i		3.09	1.86	1.66
2j		3.49	2.56	1.36
2k		5.45	3.26	1.67
2l		3.63	5.19	0.70
AZA[a]		250	12.0	20.83
IND[a]		31.0	15.0	2.06

Errors in the range of 2-5% of the shown data, from three different assays.
[a]From Ref. 1c.

We report here the inhibitory effects of sulfonamides **2a-l** on the eterase activity of hCA I and II. The sulfonamide CAI acetazolamide **AZA** and indisulam **IDA** has been used as a negative control in our experiments, and for comparison reasons. These compounds were synthesized and reported by Yaseen, R.[14] as potent anti-hyperglycemic agents in glucose fed hyperglycemic normal rats. Data of Table 1 show the following regarding inhibition of hCA I and II with compounds **2a-l**, **AZA**, and **IND** (as standards), by an esterase assay, with p-NPA (p-nitrophenyl acetate) as substrate: [9b]

(i) Against the slow cytosolic isozyme hCA I, compound **2b** behave as moderate inhibitor, with K_I value in the range of 8.5 nM. It is also interesting to note that derivatives **2c** and **7** were better hCA I inhibitor as compared to other compounds. This might indicate that hydrophobicity in the pyridazinone moiety is favorable for the inhibition of hCA I. Acetazolamide **AZA** had a K_I of 250 nM in this assay whereas compounds **2a-l** and **IND** were more powerful inhibitors than **AZA** (Table 1).

(ii) A better inhibitory activity has been observed with compounds **2a-l** investigated here for the inhibition of the rapid cytosolic isozyme hCA II (Table 1). Two derivatives, i.e., **2k** and **2l**, showed moderate hCA II inhibitory activity with K_I-s in the range of 3.26-5.19 nM, Table 1), whereas the remaining derivatives were quite effective hCA II inhibitors (Table 1). The best hCA II inhibitor in this series of derivatives was the bulky, pyridazinone substituted benzenesulfonamide derivative **2h**, which has a very low K_I value of 0.98 nM, is a better inhibitor than **AZA** and **IND**, a clinically used sulfonamides.

(iii) The rapid human blood cell isozyme (hCA II), ubiquitous in a lot of different tissues or cells,[1,2] is known to possess a high affinity for sulfonamides, we determined the selectivity ratios of the tested CAIs against human isozyme II over isozyme I (Table 1). It may be observed that most of the investigated compounds act as more potent hCA II than hCA I inhibitors, except **2c**, **2d**, **2f**, and **2l**, which showed selectivity ratios in the range of 0.39-0.96. Thus, the most hCA I selective inhibitor was compound **2d**. The most selective hCA II over hCA I inhibitors, such as derivatives **2b** and **2h**, showed selectivity ratios in the range of 4.45-4.97, which is indeed remarkable. Some other compounds also showed moderate selectivities, with ratios in the range of 1.36-2.39 (compounds **2b**, **2e**, **2g**, **2i-2l**).

Although there are several studies regarding the interactions of sulfonamide derivatives with carbonic anhydrase isozymes[12-13], it is critically important to explore further classes of potent CAIs in order to detect compounds with a different inhibition profile to find novel applications for the inhibitors of these widespread enzymes.

Especially, **2c, 2f,** and **2g** showed good activity against these hCA isozymes, more effective than the clinical used sulfonamides **AZA** and **IND**. Findings of our study indicates another class of possible CAIs of interest with strong activity, in addition to the well-known sulfonamides, the phenols/diphenols bearing bulky *ortho* moieties in their molecules.

Acknowledgments

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References and notes

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8. Detailed procedures for enzyme purification can be found in: Firstly, benzoyl chloride was stirred for four hours at room temperature in CH_2Cl_2 cellulose. After the spacer arm cellulose added as a benzyl group and finally diazotized sulfanilamide clamped to the para position of benzyl group as ligand. The hemolysate was applied to the prepared Cellulose-

