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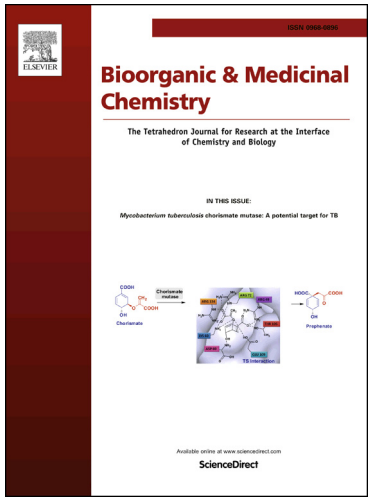

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Synthesis and carbonic anhydrase inhibition of a series of SLC-0111 analogs

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Abstract. SLC-0111 is a sulfonamide carbonic anhydrase (CA, EC 4.2.1.1) inhibitor (CAI) in Phase I/II clinical trials for the treatment of advanced hypoxic tumors complicated with metastases. Its antitumor effects are due to inhibition of the enzymatic activity of CA IX, an isoform predominantly found in tumors/metastases, but it also reduces the cancer stem cells population. Here we report the synthesis of analogs of SLC-0111, both of the sulfanilamide and metanilamide series, which possess diverse substitution patterns at the terminal ureido-phenyl moiety, thus including one or more halogens, trifluoromethyl, perchloro-/perfluorophenyl groups instead of the 4-fluorophenyl present in SLC-0111. Most of the sulfanilamide ureido derivatives were highly effective inhibitors of the tumor associated isoform and some showed selective CA IX/XII inhibitory profiles. Most of the sulfanilamide ureido derivatives were highly effective and in some cases selective CA IX/XII inhibitors, whereas the metanilamide ureido derivatives were less effective as transmembrane CA isoforms inhibitors. Structure activity relationship for this class of sulfonamides is discussed in detail.

Keywords: carbonic anhydrase; sulfonamide; inhibitor; tumor; urea derivative; SLC-0111

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1. Introduction

Many tumor types are characterized by an upregulated glucose metabolism, low levels of oxygen (hypoxia) and a dysregulated acid base balance, with the extracellular pH more acidic than the normal values (pH_e of around 6.0 – 6.5) and the intracellular pH more alkaline than the normal (pH_i of around 7.5).[1-3] This is known for decades as the Warburg effect,[4] but only in the last period it has been demonstrated that a transcription factor, the hypoxia-inducible factor (HIF) 1α, is the main player in these processes, through the overexpression of proteins involved in pH regulation, glucose metabolism, and erythropoiesis.[1-5] Several studies showed that the metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1),[6-10] also involved in several processes related to tumorigenesis, tumor progression and metastases formation, is also highly overexpressed in many tumors, as a downstream target of HIF-1α activation.[11,12]

CAs are highly effective catalysts for the reversible hydration of carbon dioxide with formation of bicarbonate and protons, being widespread in all life kingdoms, with seven genetically distinct families known to date.[6-10,13,14] Thus, CAs are involved in many physiological processes connected with pH regulation,[1-3] electrolyte secretion,[15] metabolic processes,[16] tumorigenesis,[11,12] etc., and their inhibition leads to pharmacological effects.[17,18] Indeed, sulfonamide CA inhibitors (CAIs) are clinically used as diuretics, antiglaucoma, anticonvulsant, antiobesity and antitumor agents for decades.[17-20] Many drug design strategies are presently available for obtaining effective and isoform-selective such agents belonging to various chemotypes, but the primary sulfonamides still remain among the most investigated CAIs due to their high affinity for many CA isoforms of pharmacologic interest, rather convenient pharmacology and ease of preparation.[21,22]

A sulfonamide CAI reported by one of our groups, SLC-0111,[23a] (Fig. 1) was shown to be a highly effective inhibitor of CA IX/XII, two isoforms predominantly found in tumors,[1-4] and to possess a significant antitumor/antimetastatic effect in animal models of cancer.[23-25] Furthermore, the cancer stem cell population was also diminished after treatment with this drug.[26]

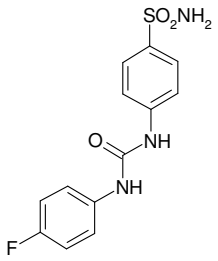


Fig. 1. Structure of SLC-0111, a sulfonamide CAI in Phase I/II clinical trials as antitumor drug.[24]

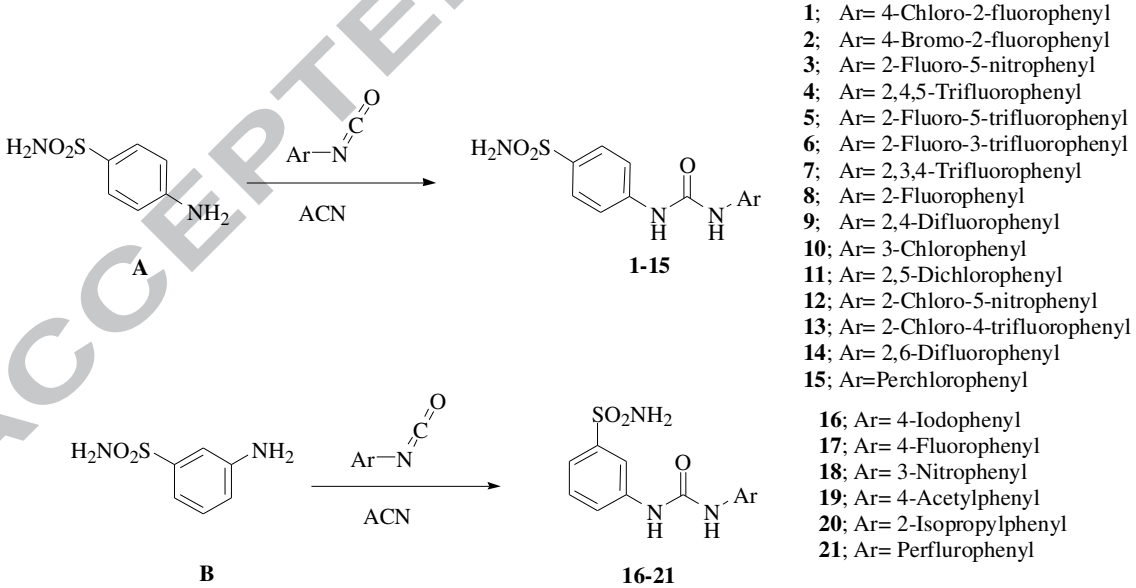
SLC-0111 entered in Phase I clinical trials in 2014 for the treatment of advanced, metastatic solid tumors overexpressing CA IX/XII.[24] The Phase I clinical trial finished successfully at the end of 2016, and the compound is currently scheduled to enter Phase II trials this year. [24]

