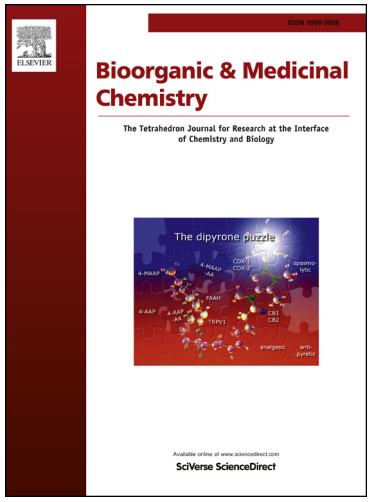

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Synthesis of 4-sulfamoylphenyl-benzylamine derivatives with inhibitory activity against human carbonic anhydrase isoforms I, II, IX and XII

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Abstract. Imine derivatives were obtained by condensation of sulfanilamide with substituted aromatic aldehydes. The Schiff bases were thereafter reduced with sodium borohydride, leading to the corresponding amines, derivatives of 4-sulfamoylphenyl-benzylamine. These sulfonamides were investigated as inhibitors of the human carbonic anhydrase (hCA, EC 4.2.1.1) isoforms hCA I and II (cytosolic isozymes), as well as hCA IX and XII (transmembrane, tumor-associated enzymes). We noted that the compounds incorporating secondary amine moieties showed a better inhibitory activity against all CA isozymes compared to the corresponding Schiff bases. Low nanomolar CA II, IX and XII inhibitors were detected, whereas the activity against hCA I was less potent. The secondary amines incorporating sulfonamide or similar zinc-binding groups, poorly investigated chemotypes for designing metalloenzyme inhibitors, may offer interesting opportunities in the field due to the facile preparation and possibility to explore a vast chemical space.

Keywords: carbonic anhydrase; sulfonamide; Schiff base; secondary amine; isoform

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1.Introduction

The carbonic anhydrase (CA, EC 4.2.1.1) family of metalloenzymes catalyzes the reversible hydration of CO_2 to HCO_3^-, and this regulatory reaction supports many physiological processes associated with pH control, ion transport, fluid secretion and several biosynthetic processes.[1-3] The inhibition of CAs has been exploited clinically for many decades, as most CA isoforms of the 15 known in humans are therapeutic targets with the potential to be inhibited or activated.[1-3] The physiologically dominant isoform is the red blood cell CA II (present in many other tissues) and its inhibitors have widespread use in a variety of applications as diuretics, antiglaucoma, and antiepileptic agents.[1-4] For such reasons, the design of novel classes of potent, possibly isoform-selective inhibitors targeting other human (h) CA isoforms may lead to clinical applications for treating a multitude of diseases such as edema, epilepsy, obesity, neuropathic pain and other neurological disorders, etc.[2–4] More recently, two CA isoforms, hCA IX and XII, have been shown to play an important role in tumor progression[5] and one CA IX/XII selective sulfonamide inhibitor is presently in Phase I clinical trials for the management of metastatic solid tumors.[5f]

The importance of sulfonamides as pharmacological agents appeared when Domagk[6] showed that sulfanilamide was the metabolite of the antibacterial drug Prontosil and possess excellent bacteriostatic effects. Later, a great number of sulfanilamide derivatives started to be employed in clinical medicine as antibacterials,[7] whereas other sulfonamides have been and are still used as pharmacological agents with a wide variety of biological actions.[8-14] All of them were designed considering sulfanilamide as lead molecule.[8] Nowadays sulfonamides have found widespread use in a variety of applications, among which as antibacterials,[9] anticancer agents,[5] diuretics,[10] CA inhibitors (CAIs) with a variety of uses,[11] hypoglycaemic agents,[12] anti-hyperthyroidism agents [13] and protease inhibitors.[14]

Schiff bases incorporating aromatic/heterocyclic sulfonamide moieties in their molecules have been extensively investigated as CAIs.[15-17] Some of these derivatives were the first ones reported to have some selectivity for the inhibition of some human (h) CA isoforms, such as hCA I, II or IV. The first Schiff bases obtained from aromatic sulfonamides and aromatic/heterocyclic aldehydes showed CA IV-selective inhibition patterns, whereas they inhibited less the off-target isoform CA II. [15-17]

Schiff base compounds have also been extensively investigated due to their role as chelating agents for main group and transition metal ions.[18,19] In the present study, we extend our previous research in the field of sulfonamide Schiff bases and their derivatives, and report

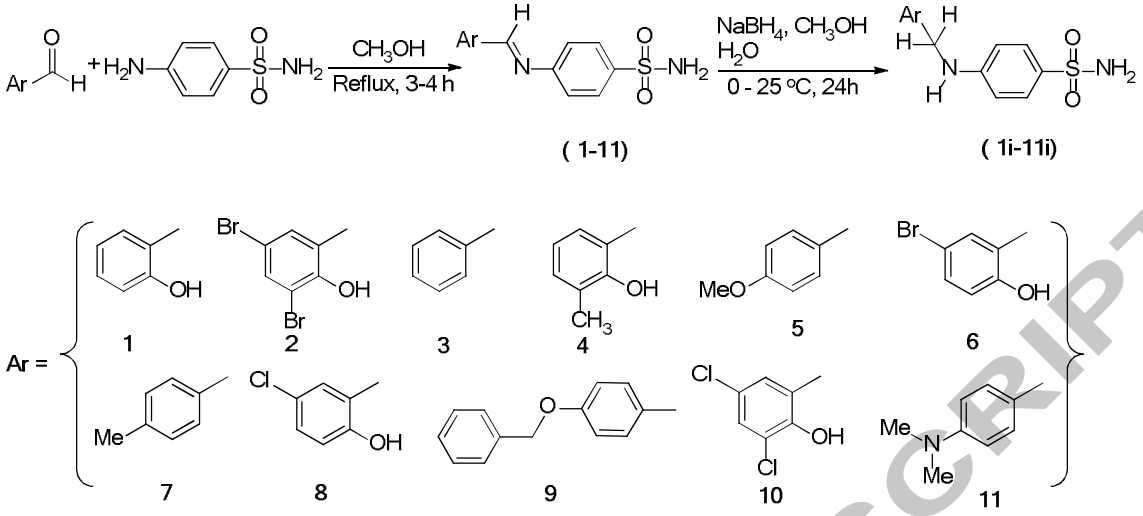
a new series of sulfanilamide-imine compounds as well as the corresponding secondary amines obtained by their reduction. These sulfonamides were investigated as inhibitors of four physiologically relevant enzymes, the cytosolic isoforms hCA I and II (drug targets for antiglaucoma agents), as well as, the transmembrane, tumor-associated ones hCA IX and XII (drug targets for antitumor agents, and respectively antiglaucoma/antitumor agents).

2. Results and Discussion

2.1. Chemistry

Schiff base derivatives (imine compounds) **1–11** were obtained by condensation of the sulfanilamide (4-aminobenzenesulfonamide) with the corresponding aromatical dehydes in methanol, with catalytic amounts of formic acid (Scheme 1) as reported earlier for many such sulfonamides by our groups.[15-17] The secondary amine sulfonamides **1i-11i** were subsequently prepared by reduction of the imine derivatives **1-11** with $NaBH_4$ in methanol. The drug design strategy for obtaining these secondary amines, which are not prone to hydrolysis is based on the fact we observed that many Schiff bases of ariomatic sulfonamides, including sulphanilamide, tend to hydrolyze to the starting materials by storage at room temperature for several weeks.[17] However, the reduced, secondary amines (suchas 1i-11i prepared here) are stable and do not undergo any hydrolytic or decomposition process even after month or years of storage.