benzylsulfanylamide affinity column equilibrated with 25 mM Tris-HCl/0.1 M Na_2SO_4 (pH 8.7). The affinity gel was washed with 25 mM Tris-HCl/22 mM Na_2SO_4 (pH 8.7). The human carbonic anhydrase (hCA I and hCA II) isozymes were eluted with 1 M NaCl/25 mM Na_2HPO_4 (pH 6.3) and 0.1 M CH_3COONa/0.5 M $NaClO_4$ (pH 5.6), respectively. All procedures were performed at 4ºC. (a) Guney, M.; Cavdar, H.; Senturk, M.; Ekinci, D. *Bioorg. Med. Chem. Lett*. **2015**, *25*, 3261; (b) Senturk, E.; Senturk, M.; Ekinci, D. *Acta Physiol.* **2015**, *215*, 99; (c) Ekinci, D.; Senturk, M.; Senturk, E. *Acta Physiol.* **2015**, *215*, 99; (d) Fidan, I.; Salmas, R.E.; Arslan, M.; Senturk, M.; Durdagi, S.; Ekinci, D.; Senturk, E.; Cosgun, S.; Supuran, C.T. *Bioorg. Med. Chem.* **2015**, *23*, 7353.

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14. Detailed synthetic procedures for the preparation of all derivatives can be found in: Melting points were determined in open capillary tubes and are uncorrected. All the Fourier TransformInfra-Red (FTIR) spectra were recorded on a Bio-rad FTS-135 spectrophotometer using KBr pellets; ν_{max} values are given in $cm^{-}1$. ^1H NMR spectra were recorded on a BrukerSpectrospin DPX 300 MHz spectrometer using deuterated DMSO as solvent and tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in δ (ppm) scale and coupling constants (*J* values) are expressed in Hz. Mass spectra (MS) were scanned by affecting FAB ionization JEOL-JMS-DX 303 system, equipped with direct inlet probe system. The m/z values of the more intense peaks are mentioned. The purity of the compounds was checked on TLC plate (Silica gel G) in the solvent system

Toluene:Ethyl acetate:Formic acid (5 : 4 : 1,TEF). Elemental analysis was carried out on CHNS Elementar (Vario EL III).

General procedure for the synthesis of 6-aryl-2-benzenesulfonamide-pyridazinones (2a-l): A mixture of appropriate β-aroylacrylic acid (**1a-l**) (0.001 mol) and 4-hydrazinobenzenesulfonamide hydrochloride (0.001 mol) in absolute ethanol (20-30 mL) was refluxed for 48 h. The solvent ethyl alcohol was removed by distillation method. The solid residue thus obtained was converted into fine powder, which was stirred with 5% sodium bicarbonate solution (25 mL). It was filtered, washed with 2% acetic acid and then with water. It was dried and crystallized from methanol. The intermediatesβ-aroylacrylic acids (**1a-l**) required for the synthesis of pyridazinones were prepared through reported procedures.[11]

*4-[6-Oxo-3-(5,6,7,8-tetrahydronaphthalen-2-yl) pyridazin-1(6H)-yl] benzenesulfonamide (**2a**):* White crystals; yield=61%; m.p. 280-281℃; R_f= 0.45 (TEF); IR ν_{max} (KBr, in cm^{-1}):3304 and 3122 (NH_2), 1652 (cyclic carbonyl),1582 (C=N), 1331 and 1148(SO_2N); ^{1}H NMR (300 MHz, DMSO-d_6, δ): 7.64 (1H, d, *J*=7.5 Hz, H-6'), 7.18 (1H, d, *J*=7.2 Hz, H-5'), 7.20 (1H, d, *J*=9.9 Hz, H-5), 7.64 (1H, d, *J*=7.5 Hz, H-2'), 7.89 (2H, d, *J*=8.4 Hz, H-3", H-5"), 7.96 (2H, d, *J*=8.7 Hz, H-2", H-6"), 8.13 (1H, d, *J*=9.9 Hz, H-4), 7.49 (2H, s, SO_2NH_2), 1.75 (4H, s, H-3'", H-4'"), 2.78 (4H, s, H-2'", H-5'"); ^{13}C NMR (75 MHz, DMSO-d_6, δ):155.12 (C=N pyridazinone), 146.21 (C-5 pyridazinone), 124.75 (C-4 pyridazinone), 154.26 (C=O pyridazinone); ESI-MS: (m/z):381[M^+], 382 [M+1], 380[M-1], 379 [M-2];CHNS Analysis for $C_{20}H_{19}N_3O_3S$: Found (Calculated) C, 63.03(63.05%); H, 5.04 (5.05%); N, 10.98 (10.97%); O, 12.58 (12.56%); S 8.40 (8.38%).