Being the first-in-class CAI with anticancer/antimetastatic action (and also acting on the cancer stem cell population in a favorable manner, diverse from all other anticancer agents used clinically, which favor the increase of this stem cell population),[26] SLC-0111 has been used as a lead compound for designing other agents with a similar pharmacologic action.[27] Here we present a new study, reporting a series of derivatives structurally related to SLC-0111, which were investigated as inhibitors of four physiologically important CA isoforms, the human (h) hCA I and II (cytosolic, off-target isoforms) and hCA IX/XII (anticancer drug targets).[1-3,11,12]

2. Results and Discussion

2.1. Chemistry

The rationale of this work is straightforward and simple. We used SLC-0111 as lead compound, and we modified its 4-fluorophenylureido fragment. (i) A first series of sulfanilamide ureido derivatives were obtained by coupling commercially available sulfanilamide **A** with aryl-isocyanates, leading to ureas **1-15**, which are structurally very similar to SLC-0111 except for the various substituents present on the aromatic tail section. Indeed, the fluorine atom present in the lead was replaced by one or more halogens (identical or different ones), trifluoromethyl and nitro moieties (Scheme 1).



1; Ar= 4-Chloro-2-fluorophenyl
2; Ar= 4-Bromo-2-fluorophenyl
3; Ar= 2-Fluoro-5-nitrophenyl
4; Ar= 2,4,5-Trifluorophenyl
5; Ar= 2-Fluoro-5-trifluorophenyl
6; Ar= 2-Fluoro-3-trifluorophenyl
7; Ar= 2,3,4-Trifluorophenyl
8; Ar= 2-Fluorophenyl
9; Ar= 2,4-Difluorophenyl
10; Ar= 3-Chlorophenyl
11; Ar= 2,5-Dichlorophenyl
12; Ar= 2-Chloro-5-nitrophenyl
13; Ar= 2-Chloro-4-trifluorophenyl
14; Ar= 2,6-Difluorophenyl
15; Ar=Perchlorophenyl

16; Ar= 4-Iodophenyl
17; Ar= 4-Fluorophenyl
18; Ar= 3-Nitrophenyl
19; Ar= 4-Acetylphenyl
20; Ar= 2-Isopropylphenyl
21; Ar= Perflurophenyl

Scheme 1. Synthesis of ureido-benzenesulfonamides **1-21** from sulfanilamide **A**, metanilamide **B** and aryl-isocyanates.

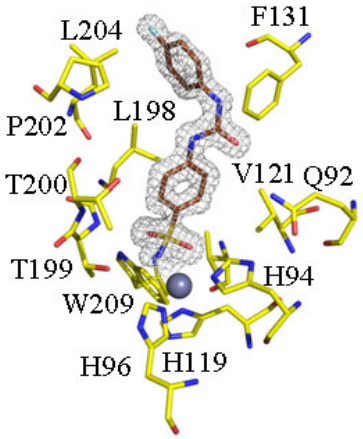


Fig. 2: X-ray crystal structure of the hCA II – SLC-0111 adduct.[23b] The zinc ion is shown as a gray sphere, its three His ligands and amino acid residues involved in the binding of the inhibitor (one letter amino acid code) are represented in yellow. The electron density of the bound inhibitor is shown.

The reasons for these changes are due to the fact that the X-ray crystal structure of SLC-0111 (and some of its analogs) bound to hCA II, [23b] showed variable orientations of the terminal aromatic ring, which, in turn, were correlated with the efficacy/selectivity as CAIs of these compounds against the various isoforms.[23]

(ii) We explored the synthesis of SLC-0111 regioisomers, of the type **16-21**, which were obtained from metanilamide **B** and arylisocyanates (Scheme 1). The main difference between these derivatives and SLC-0111/**1-15** is the *meta* position of the primary sulfonamide ($-SO_2NH_2$) moiety. As for the previously mentioned compounds (**1-15**), one or more halogens (identical or different ones), trifluoromethyl, nitro, acetyl and *iso*-propyl moieties were present in the aryl-isocyanates used as starting materials, in order to increase the explored chemical space. We stress again, X-ray crystallographic data of enzyme – SLC-0111 and analogs complexes demonstrated that the nature of the second aryl moiety is the most important factor governing CA inhibitory activity as well as selectivity towards various isoforms of pharmacological interest for this class of sulfonamide CAIs.[23]

2.2. CA inhibition

We assessed the CA inhibitory activity of compounds **1** - **21**, employing the clinically used drug acetazolamide (5-acetamido-1,3,4-thiadiazole-2-sulfonamide, **AAZ**) and SLC-0111 as positive

controls, for the inhibition of four CA isoforms of medical importance, hCA I and II (cytosolic, widely distributed enzymes) as well as hCA IX and XII (transmembrane, tumor-associated enzymes) – Table 1.

Table 1: CA inhibition data with ureidosubstituted benzenesulfonamides **1-21,** and acetazolamide (**AAZ**)/SLC-0111 as standard drugs, by a stopped-flow CO_2 hydrase assay.[28]

Compound	K_I (nM)*			
	hCA I	hCA II	hCA IX	hCA XII
1	436	162	5.1	7.9
2	373	418	6.3	7.4
3	519	276	7.3	7.6
4	569	215	7.6	6.2
5	363	170	7.4	10.1
6	395	196	8.7	8.5
7	484	335	9.6	6.4
8	550	33.0	7.7	7.3
9	275	60.6	3.7	7.1
10	158	10.0	8.4	8.5
11	747	58.2	8.9	6.8
12	307	0.94	8.2	6.1
13	192	0.97	8.4	8.2
14	158	0.93	6.1	8.3
15	538	362	6.3	7.6
16	1680	24.9	116	96.4
17	3260	17.2	219	113
18	2590	97.4	154	78.5
19	4800	258	178	66.9
20	1560	410	349	83.6
21	318	263	162	90.2
SLC-0111	5080	960	45	4.5
AAZ	250	12.0	25.2	5.6

* Errors in the range of 5 % of the reported values, from 3 different determinations (data not shown).