All the synthesized compounds were fully characterized by analytical and spectral data. Some imine compounds have been already synthesized and characterized and our results are in complete agreement with those reported in the literature. The X-ray structure of one of the 4-{[4-(dimethylamino)benzylidene]amino}benzenesulfonamide **(11)** has been published.[18d] In the study, among the aromatic aldehyde derivatives used in the synthesis of the Schiff bases were 2-hydroxybenzaldehyde **(1)**, 3,5-dibromo-2-hydroxybenzaldehyde **(2)**, benzaldehyde **(3)**, 2-hydroxy-3-methylbenzaldehyde **(4)**, 4-methoxybenzaldehyde **(5)**, 5-bromo-2-hydroxybenzaldehyde **(6)**, 4-methylbenzaldehyde **(7)**, 5-chloro-2-hydroxybenzaldehyde **(8)**, 4-(benzyloxy)benzaldehyde**(9)**, 3,5-dichloro-2-hydroxybenzaldehyde (**10)**, 4-(dimethylamino) benzaldehyde**(11)**.[19] They were chosen in such a way as to incorporate both hydrophilic (OH, dimethylamino) as well as hydrophobic (methyl, methoxy, phenethyloxy, halogens) moieties, or combination of the two types of moieties in their molecules, in order to explore structure-activity and a diverse chemical space.



Scheme 1. Synthesis of the sulfonamide derivatives **1-11** and **1i-11i**.

In general, FT-IR spectra of imine compounds **(1–11)** were characterized by the presence of a strong band atabout 1620 cm^{-1} due to the stretching of the C=N bond, while it was not observed in the spectra of amine compounds **(1i–11i)** were not observed this strong band (C=N).On the other hand, in the ^{1}H NMR spectra of **1–11** were observed a singlet at about 8.50 ppm that attributed to azomethine (CH=N) chemical shift, while in the ^{1}H NMR spectrum of **1i–11i** this peak (CH=N) was not observed. The derivatives **1i–11i** showed a peak at about 4.25 ppm due to the presence of -CH_2- group. In the ^{13}C-NMR spectrum, the signals at about 163 ppm were assigned to azomethine group (CH=N) for **1–11**. These signals were not observed for **1i-11i** (see experimental section for details).

2.2. CA inhibition

We have assayed the sulfonamides prepared here for the inhibition of four physiologically relevant CAs, the cytosolic isoforms hCA I (offtarget isoform), hCA II (drug targets for antiglaucoma agents but offtarget when other isoforms are considered for the inhibition, suichas CA VA/VB, VII, IX and XII), as well as the transmembrane, tumor-associated ones hCA IX and XII (drug targets for antitumor agents – hCA IX – and respectively antiglaucoma/antitumor agents, hCA XII).

Table 1. Inhiibtion data of hCA I, II, IX and XII with sulfonamides **1-11** and **1i-11i** reported here, and acetazolamide AAZ as standard drug, by a stopped-flow CO_2 hydrase assay.[20]

Compound	K_I (nM)*			
	hCA I	hCA II	hCA IX	hCA XII
AAZ	250	12	25	6.0
1	428	258	952	10.3
2	>50000	251	360	38.5
3	>50000	289	454	11.1
4	463	264	465	11.6
5	>50000	464	883	11.2
6	458	248	257	10.4
7	>50000	469	23.7	9.9
8	>50000	404	47.9	9.4
9	>50000	>50000	36.0	9.7
10	442	290	35.6	7.5
11	421	328	41.5	8.8
1i	48.7	8.1	2.9	3.0
2i	42.3	2.1	2.3	31.9
3i	20.0	1.7	2.2	19.8
4i	24.8	2.2	2.3	131
5i	21.9	1.8	2.2	427
6i	42.5	3.4	2.6	213
7i	17.9	1.9	2.1	121
8i	30.7	2.6	2.4	21.0
9i	37.9	3.5	2.5	404
10i	20.9	2.3	2.2	138
11i	21.5	1.9	2.4	> 50000

*Errors were in the range of ± 5-10 % of the reported values (data not shown), from 3 different assays.

The following SAR can be observed from data of Table 1:

(i) The off-target slow isoform hCA I was poorly inhibited by most Schiff bases **1-11** (K_I > 50 μM) except few derivatives (**1, 4, 6, 10** and **11**) which were weak inhibitors with inhibition constants ranging between 421 and 463 nM. On the contrary, the secondary amines **1i-11i** showed a much better inhibitory action against this isoform, with K_Is in the range of 17.9 – 48.7 nM (Table 1): Thus, the reduction of the imine functionality to the secondary amine probably allows the inhibitors to adopt a favorable conformation when bound to the enzyme, which cannot be adopted by the derivatives **1-11** which incorporate the double bond. This effect has in fact observed earlier by this group[17e] for a series of sulfonamides incorporating a longer linker between the two aromatic rings, of the type $CH_2CH_2NHCH_2$ instead of $NHCH_2$.

(ii) The dominant cytosolic isoform hCA II was weakly inhibited by the Schiff bases **1-11** (K_Is in the range of 248 – 469 nM, except **9** which was not inhibited up to 50 μM) whereas the secondary amines **1i-11i** were highly effective CAIs, with K_Is in the range of 1.7 – 8.1 nM. Thus all substitution patterns from these secondary amines lead to highly effective CAIs, with difficulty to discuss a real SAR, since the variation of the inhibition constants is minimal, as shown above.

Table 2. Selectivity ratios for inhibiting the tumor-associated isoforms hCA IX and XII over the cytosolic ones hCA I and II, with AAZ and compounds **1-11** and **1i-11i.**

Compound	Selectivity ratio			
	hCA I / hCA IX	**hCA II / hCA IX**	**hCA I / hCA XII**	**hCA II / hCA XII**
AAZ	10.00	0.48	41.67	2.00
1	0.45	0.27	41.57	25.04
2	>138.89	0.70	>1298.70	6.52
3	>110.13	0.64	>4504.50	26.04
4	1.00	0.57	39.91	22.76
5	>56.63	0.53	>4464.29	41.43
6	1.78	0.96	44.04	23.85
7	>2109.70	19.79	>5050.51	47.37
8	>1043.84	8.43	>5319.15	42.98
9	>1388.89	>1388.89	>5154.64	>5154.64
10	12.42	8.15	58.93	38.67
11	10.14	7.90	47.84	37.27
1i	16.793	2.793	16.233	2.700
2i	18.391	0.913	1.326	0.066
3i	9.091	0.773	1.010	0.086
4i	10.783	0.957	0.189	0.017
5i	9.955	0.818	0.051	0.004
6i	16.346	1.308	0.200	0.016
7i	8.524	0.905	0.148	0.016
8i	12.792	1.083	1.462	0.124
9i	15.160	1.400	0.094	0.009
10i	9.500	1.045	0.151	0.017
11i	8.958	0.792	0.0004	0.00003

(iii) The inhibition pattern of the trans-membrane isoform hCA IX with these compounds is rather similar to what discussed above for hCA II: the imines **1-11** were poorer inhibitors compared to the corresponding secondary amines **1i-11i**. For the first subseries, derivatives **1-5** showed K_Is of 257-962 nM, being thus rather weak inhibitors, whereas **7-11** were better hCA IX inhibitors, with K_Is of 23.7 – 47.9 nM, the same range as the clinically used

sulfonamide **AAZ** (Table 1). It is obvious that rather small variations in the substitution pattern at the aldehyde side of the imines (e.g., the pairs **3-7**, differing by a methyl group; **1-8**, differing by a Cl atom) lead to important differences of the CA IX inhibitory pattern. The secondary amines on the other hand were all highly similar in their inhibition behaviour towards hCA IX , again with a very limited variation of the K_Is, i.e., 2.1 – 3.9 nM (Table 1).