*4-[3-(4-Benzylphenyl)-6-oxopyridazin-1(6H)-yl] benzenesulfonamide (**2b**):*
White crystals; Yield=65%; m.p. 282-283℃; R_f= 0.48(TEF); IR ν_{max} (KBr, in cm^{-1}):3305 and 3130 (NH_2), 1647 (cyclic carbonyl),1582 (C=N), 1580 (C=N), 1335 and 1141(SO_2N);^{1}H NMR (300 MHz, DMSO-d_6, δ): 7.19-7.29 (6H, m, H-5, H-2'", H-3'", H-4'", H-5'", H-6'"), 7.36 (2H, d, *J*=8.1 Hz, H-3', H-5'), 7.49 (2H, s, SO_2NH_2), 7.84-7.96 (6H, m, H-3", H-5", H-2", H-6", H-2', H-6'), 8.13 (1H, d, *J*=9.9 Hz, H-4), 4.005 (2H, s, Ph-**CH$_2$**-Ph); ^{13}C NMR (75 MHz, DMSO-d_6, δ):154.19 (C=N pyridazinone), 147.10 (C-5 pyridazinone), 126.33 (C-4 pyridazinone), 155.12 (C=O pyridazinone);ESI-MS: (m/z):417[M^+], 418 [M+1], 416 [M-1], 415 [M-2];CHNS Analysis for $C_{23}H_{19}N_3O_3S$: Found (calculated)C, 66.10 (66.12%); H, 4.61 (4.60%); N, 10.12 (10.11%); O, 11.54 (11.52%); S, 7.72 (7.71%).

*4-[6-Oxo-3-(4-propylphenyl) pyridazin-1(6H)-yl] benzenesulfonamide (**2c**):*

White crystals; Yield=53%; m.p. 260-261℃; R_f= 0.46 (TEF); IR ν_{max} (KBr, in cm^{-1}):3297 and 3127 (NH_2), 1650(cyclic carbonyl), 1580 (C=N),1338 and 1146(SO_2N),^{1}H NMR (300 MHz, DMSO-d_6, δ): 7.21 (1H, d, *J*=9.9 Hz, H-5), 7.32 (2H, d, *J*=7.8 Hz, H-3', H-5'), 7.49 (2H, s, SO_2NH_2), 7.85 (2H, d, *J*=7.8 Hz, H-2', H-6'), 7.90 (1H, d, *J*=7.2 Hz, H-3", H-5"), 7.96 (1H, d, *J*=8.7 Hz, H-2", H-6"), 8.15 (1H, d, *J*=9.6, H-4), 0.90 (3H, t, Ph-CH_2CH_2**$\underline{CH_3}$**), 1.58-1.65 (2H, m, Ph-CH_2**$\underline{CH_2}$**CH_3), 2.61 (2H, t, Ph-**$\underline{CH_2}$**CH_2CH_3); ^{13}C NMR (75 MHz, DMSO-d_6, δ):155.11 (C=N pyridazinone), 145.14 (C-5 pyridazinone), 124.83 (C-4 pyridazinone), 154.15 (C=O pyridazinone);ESI-MS (m/z):369 [M^+], 370 [M+1], 368 [M-1], 367 [M-2];CHNS Analysis for $C_{19}H_{19}N_3O_3S$: Found (Calculated) C, 61.74 (61.75%); H, 5.19 (5.20%); N, 11.38 (11.39%); O 12.99 (13.01%); S, 8.71 (8.70%).

*4-[3-(1,2-Dihydroacenaphthylen-5-yl)-6-oxo-1,6-dihydropyridazin-1-yl]benzene-1-sulfonamide (**2d**):*

Light green crystals; Yield=25%; m.p. 250-251℃; R_f =0.54 (TEF); IR ν_{max} (KBr, in cm^{-1}):3300 and 3131 (NH_2), 1662 (cyclic carbonyl) 1595 (C=N),1329 and 1154 (SO_2N);^{1}H NMR (300 MHz, DMSO-d_6, δ): 7.11-7.92 (11H, m, aromatic protons of acenaphthyl and N-phenyl, SO_2NH_2), 7.25 (1H, d, *J*=9.6 Hz, H-5), 8.11 (1H, d, *J*=9.9 Hz, H-4), 3.29 (4H, brs, -**CH_2-CH_2**- ofacenaphthyl); ^{13}C NMR (75 MHz, DMSO-d_6, δ):154.51(C=N pyridazinone), 145.23 (C-5 pyridazinone), 124.81 (C-4 pyridazinone), 153.15 (C=O pyridazinone);ESI-MS (m/z): 403 [M^+], 404 [M+1], 402 [M-1],401 [M-2];CHNS Analysis for $C_{22}H_{17}N_3O_3S$: Found (Calculated)C, 65.50 (65.51%); H, 4.25 (4.26%); N, 10.42 (10.41%); O, 11.89 (11.88%), S 7.94 (7.95 %).