The following structure-activity relationship (SAR) can be drawn from data of Table 1:

(i) hCA I, a widely abundant cytosolic isoform in the gastro-intestinal tract and red blood cells,[6-8] was moderately inhibited by sulfonamides **1-21**, with K_Is in the range of 158-4800 nM (Table 1). It should be observed that **AAZ** is also a moderate hCA I inhibitor (K_I of 250 nM) whereas SLC-0111 is a much weaker one (K_I of 5080 nM), which is a positive feature of the candidate drug, as hCA I is in this case an off-target isoform. The sulfanilamide urea derivatives **1-15** were better hCA I inhibitors compared to the metanilamide ureas **16 – 21**. Furthermore, the presence of additional groups on the second aromatic ring from SLC-0111, as in derivatives **1-15**, had as a consequence the increase of the affinity for hCA I from the micromolar range (SLC-0111) to the high nM range for the new derivatives reported here. For example **7**, which has two more fluorine atoms compared to SLC-0111, was 10.5 times a better hCA I inhibitor compared to the lead, and this is a general trend for the other derivatives **1-15** reported here. Only the metanilamide **16-21** maintained the micromolar affinity for hCA I, similar to SLC-0111 (Table 1).

(ii) The physiologically dominant cytosolic isoform, hCA II, showed a very interesting inhibition profile with the new sulfonamides reported here. Thus, unlike SLC-0111 which is a poor inhibitor of this isoform (K_I of 960 nM), many of the new sulfonamides were low nanomolar or even subnanomolar hCA II inhibitors, others had a medium potency inhibitory profile whereas some others were medium-weak inhibitors. The K_Is against hCA II ranged between 0.93 and 418 nM (Table 1). **AAZ** is also an efficient hCA II inhibitor, with a K_I of 12.0 nM. The most efficient hCA II inhibitors in the series were **10, 12-14, 16** and **17**. The last two derivatives are metanilamide ureas whereas the first four belong to the sulfanilamide series. It is very interesting to note the substitution patterns present in the second aromatic ring of these compounds and to compare them to the one found in SLC-0111, which is a rather ineffective hCA II inhibitor, as mentioned above. Thus, **10, 12** and **13** incorporate at least one chlorine as substituent on the second aromatic ring, and eventually other groups, such as nitro and trifluoromethyl. On the other hand **14** has two fluorine atoms but in a different position compared to SLC-0111 (2,6-difluoro). This is in fact the best inhibitor in this series, being 1032 times a better hCA II inhibitor compared to the clinical candidate drug. This is a huge difference of activity, explained only by the different orientation/contacts that the 2,6-difluorophenyl moiety can do when bound to the active site, compared to the 4-fluorophenyl functionality of SLC-0111. Another interesting case is **17**, which is the *meta*-regiomer of SLC-0111. This compound is also an effective hCA II inhibitor, being 55.8 times more effective than

SLC-0111 in inhibiting hCA II. All these data show that very small structural changes in the scaffold of these inhibitors leads to huge differences of activity. Four other derivatives, **8, 9, 11** and **18**, had K_Is < 100 nM, whereas the remaining ones were medium potency hCA II inhibitors (K_Is ranging between 162 and 418 nM. The main conclusion of the SAR, mentioned above, is reinforced by these data: small structural changes in the scaffold, mainly at the second aromatic ring, influence significantly the CA inhibitory effects of these sulfonamides.

(iii) hCA IX, the main drug target for anticancer effects of the CAIs, was effectively inhibited by the sulfanilamide ureas **1-15**, with K_Is in the range of 3.7 – 9.6 nM, whereas the metanilamide **16-21** derivatives were much weaker inhibitors (K_Is in the range of 116 – 349 nM, Table 1). The SAR is almost absent in this case, as all sulfanilamide ureas showed a very compact behavior as potent CA IX inhibitors (being more effective than the lead, SLC-0111), whereas all metanilamide ureas were less effective. It is thus clear that the additional substituents present in the second aromatic ring of SLC-0111 led to an increase of affinity for hCA IX, but with a major decrease of selectivity for inhibiting hCA IX over hCA II and hCA I.

(iv) In analogy to hCA IX, the second transmembrane isoform connected to tumors, hCA XII, was also effectively inhibited by the sulfanilamide derivatives **1-15** (K_Is in the range of 6.2 – 10.1 nM), and less effectively inhibited by the metanilamides **16-21** (K_Is in the range of 66.9 – 113 nM, Table 1). SAR is thus similar to what discussed above for hCA IX.

(v) Some of the sulfonamides reported here, such as **1-7** and **15** are selective inhibitors of hCA IX/XII over hCA I/II; but the selectivity indexes are lower compared to those of the lead compound SLC-0111.

3. Conclusions

SLC-0111 is a sulfonamide CAI in Phase I/II clinical trials for the treatment of advanced hypoxic tumors complicated with metastases. Its antitumor effects are due to inhibition of the enzymatic activity of CA IX, an isoform predominantly found in tumors/metastases, but it also reduces the cancer stem cells population. Here we report the synthesis of analogs of SLC-0111, which possess diverse substitution patterns at the ureido-phenyl moiety of the candidate drug. Both sulfanilamide and metanilamide ureido derivatives were obtained, possessing one or more halogen, trifluoromethyl, perchloro-/perfluorophenyl moieties instead of the 4-fluorophenyl group found in SLC-0111. Most of the sulfanilamide ureido derivatives were highly effective and sometimes selective CA IX/XII inhibitors, whereas the amide ureido derivatives were less effective as

transmembrane CA isoforms inhibitors. Structure activity relationship for this class of sulfonamides is discussed in detail.