(iv) The most interesting inhibition profile was however observed for hCA XII. For this isoform the Schiff bases **1-11** showed a similar behaviour of potent inhibitor, with K_Is in the range of 7.5 – 38.5 nM, whereas the secondary amines **1i-11i** were generally weaker inhibitors compared to the corresponding Schiff bases, with K_Is in the range of 3.0 – 427 nM. It is not possible to explain this very intriguing behaviour of the Schiff base/cognate secondary amine, since no X-ray crystal structures for these types of CAIs were reported so far.

(v) The selectivity ratios for inhibiting the tumor-associated isoforms hCA IX and XII over the cytosolic ones hCA I and II, with **AAZ** and compounds **1-11** and **1i-11i** are shown in Table 2**.** It can be observed that some of the investigated derivatives do show some promising levels of selectrive inhibition of the transmembrane over the cytosolic isoforms, among which **7-9, 1i**. Thus, the biological activity of these sulfonamides constitute an interesting starting point for exploring in more detail the secondary amines incorporating sulfonamide or other zinc-binding group moieties, for designing isoform-selective metalloenzyme inhibitors.

3. Conclusion

In this study, Schiff base derivatives of sulfanilamide were prepared by condensation with substituted benzaldehydes incorporating hydrophilic (OH, dimethylamino) as well as hydrophobic (methyl, methoxy, phenethyloxy, halogens) moieties, or a combination of the two types of moieties in their molecules. The secondary amine sulfonamides were thereafter prepared by reduction reaction of the imine compounds with $NaBH_4$. The obtained sulfonamides were investigated as inhibitors of four CA isoforms, the cytosolic CA I and II, as well as the transmembrane, tumor-associated CA IX and XII. It is interesting to note that the compounds incorporating secondary amine moieties showed a better inhibitory activity against all CA isozymes compared to the corresponding Schiff bases. Low nanomolar CA II and IX inhibitors were detected, whereas the activity against hCA I and XII was less strong. The secondary amines incorporating sulfonamide or other zinc-binding groups, poorly investigated chemotypes for designing metalloenzyme inhibitors, may offer interesting

opportunities in the field due to the facile preparation and possibility to explore a vast chemical space.

4. Experimental

4.1.Chemistry

All the chemicals were obtained from commercial suppliers (Sigma-Aldrich, Merck) and used without further purification. Elemental analysis was carried out on a LECO CHNS model 932 elemental analyser. FT-IR spectra were performed by using with a Perkin Elmer Spectrum Two FT-IR spectrometer (400-4000 cm^{-1}) with KBr pellets.NMR spectra were recorded at 25 ^{o}C in DMSO-d_6 using TMS as an internal standard on an Agilent-VNMRS-400 spectrometer operating at 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR.Melting points were measured in open capillary tubes with an Electro thermal 9100 melting point apparatus and were uncorrected. Mass Spectra results were recorded on a Shimadzu LCMS 8040 Model spectrometer. TLC (on Merck silica gel 60 F_{254} sheets) was used to follow the course of the reaction and assess product purity.

4.1.1. General procedure for the synthesis of imine derivatives 1-11:

The Schiff bases were synthesized according to the literature method with some modifications. The benzaldehyde derivatives (1.0 mmol) in 30 mL of methanol were added dropwise to the appropriate sulfanilamide (1.0 mmol) previously dissolved in 30 mL of methanol. Catalytic amounts of formic acid were added and the reaction stirred for 3–4 h under reflux. The solvent was thenevaporated. The obtained solid was washed with ice-cold ethanol/methanol. Then, the obtained products were recrystallized from methanol (**1-9**)/ethanol (**10** and **11**) and dried under vacuum to give the corresponding imino-derivatives (**1-11**) (Scheme 1).

4.1.1.1. 4-[(2-hydroxybenzylidene)amino]benzenesulfonamide (1):

Yield: 85%; Color: Yellow; M.p: 212-213 ˚C (lit.: 212,[9b] 209-210,[15] ˚C); Anal. Calcd. For $C_{13}H_{12}N_2O_3S$ (276.31) (%): C, 56.51; H, 4.38; N, 10.14; S, 11.60.Found (%): C, 56.42; H, 4.45; N, 10.26; S, 11.45; FT-IR (KBr pellets, cm^{-1}): 3341, 3245 (NH_2), 3100-3450 (O-H···N broad), 3062 (Ar-H), 1616 (-CH=N-), 1311 (asymmetric), 1164 (symmetric) (S=O); ^{1}H-NMR

(DMSO-d_6, TMS, 400 MHz, δ ppm): 12.62 (1H, s, Ar-OH), 8.99 (1H, s, -CH=N-), 7.39 (2H, s, -SO_2NH_2), 7.87-7.89 (2H, d, *J*= 8.8, Ar-H), 7.54-7.57 (2H, d, *J*= 8.8, Ar-H), 7.69-7.71 (1H, d, *J*= 9.6, Ar-H), 7.47-7.43 (1H, t, *J*= 17.6, Ar-H), 6.98-7.02 (2H, m, Ar-H);^{13}C-NMR (DMSO-d_6, TMS, 100 MHz, δ ppm): 166.44 (-C=N-), 160.68, 151.67, 142.43, 134.36, 132.99, 127.52, 122.26, 119.81, 117.15, 112.48 (Aromatic).

4.1.1.2. 4-[(3,5-dibromo-2-hydroxybenzylidene)amino]benzenesulfonamide (2):

Yield: 85%; Color: Bright red; M.p.: 246-248 ˚C; Anal. Calcd. For $C_{13}H_{10}Br_2N_2O_3S$ (434.10) (%): C, 35.97; H, 2.32; N, 6.45; S, 7.39.Found (%): C, 35.77; H, 2.25; N, 6.55; S, 7.30.FT-IR (KBr pellets, cm^{-1}): 3313, 3237 (NH_2), 3130-3550 (O-H···N broad), 3051 (Ar-H), 1621 (-C=N-), 1325 (asymmetric), 1161 (symmetric) (S=O); ^{1}H-NMR (DMSO-d_6, TMS, 400 MHz, δ ppm): 14.12 (1H, s, -Ar-OH), 9.03 (1H, s, -CH=N-), 7.43 (2H, s, -SO_2NH_2), 7.99-7.98 (1H, d, *J*= 2.4, Ar-H), 7.91 (1H, s, Ar-H), 7.92-7.93 (2H, d, *J*= 6, Ar-C-H), 7.62-7.65 (2H, d, *J*= 8.8, Ar-C-H).^{13}C-NMR (DMSO-d_6, TMS, 100 MHz, δ ppm):164.65 (-C=N-), 157.43, 149.59, 143.29, 138.43, 135.03, 127.61, 122.53, 121.35, 112.00, 110.23 (Aromatic).