*4-[3-(2,3-Dihydro-1H-inden-5-yl)-6-oxopyridazin-1(6H)-yl] benzenesulfonamide (**2e**):*

Light yellow crystals; Yield=41%; m.p. 286-288℃; R_f =0.55 (TEF); IR ν_{max} (KBr, in cm^{-1}):3303 and 3120 (NH_2); 1655 (cyclic carbonyl), 1590 (C=N),1325 and 1149 (SO_2N);^{1}H NMR (300 MHz, DMSO-d_6, δ): 7.19 (1H, d, *J*=9.6 Hz, H-5), 7.34 (1H, d, *J*= 8.1 Hz, H-5'), 7.49 (2H, s, SO_2NH_2), 7.70 (1H, d, *J*=7.8 Hz, H-6'), 7.79 (1H, s, H-2'), 7.90 (2H, d, *J*=8.7 Hz, H-3", H-5"), 7.96 (2H, d, *J*=8.7 Hz, H-2", H-6"), 8.14 (1H, d, *J*=9.9 Hz, H-4), 2.02-2.08 (2H, m, H-3'"), 2.90 (4H, d, *J*=4.2 Hz, H-2'", H-4'"); ^{13}C NMR (75 MHz, DMSO-d_6, δ):155.58(C=N pyridazinone), 144.27 (C-5 pyridazinone), 125.81 (C-4 pyridazinone), 152.15 (C=O pyridazinone);ESI-MS (m/z): 367 [M^+], 368 [M+1], 366 [M-1], 365 [M-2];CHNS

Analysis for$C_{19}H_{17}N_3O_3S$: Found (Calculated) C, 62.07 (62.09%); H, 4.66 (4.68%); N, 11.48 (11.46%); O, 13.10 (13.08%); S, 8.77 (8.75 %).

*4-[3-(4-Ethoxyphenyl)-6-oxopyridazin-1(6H)-yl] benzenesulfonamide (**2f**):*

Light yellow crystals; Yield=55%; m.p. 248-249℃; R_f= 0.46 (TEF);IR ν_{max} (KBr, in cm^{-1}):3302 and 3121 (NH_2); 1644 (cyclic carbonyl), 1584 (C=N),1332 and 1140 (SO_2N);^{1}H NMR (300 MHz, DMSO-d_6, δ):7.03 (2H, d, *J*=8.7 Hz, H-3', H-5'), 7.19 (1H, d, *J*=9.9 Hz, H-5), 7.49 (2H, s, SO_2NH_2), 7.86-7.97 (6H, m, H-2', H-6', H-3", H-5", H-2", H-6"), 8.14 (1H, d, *J*=9.9 Hz, H-4), 1.34 (3H, t, Ar-O-CH_2-**$\underline{CH_3}$**), 4.08 (2H, q, Ar-O-**$\underline{CH_2}$**-CH_3);^{13}C NMR (75 MHz, DMSO-d_6, δ):153.81 (C=N pyridazinone), 144.46(C-5 pyridazinone), 125.13 (C-4 pyridazinone), 155.20 (C=O pyridazinone);ESI-MS (m/z):371[M^+], 372[M+1], 370 [M-1], 369[M-2];CHNS Analysis for$C_{18}H_{17}N_3O_4S$: Found (Calculated) C, 58.24 (58.25%); H, 4.60 (4.59%); N, 11.29 (11.28%); O, 17.28 (17.27%); S, 8.62 (8.61%).