4. Experimental

4.1. Chemistry

Anhydrous solvents and all reagents were purchased from Sigma-Aldrich, Alfa Aesar and TCI. All reactions involving air- or moisture-sensitive compounds were performed under a nitrogen atmosphere using dried glassware and syringes techniques to transfer solutions. Infrared (IR) spectra were recorded as KBr plates and are expressed in ν (cm^{-1}). Nuclear magnetic resonance (^{1}H-NMR, ^{13}C-NMR) spectra were recorded using a Bruker Advance III 400 MHz spectrometer in DMSO-d_6. The chemical shifts are reported in parts per million (ppm) and the coupling constants (*J*) are expressed in Hertz (Hz). Splitting patterns are designated as follows: s, singlet; d, doublet; sept, septet; t, triplet; q, quadruplet; m, multiplet; brs, broad singlet; dd, double of doublets. The assignment of exchangeable protons (O*H* and N*H*) was confirmed by the addition of D_2O. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel F-254 plates. Flash chromatography purifications were performed on Merck Silica gel 60 (230-400 mesh ASTM) as the stationary phase and ethyl acetate/*n*-hexane were used as eluents.

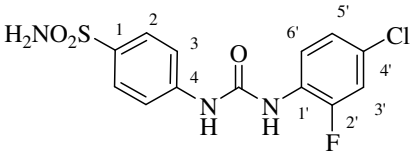
General procedure for the synthesis of compounds **1-21**. [23a]

A; *p*-SO_2NH_2
B; *m*-SO_2NH_2

1-21

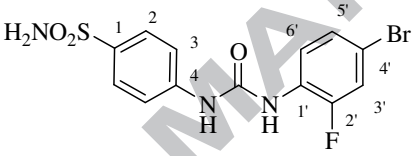
Sulfanilamide **A** or metanilamide **B** (1.0 eq) was dissolved in acetonitrile (ACN) and then treated with a stoichiometric amount of the proper, commercially available isocyanate ArNCO. The reaction mixture was stirred at room temperature until completion (TLC monitoring). The heavy precipitate formed was filtered-off, washed with diethyl ether, and dried under vacuo to afford the title compounds **1-21** which did not require further purification.

4-(3-(4'-chloro-2-fluorophenyl)ureido)benzenesulfonamide **1**



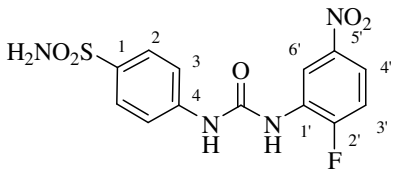
4-(3-(4'-Chloro-2-fluorophenyl)ureido)benzenesulfonamide **1**: ν_{max} (KBr) cm^{-1} 3270, 1650, 1560; δ_H (400 MHz, DMSO-*d*$_6$) 7.26-7.30 (3H, brs, SO_2NH_2, exchange with D_2O, 5'/6'-H), 7.52 (1H, dd, *J*, 8.2, 2.1, 5'/6'-H), 7.64 (2H, d, *J* 8.2, 2 x 2/3-H), 7.78 (2H, d, *J* 8.2, 2 x 2/3-H), 8.19 (1H, dd, *J* 9.0, 8.2 3'-H), 8.79 (1H, s, N*H*, exchange with D_2O), 9.47 (1H, s, N*H*, exchange with D_2O); δ_C (100 MHz, DMSO-*d*$_6$) 152.8 (d, J^1_{C-F} 245, C-2'), 152.7 (C=O), 143.2, 138.2, 127.8, 127.4, (d, J_{C-F} 10), 126.7 (d, J_{C-F} 10), 125.6, 122.5, 118.4, 116.6 (d, J_{C-F} 23); δ_F (376 MHz, DMSO-*d*$_6$) –126.38; Anal. Elem. Cal.: C, 45.42; H, 3.23; N, 12.22), Found: C, 45.39; H, 3.18; N, 12.08.

4-(3-(4'-bromo-2'-fluorophenyl)ureido)benzenesulfonamide **2**



4-(3-(4'-Bromo-2'-fluorophenyl)ureido)benzenesulfonamide **2**: ν_{max} (KBr) cm^{-1}, 3272, 3265, 1643, 1590; δ_H (400 MHz, DMSO-*d*$_6$) 7.27 (2H, s, SO_2NH_2, exchange with D_2O), 7.40 (1H, d, *J*, 9.2 5'/6'-H), 7.64 (3H, m, 2 x 2/3-H, 5'/6'-H), 7.78 (2H, d, *J* 9.2, 2 x 2/3-H), 8.16 (1H, t, *J* 8.8 3'-H), 8.79 (1H, s, N*H*, exchange with D_2O), 9.48 (1H, s, N*H*, exchange with D_2O); δ_C (100 MHz, DMSO-*d*$_6$) 152.8 (d, J^1_{C-F} 245, C-2'), 152.7 (C=O), 143.2, 138.2, 128.5, 127.9, 127.7 (d, J_{C-F} 6), 122.8, 119.2 (d, J_{C-F} 22), 118.4, 114.0 (d, J_{C-F} 9); δ_F (376 MHz, DMSO-*d*$_6$) –126.38; Anal. Elem. Cal.: C, 40.22; H, 2.86; N, 10.82), Found: C, 39.97; H, 2.64; N, 10.99.

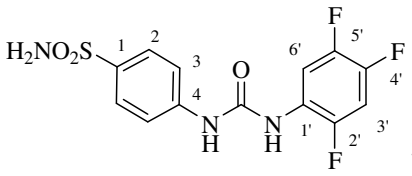
4-(3-(2'-fluoro-5'-nitrophenyl)ureido)benzenesulfonamide **3**



4-(3-(2'-Fluoro-5'-nitrophenyl)ureido)benzenesulfonamide **3**: ν_{max} (KBr) cm^{-1}, 3300, 3269, 1648, 1600; δ_H (400 MHz, DMSO-*d*$_6$) 7.28 (2H, s, SO_2NH_2, exchange with D_2O), 7.60 (1H, dd, *J*, 9.2 6.8,

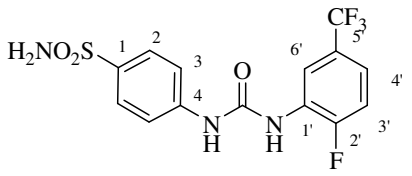
6'-H), 7.70 (2H, d, *J* 9.2, 2 x 2/3-H), 7.80 (2H, d, *J* 9.2, 2 x 2/3-H), 7.96 (1H, m, 4'-H), 9.13 (1H, s, N*H*, exchange with D_2O), 9.18 (1H, m, 6'-H), 9.57 (1H, s, N*H*, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 156.0 (d, $J^1_{C\text{-}F}$ 251, C-2'), 154.8 (C=O), 144.9, 142.9, 138.6, 129.3 (d, $J_{C\text{-}F}$ 12), 127.9, 119.0 (d, $J_{C\text{-}F}$ 9), 118.7, 117.0 (d, $J_{C\text{-}F}$ 22), 115.7; δ_F (376 MHz, DMSO-d_6) –119.43; Anal. Elem. Cal.: C, 44.07; H, 3.13; N, 15.81), Found: C, 43.82; H, 2.95; N, 15.95.