4.1.1.3. 4-(benzylideneamino)benzenesulfonamide (3):

Yield: 80%; Color: White; M.p.: 189 ˚C (lit.: 188-190,[19a]190 [9b]°C); Anal. Calcd. For $C_{13}H_{12}N_2O_2S$ (260.31) (%): C, 59.98; H, 4.65; N, 10.76; S, 12.32. Found (%): C, 59.78; H, 4.73; N, 10.93; S, 12.21; FT-IR (KBr pellets, cm^{-1}): 3295, 3165 (NH_2), 3002 (Ar-C-H), 1622 (-C=N-), 1334 (asymmetric), 1151 (symmetric) (S=O); ^{1}H-NMR (DMSO-d_6, TMS, 400 MHz, δ ppm): 8.55 (1H, s, -CH=N-), 7.33 (2H, s, -SO_2NH_2), 7.92-7.90 (2H, d, *J*= 8.8, Ar-H), 7.82-7.84 (2H, d, *J*= 8.4, Ar-H)), 7.34-7.36 (3H, m, Ar-H), 7.16-7.18 (2H, d, *J*= 8.8, Ar-H); ^{13}C-NMR (DMSO-d_6, TMS, 100 MHz, δ ppm): 162.26 (-C=N-), 155.14, 141.25, 137.03, 131.31, 128.94, 128.28, 127.37, 121.64 (Aromatic). MS (m/z): 260.062[19a].

4.1.1.4. 4-[(2-hydroxy-3-methylbenzylidene)amino]benzenesulfonamide (4):

Yield: 85%; Color: Yellow; M.p.: 168-170 ˚C; Anal. Calcd. For $C_{14}H_{14}N_2O_3S$(290.34) (%): C, 57.92; H, 4.86; N, 9.65; S, 11.04. Found (%): C, 57.78; H, 4.75; N, 9.76; S, 11.10; FT-IR (KBr pellets, cm^{-1}):3356, 3259 (NH_2), 3160-3450 (O-H···N broad), 3061 (Ar-H), 2932 (Ar-CH_3), 1614 (-C=N-), 1318 (asymmetric), 1159 (symmetric) (S=O).^{1}H-NMR (DMSO-d_6, TMS, 400 MHz, δ ppm): 13.244 (1H, s, Ar-OH), 8.983 (1H, s, -CH=N-), 7.39 (2H, s, SO_2NH_2), 7.89-7.87 (2H, d, *J*= 8.8, Ar-H), 7.59-7.56 (2H, d, *J*= 8.8, Ar-H), 7.49-7.47 (1H, d, *J*= 8, Ar-H), 7.34-7.32 (1H, d, *J*= 7.2, Ar-H), 6.92-6.88 (1H, t, *J*= 14.8, Ar-H), 2.21 (3H, s,

Ar-CH_3);^{13}C-NMR (DMSO-d_6, TMS, 100 MHz, δ ppm): 166.50 (-C=N-), 159.24, 151.01, 142.54, 135.15, 131.45, 127.54, 125.67, 122.30, 119.29, 118.62 (Aromatic), 15.64 (Ar-CH_3).LC-MS/MS, MS (m/z): 291.05 [M+H].

4.1.1.5. 4-[(4-methoxybenzylidene)amino]benzenesulfonamide (5):

Yield: 80%; Color: White; M.p.: 197-198 ˚C, (lit:195-197[19a], 199-201[19b], 205[9b] ˚C); Anal. Calcd. for $C_{14}H_{14}N_2O_3S$ (290.34) (%): C, 57.92; H, 4.86; N, 9.65; S, 11.04.Found (%): C, 57.78; H, 4.78; N, 9.73; S, 11.12; FT-IR (KBr pellets, cm^{-1}): 3277, 3146 (NH_2), 3068, 3025 (Ar-H), 2960-2840 (Al-H), 1610 (-C=N-), 1332 (asymmetric), 1151 (symmetric) (S=O), 1269 (Ar-O-C);^{1}H-NMR (DMSO-d_6, TMS, 400 MHz, δ ppm): 8.53 (1H, s, -CH=N-), 7.32 (2H, s, -SO_2NH_2), 7.90-7.88 (2H, d, *J*= 8.8, Ar-H), 7.35-7.32 (2H, d, *J*= 8.8, Ar-H), 7.83-7.81 (2H, d, *J*= 8.8, Ar-H), 7.08-7.06 (2H, *J*= 8.8, Ar-H), 3.83 (3H, s, -O-$\underline{C}H_3$);^{13}C-NMR (DMSO-d_6, TMS, 100 MHz, δ ppm): 162.75 (-C=N-), 162.27, 155.14, 141.21, 131.30, 128.98, 127.36, 121.62, 114.81 (Aromatic), 55.92 (-O-$\underline{C}H_3$); MS (m/z): 290.073.

4.1.1.6. 4-[(5-bromo-2-hydroxybenzylidene)amino]benzenesulfonamide (6):

Yield: 85%; Color: Orange; M.p.: 217-219 ˚C (lit.[19]: 212-214°C); Anal. Calcd. For $C_{13}H_{11}BrN_2O_3S$ (355.21) (%):C, 43.96; H, 3.12; N, 7.89; S, 9.03. Found (%): C, 43.90; H, 3.07; N, 7.98; S, 8.96; FT-IR (KBr pellets, cm^{-1}): 3360, 3270 (NH_2), 3150-3450 (O-H···N broad), 3094, 3068 (Ar-C-H), 1616 (-C=N-), 1336 (asymmetric), 1154 (symmetric) (S=O); ^{1}H-NMR (DMSO-d_6, TMS, 400 MHz, δ ppm): 12.50 (1H, s, Ar-OH), 8.94 (1H, s, -CH=N-), 7.40 (2H, s, -SO_2NH_2), 7.92-7,91 (1H, d, *J*= 2.8, Ar-H), 7.90-7.88 (2H, d, *J*= 8.4, Ar-H), 7.60-7.57 (1H, m, Ar-H), 7.547-7.525 (2H, d, *J*= 8.8, Ar-H), 6.99-6.97 (1H, d, *J*= 8.8, Ar-H);^{13}C-NMR (DMSO-d_6, TMS, 100 MHz, δ ppm): 163.54 (-C=N-), 159.64, 151.66, 142.66, 136.51, 134.13, 127.54, 122.27, 121.82, 119.63, 110.61 (Aromatic); MS (ESI) $[M]^+$ = 354.5[19c].

4.1.1.7. 4-[(4-methylbenzylidene)amino]benzenesulfonamide (7):

Yield: 85%; Color: White; M.p: 196-198 ˚C(lit.[19]:198-200 ˚C); Anal. Calcd. for $C_{14}H_{14}N_2O_2S$ (274.34) (%): C, 61.29; H, 5.14; N, 10.21; S, 11.69. Found (%): C, 61.21; H, 5.06; N, 10.27; S, 11.63; FT-IR (KBr pellets, cm^{-1}): 3286, 3186 (NH_2), 3085, 3043 (Ar-H), 1622 (-C=N-), 1334 (asymmetric), 1154 (symmetric) (S=O);^{1}H-NMR (DMSO-d_6, TMS, 400 MHz, δ ppm): 8.57 (1H, s, -CH=N-), 7.33 (2H, s, -SO_2NH_2), 7.84-7.84 (2H, d, *J*= 2.8, Ar-H), 7.82-7.82 (2H, d, *J*= 3.2, Ar-H), 7.37-7.36 (2H, d, *J*= 2, Ar-H), 7.35-7.34(2H, *J*= 2, Ar-H), 2.37 (3H, s, Ar-

$\underline{C}H_3$);^{13}C-NMR (DMSO-d_6, TMS, 100 MHz, δ ppm): 162.93 (-C=N-), 154.96, 142.60,141.46, 133.56, 129.96, 129.44, 127.36,121.65 (Aromatic), 21.67(Ar-$\underline{C}H_3$); MS (m/z): 274.077[19a].