*4-[6-Oxo-3-(4-phenoxyphenyl) pyridazin-1(6H)-yl] benzenesulfonamide (**2g**):*

Light yellow crystals; Yield=58%; m.p. 260-261℃; R_f= 0.51 (TEF);IR ν_{max} (KBr, in cm^{-1}):3305 and 3132 (NH_2); 1647 (cyclic carbonyl), 1580 (C=N),1334 and 1140(SO_2N); ^{1}H NMR (300 MHz, DMSO-d_6, δ):7.07-7.98 (14H, m, H-2', H-6', H-3', H-5', H-3", H-5", H-2", H-6", H-2"', H-3"', H-4"', H-5"', H-6"', H-5), 8.15 (1H, d, *J*=9.6 Hz, H-4), 7.49 (2H, s, SO_2NH_2); ^{13}C NMR (75 MHz, DMSO-d_6):156.02 (C=N pyridazinone), 144.76(C-5 pyridazinone), 125.15 (C-4 pyridazinone), 154.74(C=O pyridazinone);ESI-MS (m/z):419 [M^+], 420 [M+1], 418 [M-1], 417 [M-2];CHNS Analysis for$C_{22}H_{17}N_3O_4S$: Found (Calculated)C, 62.96 (62.98%); H, 4.09 (4.11%); N, 10.05 (10.04%); O, 15.30 (15.28%); S 7.68 (7.66%).

*4-[3-(4-Cyclohexylphenyl)-6-oxo-1,6-dihydropyridazin-1-yl]benzene-1-sulfonamide (**2h**):*

Yellow crystals; Yield=45%, m.p. 234-235℃; R_f= 0.52 (TEF);IR ν_{max} (KBr, in cm^{-1}):3298 and 3135 (NH_2), 1641 (cyclic carbonyl),1599 (C=N), 1328 and 1137(SO_2N),^{1}H NMR (300 MHz, DMSO-d_6, δ):7.21 (1H, d, *J*=9.9 Hz, H-5), 7.35 (2H, d, *J*=8.1 Hz, H-3', H-5'), 7.48 (2H, s, SO_2NH_2), 7.84 (2H, d, *J*=7.8 Hz, H-2', H-6'), 7.90 (2H, d, *J*=8.4 Hz, H-3", H-5"), 7.96 (2H, d, *J*=7.8 Hz, H-2", H-6"), 8.14 (1H, d, *J*=9.6 Hz, H-4), 3.1 (1H, s,cyclohexyle ring), 1.31-1.81 (9H, m, cyclohexyl ring);^{13}C NMR (75 MHz, DMSO-d_6, δ):154.12 (C=N pyridazinone), 144.96(C-5 pyridazinone), 124.85 (C-4 pyridazinone), 156.24 (C=O pyridazinone);MALDI (m/z):409 [M^+], 410 [M+1], 408 [M-1], 407 [M-2];CHNS Analysis for$C_{22}H_{23}N_3O_3S$: Found

(Calculated)C, 64.48 (64.50%); H, 5.68 (5.69%); N, 10.28 (10.29%); O, 11.74 (11.75%); S 7.85 (7.86%).

*4-[3-(4-Iodophenyl)-6-oxopyridazin-1(6H)-yl]benzenesulfonamide (**2i**):*

Light brown crystals; Yield=31%; 269-270℃; R_f= 0.5 (TEF);IR ν_{max} (KBr, in cm^{-1}):3301 and 3140 (NH_2), 1638 (cyclic carbonyl), 1591 (C=N),1326 and 1133(SO_2N); ^1H NMR (300 MHz, DMSO-d_6, δ): 7.23 (1H, d, *J*=9.9 Hz, H-5), 7.48 (2H, s, SO_2NH_2), 7.52-7.98 (8H, m, H-2', H-3', H-5', H-6', H-2'', H-3'', H-5'', H-6''), 8.16 (1H, d, *J*=9.9 Hz, H-4); ^{13}C NMR (75 MHz, DMSO-d_6, δ):156.11 (C=N pyridazinone), 144.91 (C-5 pyridazinone), 125.16 (C-4 pyridazinone), 155.18 (C=O pyridazinone);ESI-MS (m/z):453 [M^+], 454 [M+1], 452[M-1], 451 [M-2];CHNS Analysis for $C_{16}H_{12}IN_3O_3S$: Found (Calculated) C, 42.41 (42.40%); H, 2.68 (2.67%); I, 28.01 (28.00%); N, 9.26(9.27%); O, 10.60 (10.59%); S, 7.06 (7.07%).