4-(3-(2',4',5'-trifluorophenyl)ureido)benzenesulfonamide **4**



4-(3-(2',4',5'-Trifluorophenyl)ureido)benzenesulfonamide **4**: ν_{max} (KBr) cm^{-1}, 3270, 3163, 1638, 1580; δ_H (400 MHz, DMSO-d_6) 7.27 (2H, s, SO_2NH_2, exchange with D_2O), 7.68 (3H, m, 2 x 2/3-H, 3'-H), 7.78 (2H, d, *J* 9.2, 2 x 2/3-H), 8.22 (1H, m, 6'-H), 8.58 (1H, s, N*H*, exchange with D_2O), 9.47 (1H, s, N*H*, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 152.7 (C=O), 148.2 (dd, $J^1_{C\text{-}F}$ 240, 12.2), 146.4 (d, $J^1_{C\text{-}F}$ 237, 15.8), 144.4 (dd, $J^1_{C\text{-}F}$ 250, 12.0), 143.3, 138.3, 127.8, 125.0 (m), 118.5, 109.5 (dd, $J_{C\text{-}F}$ 24.5, 2.9), 106.4 (dd, $J_{C\text{-}F}$ 25.6, 22.0); δ_F (376 MHz, DMSO-d_6) –130.64 (d, $J_{F\text{-}F}$ 13.9), –141.75 (m), –143.06 (d, $J_{F\text{-}F}$ 24.4); Anal. Elem. Cal.: C, 45.22; H, 2.92; N, 12.17), Found: C, 45.41; H, 2.84; N, 12.25.

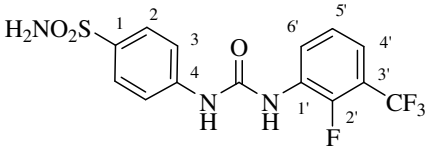
4-(3-(2'-fluoro-5'-(trifluoromethyl)phenyl)ureido)benzenesulfonamide **5**



4-(3-(2'-Fluoro-5'-(trifluoromethyl)phenyl)ureido)benzenesulfonamide **5**: ν_{max} (KBr) cm^{-1} 3270, 1660, 1560; δ_H (400 MHz, DMSO-d_6) 7.28 (2H, s, SO_2NH_2, exchange with D_2O), 7.47 (1H, m, 4'-H), 7.55 (1H, dd, *J* 10.8 8.8, 3'-H), 7.67 (2H, d, *J* 8.8, 2 x 2/3-H), 7.79 (2H, d, *J* 8.8, 2 x 2/3-H), 8.63 (1H, m, 6'-H), 9.03 (1H, s, N*H*, exchange with D_2O), 9.56 (1H, s, N*H*, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 154.0 (d, $J^1_{C\text{-}F}$ 247, C-2'), 152.8 (C=O), 143.0, 138.5, 129.30 (d, $J_{C\text{-}F}$ 11.3), 127.9, 126.2 (dd, $J_{C\text{-}F}$ 31.8, 3.3), 123.5, 120.7 (m), 118.6, 117.7, 117.0 (d, $J_{C\text{-}F}$ 20.5); δ_F (376 MHz,

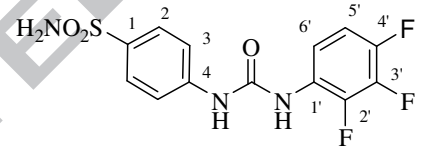
DMSO-d_6) –60.7 (3F, C-F_3), -123.8 (1F, 2'-*F*); Anal. Elem. Cal.: C, 44.56; H, 2.94; N, 11.14), Found: C, 44.63; H, 2.86; N, 11.00.

4-(3-(2'-fluoro-3'-(trifluoromethyl)phenyl)ureido)benzenesulfonamide **6**



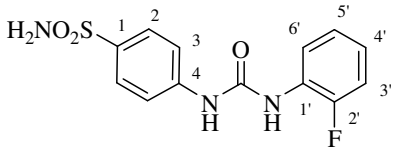
4-(3-(2'-fluoro-3'-(trifluoromethyl)phenyl)ureido)benzenesulfonamide **6**: ν_{max} (KBr) cm^{-1}, 3320, 1645, 1570; δ_H (400 MHz, DMSO-d_6) 7.27 (2H, s, SO_2NH_2, exchange with D_2O), 7.43 (2H, m, 5'-H, 6'-H), 7.65 (2H, d, *J* 8.8, 2 x 2/3-H), 7.79 (2H, d, *J* 8.8, 2 x 2/3-H), 8.46 (1H, m, 4'-H), 8.96 (1H, s, N*H*, exchange with D_2O), 9.53 (1H, s, N*H*, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 152.7 (C=O), 150.0 (d, J^1_{C-F} 250, C-2'), 143.1, 138.3, 129.4 (d, J_{C-F} 9), 127.8, 125.9 (d, J_{C-F} 43.9), 124.9, 122.2, 120.4, 118.7, 117.5 (dd, J_{C-F} 32.1, 3.2); δ_F (376 MHz, DMSO-d_6) –59.8 (3F,d, J_{F-F} 13.2, C-F_3), -132.2 (1F, q, *J* 13.2, 2'-*F*); Anal. Elem. Cal.: C, 44.56; H, 2.94; N, 11.14), Found: C, 44.18; H, 2.78; N, 11.21.