4.1.1.8. 4-[(5-chloro-2-hydroxybenzylidene)amino]benzenesulfonamide (8):

Yield: 85%; Color: Bright orange; M.p: 199-201 ˚C (lit. [19d]: 196-198˚C); Anal. Calcd. For $C_{13}H_{11}ClN_2O_3S$ (310.76) (%): C, 50.24; H, 3.57; N, 9.01; S, 10.32.Found (%):C, 50.16; H, 3.53; N, 9.13; S, 10.23; FT-IR (KBr pellets, cm^{-1}): 3359, 3270 (NH_2), 3150-3550 (O-H···N broad), 3107, 3077 (Ar-H), 1618 (-C=N-), 1336 (asymmetric), 1154 (symmetric) (S=O); ^{1}H-NMR (DMSO-d_6, TMS, 400 MHz, δ ppm): 12.48 (1H, s, Ar-OH), 8.95 (1H, s, -CH=N-), 7.40 (2H, s, -SO_2NH_2), 7.90-7.88 (2H, d, *J*= 8.8, Ar-H), 7.792-7.785 (1H, d, *J*= 2.8, Ar-H), 7.55-7.53 (2H, d, *J*= 8.8, Ar-H), 7.49-7.46 (1H, m, Ar-H), 7.04-7.02 (1H, d, *J*= 8.8, Ar-H); ^{13}C-NMR (DMSO-d_6, TMS, 100 MHz, δ ppm): 163.61 (-C=N-), 159.24, 151.67, 142.66, 133.75, 131.15, 127.54, 122.27, 123.25, 121.21, 119.22 (Aromatic). MS (ESI) $[M]^+$ = 310[19d].

4.1.1.9. 4-{[4-(benzyloxy)benzylidene]amino}benzenesulfonamide (9):

Yield: 75%; Color: white; M.p: 217-219 ˚C; Anal. Calcd. For $C_{20}H_{18}N_2O_3S$ (366.43) (%): C, 65.55; H, 4.95; N, 7.64; S, 8.75. Found (%): C, 65.47; H, 5.01; N, 7.77; S, 8.69; FT-IR (KBrpellets, cm^{-1}): 3337, 3253 (NH_2), 3068, 3025 (Ar-H), 2950-2850 (Al-H), 1627 (-C=N-), 1293 (asymmetric), 1156 (symmetric) (S=O), 1247 (-O-C-); ^{1}H-NMR (DMSO-d_6, TMS, 400 MHz, δ ppm): 8.59 (1H, s, -CH=N-), 7.33 (2H, s, -SO_2NH_2), 7.92-7.90 (2H, d, *J*= 8.8, Ar-H), 7.84-7.82 (2H, d, *J*= 8.4, Ar-H), 7.49-7.39 (5H, m, Ar-H), 7.34-7.36 (2H, d, *J*= 8.4, Ar-H), 7.18-7.16 (2H, *J*= 8.8, Ar-H), 5.21 (2H, s, Ar-O-$\underline{C}H_2$-Ar);^{13}C-NMR (DMSO-d_6, TMS, 100 MHz, δ ppm): 162.26 (-C=N-), 161.84, 155.13, 141.25, 137.03, 129.17, 128.45, 131.31, 127.37, 128.94, 128.28, 121.64, 115.94 (Aromatic), 69.93 (Ar-$\underline{C}H_2$-O-Ar).LC-MS/MS (m/z): 367.05 [M+H].

4.1.1.10. 4-[(3,5-dichloro-2-hydroxybenzylidene)amino]benzenesulfonamide (10):

Yield: 85%; Color: Bright red; M.p.: 242-244 ˚C; Anal. Calcd. for $C_{13}H_{10}Cl_2N_2O_3S$ (345.20) (%): C, 45.23; H, 2.92; N, 8.12; S, 9.29, Found (%): C, 45.19; H, 2.80; N, 8.25; S, 9.20;FT-IR (KBr pellets, cm^{-1}): 3344, 3245 (NH_2), 3120-3600 (O-H···N broad), 3063 (Ar-C-H), 1619 (-C=N-), 1324 (asymmetric), 1159 (symmetric) (S=O); ^{1}H-NMR (DMSO-d_6, TMS, 400 MHz, δ ppm): 13.99 (1H, s, -Ar-OH), 9.54 (1H, s, -CH=N-), 7.43 (2H, s, -SO_2NH_2), 7.93-7.91 (2H, d, *J*=8.8, Ar-H), 7.80-7.79 (1H, d, *J*=2.8, Ar-H), 7.772-7.765 (1H, d, *J*= 2.8, Ar-H), 7.65-7.62 (2H, d, *J*= 8.8, Ar-H); ^{13}C-NMR (DMSO-d_6, TMS, 100 MHz, δ ppm): 164.99 (-C=N-),

156.15, 149.77, 143.29, 133.18, 131.36, 127.61, 122.85, 122.51, 122.21, 121.00 (Aromatic); LC-MS/MS (m/z): 342.95 [M-H].

4.1.1.11. 4-{[4-(dimethylamino)benzylidene]amino}benzenesulfonamide (11):

Yield: 85%; Color: Lightyellow; M.p: 230-232 ˚C (lit: 226-227[15] , 212-214[19a], 222-225[19e] ˚C); Anal. Calcd. for $C_{15}H_{17}N_3O_2S$ (303.38) (%): C, 59.38; H, 5.65; N, 13.85; S, 10.57.Found (%):C, 59.31; H, 5.60; N, 13.96; S, 10.48. FT-IR (KBr pellet, cm^{-1}): 3321, 3282 (NH_2), 3086, 3047 (Ar-C-H), 2950-2800 ($-CH_3$), 1605 (-C=N-), 1331 (asymmetric), 1149 (symmetric) (S=O), 1231 (-N-C). ^{1}H-NMR (DMSO-d_6, TMS, 400 MHz, δ ppm): 8.41 (1H, s, -CH=N-), 7.27 (2H, s, $-SO_2NH_2$), 7.79-7.77 (2H, d, *J*= 8.8, Ar-H), 7.75-7.73 (2H, d, *J*= 9.2, Ar-H), 7.30-7.28 (2H, d, *J*= 8.8, Ar-H), 6.79-6.77 (2H, *J*= 8.8, Ar-H), 3.01 (6H, s, N-($\underline{C}H_3)_2$). ^{13}C-NMR (DMSO-d_6, TMS, 100 MHz, δ ppm): 162.15 (-C=N-), 155.73, 153.14, 140.54, 131.14, 127.32, 123.78, 121.53, 111.87 (Aromatic), 40.14 (-N-($\underline{C}H_3)_2$). MS (m/z): 303.1041[19a].