*4-[3-(4-Fluorophenyl)-6-oxopyridazin-1(6H)-yl] benzenesulfonamide (**2j**):*

Dark yellow crystals; Yield=45%; m.p. 288-289 ℃; R_f = 0.44(TEF);IR ν_{max} (KBr, in cm^{-1}):3299 and 3130 (NH_2); 1659 (cyclic carbonyl), 1599 (C=N),1328 and 1156 (SO_2N);^1H NMR (300 MHz, DMSO-d_6, δ):7.23 (1H, d, *J*=9.9 Hz, H-5), 7.36 (2H, d, *J*=8.7 Hz, H-2', H-6'), 7.50 (2H, s, SO_2NH_2), 7.93-8.03 (6H, m, H-3', H-5', H-3'', H-5'', H-2', H-6''), 8.17 (1H, d, *J*=9.9 Hz, H-4); ^{13}C NMR (75 MHz, DMSO-d_6, δ):157.09 (C=N pyridazinone), 146.13 (C-5 pyridazinone), 125.11 (C-4 pyridazinone), 156.17 (C=O pyridazinone);ESI-MS (m/z):345[M^+], 346 [M+1], 344[M-1], 343 [M-2]; CHNS Analysis for$C_{16}H_{12}FN_3O_3S$: Found (Calculated) C, 55.65 (55.67%); H, 3.53 (3.51%); F, 5.54 (5.52%); N, 12.19 (12.18%); O, 13.88 (13.87%); S; 9.27 (9.26%).

*4-[3-(4-Bromophenyl)-6-oxopyridazin-1(6H)-yl] benzenesulfonamide (**2k**):*

Light yellow crystals; Yield=35%; m.p. 278-279 ℃; R_f= 0.4 (TEF);IR ν_{max} (KBr, in cm^{-1}):3301 and 3135 (NH_2), 1641 (cyclic carbonyl),1588 (C=N)1328 and 1137 (SO_2N); ^1H NMR (300 MHz, DMSO-d_6, δ): 7.24 (1H, d, *J*=9.9 Hz, H-5), 7.49 (2H, s, SO_2NH_2), 7.71 (2H, d, *J*=8.4 Hz, H-2', H-6'), 7.93 (6H, m, H-2'', H-3'', H-5'', H-6'', H-3', H-5'), 8.18 (1H, d, *J*=9.9 Hz, H-4); ^{13}C NMR (75 MHz, DMSO-d_6, δ):154.34 (C=N pyridazinone), 144.87 (C-5 pyridazinone), 124.96 (C-4 pyridazinone), 156.35 (C=O pyridazinone); ESI-MS (m/z):406 [M^+], 408 [M+2], 405 [M-1], 404 [M-2]; CHNS Analysis for $C_{16}H_{12}BrN_3O_3S$: Found (Calculated) C, 47.31 (47.32%); H, 2.97 (2.95%); Br, 19.71 (19.70%); N, 10.37 (10.35%), O, 11.82 (11.80%); S 7.89 (7.90%).

*4-[3-(3, 4-Difluorophenyl)-6-oxopyridazin-1(6H)-yl] benzenesulfonamide (**2l**):*

White crystals; Yield=37%; m.p. 270-271℃; R_f = 0.46; (TEF);IR ν_{max} (KBr, in cm^{-1}):3298 and 3121 (NH_2), 1657 (cyclic carbonyl), 1589 (C=N),1322 and 1152 (SO_2N);^{1}H NMR (300 MHz, DMSO-d_6, δ): 7.25 (1H, d,*J*=9.6 Hz, H-5), 7.49 (1H, s, SO_2NH_2), 7.58 (1H, d, *J*=9.6 Hz, H-5'), 7.83 (1H, d, *J*=5.7 Hz, H-2'), 7.92 (2H, d,*J*=9 Hz, H-3", H-5"), 7.97 (2H, d, *J*=8.7 Hz, H-2", H-6"), 8.004-8.039 (1H, m, H-6'), 8.19 (1H, d, *J*=9.9 Hz, H-4); ^{13}C NMR (75 MHz, DMSO-d_6, δ):154.41 (C=N pyridazinone), 143.77 (C-5 pyridazinone), 125.20 (C-4 pyridazinone), 154.13 (C=O pyridazinone); ESI-MS (m/z):363[M^+], 364 [M+1], 362[M-1], 361 [M-2]; CHNS Analysis for $C_{16}H_{11}F_2N_3O_3S$: Found (Calculated): C, 52.87 (52.86%); H, 3.12 (3.11%); F, 10.46 (10.45%); N, 11.56 (11.55%); O, 13.19 (13.20%); S, 8.81 (8.83%).

Pyridazinone substituted benzenesulfonamides as potent carbonic anhydrase inhibitors

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