4-(3-(2',3',4'-trifluorophenyl)ureido)benzenesulfonamide **7**



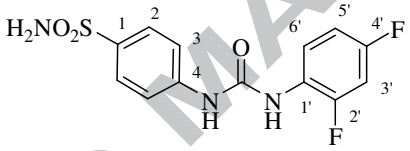
4-(3-(2',3',4'-Trifluorophenyl)ureido)benzenesulfonamide **7**: ν_{max} (KBr) cm^{-1} 3270, 1640, 1592; δ_H (400 MHz, DMSO-d_6) 7.27 (2H, s, SO_2NH_2, exchange with D_2O), 7.34 (2H, m, 5'/6'-H), 7.65 (2H, d, *J* 8.8, 2 x 2/3-H), 7.77 (2H, d, *J* 8.8, 2 x 2/3-H), 7.88 (1H, m, 5'/6'-H), 8.82 (1H, s, N*H*, exchange with D_2O), 9.45 (1H, s, N*H*, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 152.9 (C=O), 146.6 (d, J^1_{C-F} 237), 143.2, 141.8 (d, J^1_{C-F} 240), 139.6 (d, J^1_{C-F} 242), 138.3, 127.8, 126.0 (m), 118.5, 116.7 (m), 112.6 (dd, J_{C-F} 18, 4); δ_F (376 MHz, DMSO-d_6) –143.1 (1F,d, J_{F-F} 21.7, 2'/4'-*F*), -148.4 (1F,d, J_{F-F} 21.7, 2'/4'-*F*), -161.1 (1F,t, J_{F-F} 21.7, 3'-*F*); Anal. Elem. Cal.: C, 45.22; H, 2.92; N, 12.17), Found: C, 45.59; H, 2.65; N, 12.53.

4-(3-(2'-fluorophenyl)ureido)benzenesulfonamide **8**



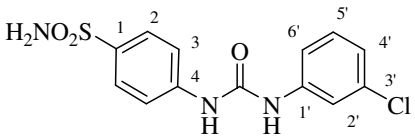
4-(3-(2'-Fluorophenyl)ureido)benzenesulfonamide **8**: ν_{max} (KBr) cm^{-1} 3272, 1643, 1600; δ_H (400 MHz, DMSO-d_6) 7.07 (1H, m, 3'-H), 7.20 (1H, m, 4'-H), 7.26 (3H, brs, SO_2NH_2, exchange with D_2O, 5'-H), 7.64 (2H, d, *J* 8.8, 2 x 2/3-H), 7.80 (2H, d, *J* 8.8, 2 x 2/3-H), 8.20 (1H, m, 6'-H)8.70 (1H, s, N*H*, exchange with D_2O), 9.46 (1H, s, N*H*, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 153.1 (d, J^1_{C-F} 240), 152.9 (C=O), 143.4, 138.1, 128.1, 127.8, 125.4, 123.8, 121.7, 118.3, 116.6 (d, J_{C-F} 19); δ_F (376 MHz, DMSO-d_6) –129.59; Anal. Elem. Cal.: C, 50.48; H, 3.91; N, 13.58), Found: C, 50.32; H, 4.13; N, 13.68.

4-(3-(2',4'-difluorophenyl)ureido)benzenesulfonamide **9**



4-(3-(2',4'-Difluorophenyl)ureido)benzenesulfonamide **9**: ν_{max} (KBr) cm^{-1} 3270, 1642, 1598; δ_H (400 MHz, DMSO-d_6) 7.08 (1H, m, 3'/5'-H), 7.25 (2H, s, SO_2NH_2, exchange with D_2O), 7.36 (1H, m, 3'/5'-H), 7.64 (2H, d, *J* 8.8, 2 x 2/3-H), 7.78 (2H, d, *J* 8.8, 2 x 2/3-H), 8.11 (1H, m, 6'-H), 8.64 (1H, s, N*H*, exchange with D_2O), 9.41 (1H, s, N*H*, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 158.1 (dd, J_{C-F} 243, 13), 153.4 (dd, J_{C-F} 244, 12), 153.0 (C=O), 143.4, 138.1, 128.1, 127.8, 124.5 (d, J_{C-F} 9), 123.2 (d, J_{C-F} 8), 118.4, 112.0 (d, J_{C-F} 21), 104.8 (t, J_{C-F} 25); δ_F (376 MHz, DMSO-d_6) –117.52, -124.3; Anal. Elem. Cal.: C, 47.70; H, 3.39; N, 12.84), Found: C, 47.60; H, 3.09; N, 13.14.

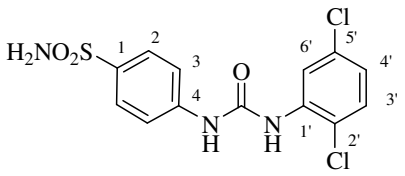
4-(3-(3'-chlorophenyl)ureido)benzenesulfonamide **10**



4-(3-(3'-chlorophenyl)ureido)benzenesulfonamide **10**: ν_{max} (KBr) cm^{-1}, 3274, 1641, 1591; δ_H (400 MHz, DMSO-d_6) 7.07 (1H, d, *J* 8.8, 4'-H), 7.25 (2H, s, SO_2NH_2, exchange with D_2O), 7.33 (2H, m,

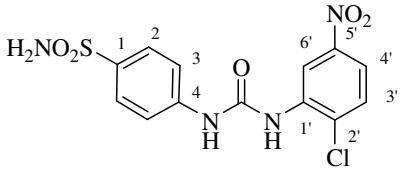
5'-H, 6'-H), 7.64 (2H, d, *J* 8.8, 2 x 2/3-H), 7.77 (3H, m, *J* 8.8, 2 x 2/3-H, 2'-H), 9.04 (1H, s, N*H*, exchange with D_2O), 9.17 (1H, s, N*H*, exchange with D_2O); δ_C (100 MHz, DMSO-*d*$_6$) 153.1 (C=O), 143.5, 141.8, 138.0, 134.1, 131.3, 127.7, 122.7, 118.7, 118.6, 117.8; Anal. Elem. Cal.: C, 47.93; H, 3.71; N, 12.90), Found: C, 47.50; H, 3.44; N, 13.13.