4.1.2. General procedure for the synthesis of amine derivatives 1i-11i:

To a stirred suspension of the imino-compound (**1-11**) (1.0 mmol) in methanol (30 mL) at 0 ^{o}C was added solid sodium borohydride ($NaBH_4$) (6.0 mmol) in small portions, over 1h. The mixture is left under stirring for 24 more hours at room temperature. After the reduction was complete, half of the solvent in the reaction mixture was evaporated and the remaining mixture was poured on ice. The resulting fine white precipitate was filtered, washed with cold water and recrystallized from absolute methanol (**1i-4i**), acetonitrile (**5i-7i**), acetone (**8i, 9i**), chloroform (**10i**) or dichloromethane (**11i**) and dried under vacuum to give the corresponding amino-derivative (**1i-11i**) in high yields.

4.1.2.1.4-[(2-hydroxybenzyl)amino]benzenesulfonamide (1i):

Yield: 65%; Color: white; M.p.: 185-187 ˚C; Anal. Calcd. For $C_{13}H_{14}N_2O_3S$ (278.33) (%): C, 56.10; H, 5.07; N, 10.06; S, 11.52.Found (%): C, 56.25; H, 4.98; N, 10.20; S, 11.45;FT-IR (KBr pellet, cm^{-1}): 3337 (NH, NH_2),3253 (OH), 3077 (Ar-C-H), 2853, 2942 (Al-H), *1616 (-C=N-)(disappeared),*1295 (asymmetric), 1154 (symmetric) (S=O); ^{1}H-NMR (DMSO-d_6, TMS, 400 MHz, δ ppm): 9.54 (1H, s, -Ar-OH), 6.86 (2H, s, $-SO_2NH_2$), 7.44-7.47 (2H, d, *J*= 8.8, Ar-H), 6.58-6.60 (2H, d, *J*= 8.8, Ar-H), 6.76 (1H, s, -Ar-NH), 7.11-7.09 (1H, d, *J*= 8.8, Ar-H), 6.69-6.73 (1H, t, *J*= 16, Ar-H), 7.01-7.05 (1H, t, *J*= 15.2, Ar-H), 6.79-6.82 (1H, d, *J*= 9.2, Ar-H), 4,23-421 (2H, d, Ar-CH_2);^{13}C-NMR (DMSO-d_6, TMS, 100 MHz, δ ppm): 155.44,

151.86, 130.41, 128.46, 128.14, 127.72, 111.30, 119.23, 125.26, 115.36 (Aromatic), 41.28 (Ar-$\underline{C}H_2$-N-).

4.1.2.2. 4-[(3,5-dibromo-2-hydroxybenzyl)amino]benzenesulfonamide (2i):

Yield: 55%; Color: white; M.p.: 163 ˚C; Anal. Calcd. For $C_{13}H_{12}Br_2N_2O_3S$ (436.12) (%): C, 35.80; H, 2.77; N, 6.42; S, 7.35.Found (%): C, 35.72; H, 2.55; N, 6.55; S, 7.28; FT-IR (KBr pellet, cm^{-1}): 3438, 3353(NH, NH_2), 3266 (O-H), 3116, 3085 (Ar-H), 2830-2965 (Al-H), *1621 (-C=N-)(disappeared),*1305 (asymmetric), 1138 (symmetric) (S=O); ^{1}H-NMR (DMSO-d_6, TMS, 400 MHz, δ ppm): 9.65 (1H, s, Ar-OH), 6.92 (2H, s, -SO_2NH_2), 6.90 (1H, broad, N-H), 7.614-7.608 (1H, d, *J*= 2.4, Ar-H), 7.254-7.248 (1H, d, *J*= 2.4, Ar-H), 7.50-7.52 (2H, d, *J*= 8.8, Ar-H), 6.59-6.61 (2H, d, *J*= 8.8, Ar-H), 4.32 (2H, s, -Ar-CH_2); ^{13}C-NMR (DMSO-d_6, TMS, 100 MHz, δ ppm): 151.42, 151.26, 133.13, 129.98, 131.32, 131.20, 127.89, 112.85, 111.77, 111.52 (Aromatic), 41.82 (Ar-CH_2-N).LC-MS/MS MS (m/z): 434.85 [M-H].

4.1.2.3. 4-(benzylamino)benzenesulfonamide (3i):

Yield: 80%; Color: white; M.p.: 174-176 ˚C; Anal. Calcd. For $C_{13}H_{12}N_2O_2S$ (262.33) (%): C, 59.52; H, 5.38; N, 10. 68; S, 12.22.Found (%):C, 59.65; H, 5.27; N, 10.90; S, 12.11; FT-IR (KBr pellet, cm^{-1}):3395, 3343, 3264 (NH, NH_2), 3106, 3029 (Ar-H), 2868-2954 (Al-H),*1622 (-C=N-)(disappeared),*1294 (asymmetric), 1147 (symmetric) (S=O);^{1}H-NMR (DMSO-d_6, TMS, 400 MHz, δ ppm): 7.48-7.46 (2H, d, *J*= 8.8, Ar-H), 7.31-7.320 (4H, m, Ar-H), 7.25 (1H, broad, Ar-NH-C); 6.96-6.99 (1H, t, Ar-H), 6.883 (2H, s, -SO_2NH_2), 6.60-6.63 (2H, d, *J*= 9.2, Ar-H), 4.33-4.31 (2H, d, *J*= 6, Ar-CH_2-N);^{13}C-NMR (DMSO-d_6, TMS, 100 MHz, δ ppm): 151.66, 139.82, 130.72, 128.81, 127.74, 127.53, 127.23, 111.54(Aromatic), 46.29 (Ar-$\underline{C}H_2$-N); LC-MS/MS (m/z): 263.10 [M+H].

4.1.2.4. 4-[(2-hydroxy-3-methylbenzyl)amino]benzenesulfonamide (4i):

Yield: 75%; Color: white; M.p.: 177 ˚C; Anal. Calcd. For $C_{14}H_{16}N_2O_3S$ (292.35) (%): C, 57.52; H, 5.52; N, 9.58; S, 10.97. Found (%): C, 57.45; H, 5.55; N, 9.66; S, 11.10; FT-IR (KBr pellet, cm^{-1}): 3358, 3257(NH, NH_2), 3123(OH), 3034 (Ar-H), 2861-2953 (Al-H), *1614 (-C=N-)(disappeared),*1327 (asymmetric), 1156 (symmetric) (S=O); ^{1}H-NMR (DMSO-d_6, TMS, 400 MHz, δ ppm): 8.43(1H, broad, Ar-OH), 6.75 (1H, broad,-N-$\underline{H}$-), 6.86 (2H, s, -SO_2NH_2), 7.47-7.44 (2H, d, *J*= 8.8, Ar-H), 6.59-6.57 (2H, d, *J*= 8.8, Ar-H), 6.96-6.94 (2H, d, *J*= 7.6, Ar-H), 6.684-6.647 (1H, t, *J*= 14.8, Ar-H), 4.27-4.260 (2H, d, *J*= 5.2, Ar-CH_2-N-), 2.17 (3H, s, Ar-CH_3); ^{13}C-NMR (DMSO-d_6, TMS, 100 MHz, δ ppm):153.15, 151.85, 130.48,

129.64, 127.72, 126.04, 125.03, 119.71, 111.37 (Aromatic), 41.95 (Ar-$\underline{C}H_2$-N), 16.96 (Ar-$\underline{C}H_3$); LC-MS/MS (m/z): 291.10 [M-H].

4.1.2.5. 4-[(4-methoxybenzyl)amino]benzenesulfonamide (5i):

Yield: 70%; Color: white; M.p: 181-183 ˚C ; Anal. Calcd. for $C_{14}H_{16}N_2O_3S$ (292.35) (%): C, 57.52; H, 5.52; N, 9.58; S, 10.97.Found (%): C, 57.48; H, 5.58; N, 9.73; S, 11.05. FT-IR (KBr pellet, cm^{-1}): 3388, 3333, 3258 (NH, NH_2), 3074, 3035 (Ar-H), 2955-2835 (Al-H), *1610 (-C=N-)(disappeared),* 1305 (asymmetric), 1161(symmetric(S=O), 1247 (Ar-O-C). ^{1}H-NMR (DMSO-d_6, TMS, 400 MHz, δ ppm): 6.86 (2H, s, -SO_2NH_2), 6.89 (1H, s, broad, N-H), 7.46-7.44 (2H, d, *J*= 8.8, Ar-H), 7.25-7.22 (2H, d, *J*= 8.8, Ar-H), 6.88-6.86 (2H, d, *J*= 9.2, Ar-H), 6.61-6.59(2H, *J*= 8.8, Ar-H), 4.24-4.22 (2H, d, Ar-CH_2-N), 3.698 (3H, s, -O-$\underline{C}H_3$). ^{13}C-NMR (DMSO-d_6, TMS, 100 MHz, δ ppm): 158.64, 151.65, 131.54, 130.59, 128.80, 127.69, 114.22, 111.53(Aromatic), 55.48 (-O-$\underline{C}H_3$), 45.75 (Ar-$\underline{C}H_2$-N). LC-MS/MS (m/z): 291.10 [M+H].

4.1.2.6. 4-[(5-bromo-2-hydroxybenzyl)amino]benzenesulfonamide (6i):

Yield: 60%; Color: white; M.p: 213-215 ˚C; Anal. Calcd. For $C_{13}H_{13}BrN_2O_3S$ (357.22) (%):C, 43.71; H, 3.67; N, 7.84; S, 8.98.Found (%):C, 43.61; H, 3.59; N, 7.94; S, 8.92; FT-IR (KBr pellet, cm^{-1}): 3370, 3278 (NH, NH_2), 3235 (OH), 3073, 3047 (Ar-C-H), 2820-2965 (Al-H), *1616 (-C=N-)(disappeared),*1342 (asymmetric), 1142 (symmetric) (S=O);^{1}H-NMR (DMSO-d_6, TMS, 400 MHz, δ ppm):9.94 (1H, s, -Ar-OH), 6.88 (2H, s, -SO_2NH_2), 6.82 (1H, broad, N-H), 7.49-7.46 (2H, d, *J*= 8.8, Ar-H), 7.21-7.18 (2H, m, *J*= 2.4, Ar-H), 6.78-6.76 (1H, d, *J*= 8.4, Ar-H), 6.60-6.58 (2H, d, *J*= 8.8, Ar-H)), 4.20-4.22 (2H, d, *J*= 6, -Ar-CH_2); ^{13}C-NMR (DMSO-d_6, TMS, 100 MHz, δ ppm): 154.82, 151.53, 130.82, 130.65, 130.54, 128.36, 127.83,117.50, 111.36, 110.49 (Aromatic), 40.85 (Ar-$\underline{C}H_2$-N).LC-MS/MS (m/z): 357.00 [M-H].

4.1.2.7. 4-[(4-methylbenzyl)amino]benzenesulfonamide (7i):

Yield: 70%; Color: white; M.p: 194-196 ˚C; Anal. Calcd. for $C_{14}H_{16}N_2O_2S$ (276.35) (%): C, 60.85; H, 5.84; N, 10.14; S, 11.60.Found (%): C, 60.79; H, 5.88; N, 10.23; S, 11.54. FT-IR (KBr pellet, cm^{-1}): 3387, 3329, 3257 (NH, NH_2), 3096, 3028 (Ar-C-H), 2850-2950 (Al-C-H), *1622 (-C=N-) disappeared,* 1301 (asymmetric), 1156 (symmetric) (S=O). ^{1}H-NMR (DMSO-d_6, TMS, 400 MHz, δ ppm): 6.86(2H, s, -SO_2NH_2), 6.93 (1H, s, N-H), 7.46-7.43 (2H, d, *J*= 8.8, Ar-C-H (C_2, C_6)), 7.21-7.19 (2H, d, *J*= 8, Ar-C-H (C'_2, C'_6)), 7.12-7.10 (2H, *J*= 7.6, Ar-

C-H (C'$_3$, C'$_5$)), 6.60-6.58 (2H, d, *J*= 8.8, Ar-C-H (C_3, C_5)), 4.27-4.25 (2H, d, *J*= 4.8, Ar-CH_2-N), 2.24 (3H, s, Ar-$\underline{C}H_3$). ^{13}C-NMR (DMSO-d_6, TMS, 100 MHz, δ ppm): 151.66, 136.69, 136.24, 130.62, 129.36, 127.69, 127.49, 111.53(Aromatic), 46.05 (Ar-$\underline{C}H_2$-N), 21.10 (Ar-$\underline{C}H_3$). LC-MS/MS (m/z): 277.10 [M+H].

4.1.2.8. 4-[(5-chloro-2-hydroxybenzyl)amino]benzenesulfonamide (8i):

Yield: 60%; Color: white; M.p: 195-197 ˚C; Anal. Calcd. For $C_{13}H_{13}ClN_2O_3S$ (312.77) (%): C, 49.92; H, 4.19; N, 8.96; S, 10.25. Found (%):C, 50.02; H, 4.11; N, 9.02; S, 10.17; FT-IR (KBr pellet, cm^{-1}): 3368, 3337(NH, NH_2), 3262 (OH), 3077, 3051 (Ar-C-H), 2850-2965 (Al-H), *1618 (-C=N-) disappeared,*1323 (asymmetric), 1138 (symmetric) (S=O);^{1}H-NMR (DMSO-d_6, TMS, 400 MHz, δ ppm): 9.93 (1H, s, -Ar-OH), 6.89 (2H, s, -SO_2NH_2), 6.86 (1H, broad, N-H), 7.50-7.48 (2H, d, *J*= 8.8, Ar-H), 7.10-7.07 (2H, m, *J*= 2.4, Ar-H), 6.84-6.82 (1H, d, *J*= 8.8, Ar-H), 6.620-6.59 (2H, d, *J*= 8.8, Ar-H), 4.23-4.22 (2H, d, *J*= 6, -Ar-CH_2-N); ^{13}C-NMR (DMSO-d_6, TMS, 100 MHz, δ ppm): 154.38, 151.56, 130.83, 127.84, 127.76, 127.70, 122.85, 116.95, 111.38(Aromatic), 40.92 (Ar-CH_2-N); LC-MS/MS (m/z): 311.00 [M-H].