4-(3-(2',5'-dichlorophenyl)ureido)benzenesulfonamide **11**



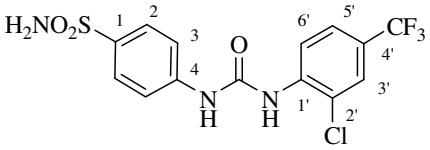
4-(3-(2',5'-Dichlorophenyl)ureido)benzenesulfonamide **11**: ν_{max} (KBr) cm^{-1} 3275, 1650, 1578; δ_H (400 MHz, DMSO-*d*$_6$) 7.15 (1H, dd, *J* 8.8 2.8, 4'-H), 7.28 (2H, s, SO_2NH_2, exchange with D_2O), 7.55 (1H, dd, *J* 8.8 2.8, 3'-H), 7.64 (2H, d, *J* 8.8, 2 x 2/3-H), 7.79 (2H, d, *J* 8.8, 2 x 2/3-H), 8.35 (1H, d, *J* 2.8, 6'-H), 8.60 (1H, s, N*H*, exchange with D_2O), 9.90 (1H, s, N*H*, exchange with D_2O); δ_C (100 MHz, DMSO-*d*$_6$) 152.7 (C=O), 143.1, 138.4, 137.8, 132.9, 131.5, 127.9, 124.0, 121.3, 121.1, 118.7; Anal. Elem. Cal.: C, 43.35; H, 3.08; N, 11.67), Found: C, 43.64; H, 3.08; N, 11.66.

4-(3-(2'-Chloro-5'-nitrophenyl)ureido)benzenesulfonamide **12**



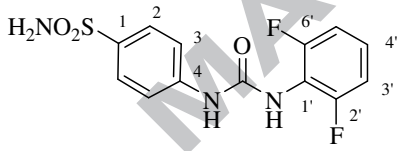
4-(3-(2'-Chloro-5'-nitrophenyl)ureido)benzenesulfonamide **12**: ν_{max} (KBr) cm^{-1}, 3164, 3271, 1641, 1592; δ_H (400 MHz, DMSO-*d*$_6$) 7.29 (2H, s, SO_2NH_2, exchange with D_2O), 7.70 (2H, d, *J* 8.8, 2 x 2/3-H), 7.82 (3H, m, 2 x 2/3-H, 3'-H), 7.93 (1H, dd, *J* 8.9 2.2, 4'-H), 8.84 (1H, s, N*H*, exchange with D_2O), 9.20 (1H, d, *J* 2.2, 6'-H), 9.99 (1H, s, N*H*, exchange with D_2O); δ_C (100 MHz, DMSO-*d*$_6$) 152.7 (C=O), 147.5. 142.9, 138.6, 137.7, 131.3, 128.9, 127.9, 118.8, 118.5, 115.6; Anal. Elem. Cal.: C, 42.11; H, 2.99; N, 15.11), Found: C, 42.38; H, 3.24; N, 15.43.

4-(3-(2'-Chloro-4'-(trifluoromethyl)phenyl)ureido)benzenesulfonamide **13**



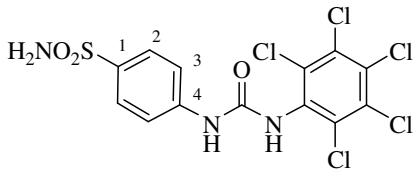
4-(3-(2'-Chloro-4'-(trifluoromethyl)phenyl)ureido)benzenesulfonamide **13**: ν_{max} (KBr) cm^{-1}, 3169, 1639, 1560; δ_H (400 MHz, DMSO-d_6) 7.29 (2H, s, SO_2NH_2, exchange with D_2O), 7.69 (2H, d, *J* 8.8, 2 x 2/3-H), 7.74 (1H, dd, *J* 8.8 4.0, 5'-H), 7.80 (2H, d, *J* 8.8, 2 x 2/3-H), 7.92 (1H, dd, *J* 4.0, 3'-H), 8.50 (1H, d, *J* 8.8, 6'-H), 8.76 (1H, s, N*H*, exchange with D_2O), 10.0 (1H, s, N*H*, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 152.5, 143.0, 140.4, 138.5, 128.7, 127.9, 127.2 (m), 125.8 (m), 124.0 (d, J_{C-F} 33.0), 13.2, 122.7, 121.4; δ_F (376 MHz, DMSO-d_6) –60.42; Anal. Elem. Cal.: C, 42.70; H, 2.82; N, 10.67), Found: C, 42.92; H, 2.76; N, 11.70.

4-(3-(2',6'-difluorophenyl)ureido)benzenesulfonamide **14**



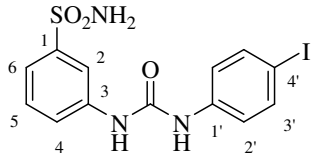
4-(3-(2',6'-Difluorophenyl)ureido)benzenesulfonamide **14**: ν_{max} (KBr) cm^{-1}, 3164, 3270, 1641, 1590; δ_H (400 MHz, DMSO-d_6) 7.33 (4H, m, SO_2NH_2, exchange with D_2O, 2 x 5'-H), 7.37 (1H, m, 4'-H), 7.64 (2H, d, *J* 8.8, 2 x 2/3-H), 7.76 (2H, d, *J* 8.8, 2 x 2/3-H), 8.30 (1H, s, N*H*, exchange with D_2O), 9.38 (1H, s, N*H*, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 158 (d, J^1_{C-F} 247, C-2', C-6'), 153.27 (C=O), 143.7, 138.0, 128.3 (m), 127.7, 118.5, 115.9 (t, J_{C-F} 16, C-4'), 112.6 (d, J_{C-F} 23, 2 x C-3'); δ_F (376 MHz, DMSO-d_6) –118.73; Anal. Elem. Cal.: C, 47.70; H, 3.39; N, 12.84), Found: C, 47.45; H, 2.91; N, 13.17.

4-(3-(perchlorophenyl)ureido)benzenesulfonamide **15**



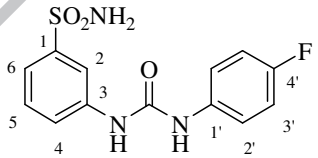
4-(3-(perchlorophenyl)ureido)benzenesulfonamide **15**: ν_{max} (KBr) cm^{-1}, 3168, 3275, 1641, 1590; δ_H (400 MHz, DMSO-*d*$_6$) 7.24 (2H, m, SO_2NH_2, exchange with D_2O), 7.64 (2H, d, *J* 8.8, 2 x 2/3-H), 7.75 (2H, d, *J* 8.8, 2 x 2/3-H), 8.29 (1H, s, N*H*, exchange with D_2O), 9.38 (1H, s, N*H*, exchange with D_2O); δ_C (100 MHz, DMSO-*d*$_6$) 153.2 (C=O), 142.0, 139.2, 130.1 (overlapping signals), 127.4, 125.5; Anal. Elem. Calc.: C, 33.68; H, 1.74; N, 9.06), Found: C, 34.01; H, 1.81; N, 9.10.