4.1.2.9. 4-{[4-(benzyloxy)benzyl]amino}benzenesulfonamide (9i):

Yield: 70%; Color: white; M.p: 183-185 ˚C; Anal. Calcd. For $C_{20}H_{20}N_2O_3S$ (368.45) (%): C, 65.20; H, 5.47; N, 7.60; S, 8.70.Found (%):C, 65.11; H, 5.39; N, 7.67; S, 8.63; FT-IR (KBr pellet, cm^{-1}): 3339, 3253 (NH, NH_2), 3060, 3032 (Ar-H), 2950-2850 (Al-H), *1627(-C=N-) disappeared,*1323 (asymmetric), 1148 (symmetric) (S=O), 1245 (Ar-O-C); ^{1}H-NMR (DMSO-d_6, TMS, 400 MHz, δ ppm): 7.36 (2H, s, -SO_2NH_2), 7.48-7.45 (2H, d, *J*= 8.8, Ar-H), 7.41-7.31 (5H, m, Ar-H), 7.26-7.24 (2H, d, *J*= 8.8, Ar-H), 6.97-6.95 (2H, d, *J*= 8.8, Ar-H), 6.62-6.60 (2H, *J*= 9.2, Ar-H), 6.91 (1H, broad, -N$\underline{H}$-Ar), 5.07 (2H, s, Ar-O-CH_2-Ar), 4.25-4.24 (2H, d, *J*= 5.6, Ar-CH_2-N-).^{13}C-NMR (DMSO-d_6, TMS, 100 MHz, δ ppm): 157.74, 151.68, 137.62, 131.83, 130.62, 128.23, 128.86, 128.09,127.83, 127.71, 115.14, 111.55 (Aromatic), 69.61(Ar-$\underline{C}H_2$-O-Ar), 45.76 (Ar-CH_2-N-).LC-MS/MS (m/z): 369.05 [M+H].

4.1.2.10. 4-[(3,5-dichloro-2-hydroxybenzyl)amino]benzenesulfonamide (10i):

Yield: 85%; Color: white; M.p: 174-176 ˚C; Anal. Calcd. For $C_{13}H_{12}Cl_2N_2O_3S$ (347.22) (%): C, 44.97; H, 3.48; N, 8.07; S, 9.23.Found (%): C, 44.95; H, 3.43; N, 8.13; S, 9.19; FT-IR (KBr pellet, cm^{-1}): 3352, 3263 (NH, NH_2), 3245 (OH), 3120, 3092 (Ar-H), 2850-2950 (Al-H), *1619(-C=N-) disappeared,*1325 (asymmetric), 1156 (symmetric) (S=O); ^{1}H-NMR (DMSO-

d_6, TMS, 400 MHz, δ ppm): 9.77 (2H, broad, Ar-OH, N-H), 6.89 (2H, s, -SO_2NH_2), 7.50-7.48 (2H, d, *J*= 8.8, Ar-H), 7.37-7.36 (1H, d, *J*= 2.8, Ar-H), 7.083-7.077 (1H, d, *J*= 2.4, Ar-H), 7.60-7.58 (2H, d, *J*= 8.8, Ar-H), 4,30 (2H, s, -Ar-CH_2); ^{13}C-NMR (DMSO-d_6, TMS, 100 MHz, δ ppm): 151.29, 150.14, 131.14, 130.83, 127.87, 127.60, 126.55, 123.61, 122.17, 111.48 (Aromatic), 41.61 (Ar-CH_2-N).LC-MS/MS (m/z): 344.95 [M-H].

4.1.2.11. 4-{[4-(dimethylamino)benzyl]amino}benzenesulfonamide (11i):

Yield: 70%; Color: whitish; M.p: 181-183 ˚C; Anal. Calcd. For $C_{14}H_{16}N_3O_2S$ (305.40) (%): C, 58.99; H, 6.27; N, 13.76; S, 10.50.Found (%): C, 58.90; H, 6.21; N, 13.88; S, 10.47. FT-IR (KBr pellet, cm^{-1}): 3384, 3356, 3252 (NH, NH_2), 3107, 3073 (Ar-H), 2950-2800 (Al-H), *1605 (-C=N-) disappeared,*1308 (asymmetric), 1152(symmetric) (S=O), 1239 (Ar-N-C). ^{1}H-NMR (DMSO-d_6, TMS, 400 MHz, δ ppm): 6.87 (2H, s, -SO_2NH_2), 5.90 (1H, s, N-H), 7.46-7.44 (2H, d, *J*= 8.8, Ar-H), 7.16-7.14 (2H, d, *J*= 8.4, Ar-H), 6.69-6.67 (2H, d, *J*= 8.8, Ar-H), 6.62-6.60 (2H, *J*= 8.8, Ar-H), 4.19-4.17 (2H, d, *J*= 6, Ar-CH_2-N), 2.84 (6H, s, N-($C\underline{H_3}$)$_2$). ^{13}C-NMR (DMSO-d_6, TMS, 100 MHz, δ ppm): 151.78, 150.03, 130.39, 128.99, 128.49, 127.66, 112.93, 111.50 (Aromatic), 45.95 (Ar-$\underline{C}H_2$-N), 40.75 (-N-($\underline{C}H_3$)$_2$); LC-MS/MS (m/z): 306.10 [M+H].

4.2. Carbonic anhydrase inhibition assay

An Applied Photophysics stopped-flow instrument has been used for assaying the CA-catalyzed CO_2 hydration activity.[20] Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes buffer (pH 7.5) and 20 mM $NaClO_4$ for maintaining constant ionic strength, following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10-100 s, at 20 °C. 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 have been used to determine the initial velocity. The uncatalyzed rates were measured in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (1 mM) were prepared in distilled-deionized water and dilutions down to 0.01 nM were made thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E-I complex. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3 and the Cheng-Prusoff equation as reported earlier,[21] and represent the mean from

at least three different determinations. All CA isoforms were recombinant ones obtained in huse as reported earlie,[22-24] and their concentrations in the assay system were: 11.4 nM for hCA I, 9.0 nM for hCA II; 7.8 nM for hCA IX and 14.7 nM for hCA XII, respectively.

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Synthesis of 4-sulfamoylphenyl-benzylamine derivatives with inhibitory activity against human carbonic anhydrase isoforms I, II, IX and XII

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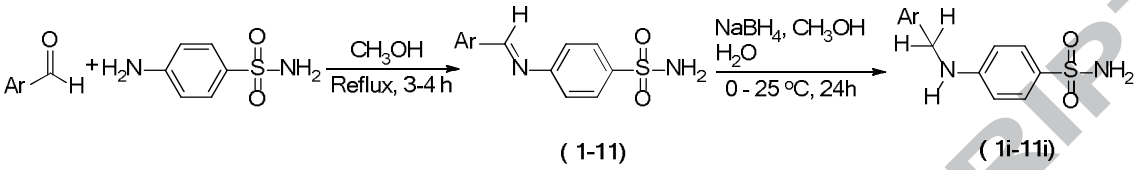

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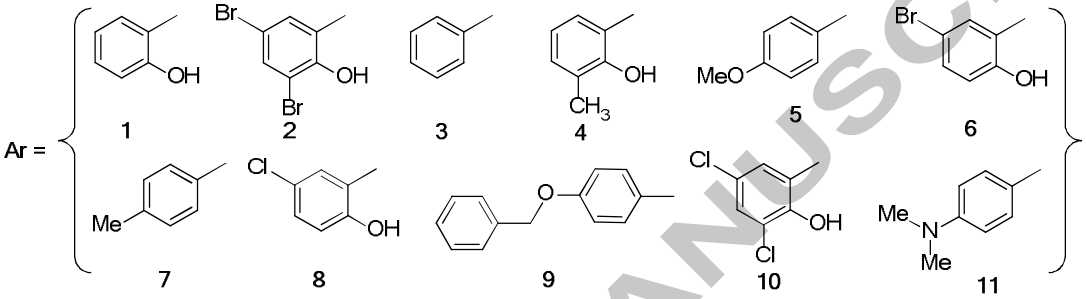

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