3-(3-(4'-Iodophenyl)ureido)benzenesulfonamide **16**



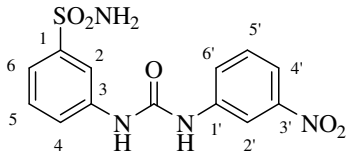
3-(3-(4'-Iodophenyl)ureido)benzenesulfonamide **16**: m.p. 256-258 °C; ν_{max} (KBr) cm^{-1}, 3165, 3265, 1643, 1589; δ_H (400 MHz, DMSO-*d*$_6$) 7.34-7.66 (9H, m, Ar-H, SO_2NH_2, exchange with D_2O), 8.10 (1H, d, *J*, 2.1, 2-H), 8.79 (1H, s, N*H*, exchange with D_2O), 9.08 (1H, s, N*H*, exchange with D_2O); δ_C (100 MHz, DMSO-*d*$_6$) 153.1 (C=O), 145.6, 140.9, 140.3, 138.3, 130.3, 122.1, 121.6, 119.9, 116.1, 85.9; Elem. Anal. Calc. C, 37.42; H, 2.90; N, 10.07; Found; C, 37.36; H, 2.79; N, 9.82; *m/z* (ESI^+) 418 $(M+H)^+$.

3-(3-(4'-Fluorophenyl)ureido)benzenesulfonamide **17**



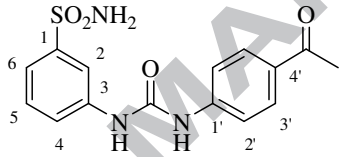
3-(3-(4'-Fluorophenyl)ureido)benzenesulfonamide **17**: m.p. 233-235 °C; ν_{max} (KBr) cm^{-1}, 3377, 3352, 1685, 1557; δ_H (400 MHz, DMSO-*d*$_6$) 7.17 (1H, dd, J 8.8, Ar-H), 7.45 (2H, s, SO_2NH_2, exchange with D_2O), 7.47-7.60 (5H, m, Ar-H), 8.10 (1H, d, *J*, 2.1, 2-H), 8.78 (1H, s, N*H*, exchange with D_2O), 9.04 (1H, s, N*H*, exchange with D_2O); δ_C (100 MHz, DMSO-*d*$_6$) 158.4 (d, J_{C-F} 237, C-4'), 153.4 (C=O), 145.6, 141.1, 136.6, 130.3, 122.0, 121.2, 119.8, 116.3, 116.1; δ_F (376 MHz, DMSO-*d*$_6$) –121.14; Elem. Anal. Calc. C, 50.48; H, 3.91; N, 13.58; Found: C, 49.98; H, 3.79; N, 13.49]; *m/z* (ESI^+) 311 $(M+H)^+$.

3-(3-(3'-Nitrophenyl)ureido)benzenesulfonamide **18**



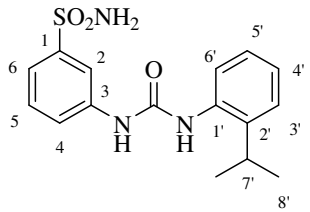
3-(3-(3'-Nitrophenyl)ureido)benzenesulfonamide **18**: m.p. 252-255 °C; ν_{max} (KBr) cm^{-1}, 3380, 3350, 1689, 1550; δ_H (400 MHz, DMSO-d_6) 7.41 (2H, s, SO_2NH_2, exchange with D_2O), 7.48-7.64 (4H, m, Ar-H), 7.77 (1H, dd, *J* 7.2, 2.1, Ar-H), 7.87 (1H, dd, *J* 7.2, 2.1, Ar-H), 8.14 (1H, d, *J*, 2.1, 2-H), 8.63 (1H, d, *J*, 2.1, 2'-H), 9.23 (1H, s, N*H*, exchange with D_2O), 9.31 (1H, s, N*H*, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 153.3 (C=O), 149.1, 145.7, 141.7, 140.6, 131.0, 130.3, 125.4, 122.4, 120.2, 117.4, 116.4, 113.2; Elem. Anal. Calc. C, 46.78; H, 3.60; N, 16.66; Found C, 46.91; H, 3.55; N, 16.94; *m/z* (ESI^+) 337 $(M+H)^+$.

3-(3-(4'-Acetylphenyl)ureido)benzenesulfonamide **19**



3-(3-(4'-Acetylphenyl)ureido)benzenesulfonamide **19**: m.p. 267-269 °C; ν_{max} (KBr) cm^{-1}, 3402, 3351, 2014, 1933, 1912, 1593; δ_H (400 MHz, DMSO-d_6) 2.56 (3H, s, CH_3), 7.41 (2H, s, SO_2NH_2, exchange with D_2O), 7.48-7.55 (3H, m, 4-H, 5-H, 6-H), 7.60 (2H, d, *J* 7.2, 2 x 2'-H), 7.97 (2H, d, *J* 7.2, 2 x 3'-H), 8.13 (1H, t, *J* 2.0, 2-H), 9.18 (1H, s, N*H*, exchange with D_2O), 9.19 (1H, s, N*H*, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 197.2 (CH_3C=O), 153.0 (C=O), 145.7, 145.0, 140.7, 131.6, 130.5, 130.4, 122.2, 120.2, 118.2, 116.2, 27.2; Elem. Anal. Calc. C, 54.04; H, 4.54; N, 12.60; Found C, 54.78; H, 4.47; N, 12.96; *m/z* (ESI^+) 334 $(M+H)^+$.

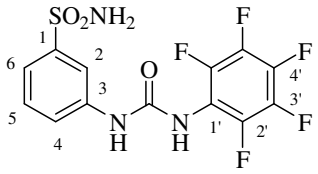
3-(3-(2'-Isopropylphenyl)ureido)benzenesulfonamide **20**



3-(3-(2'-Isopropylphenyl)ureido)benzenesulfonamide **20**: m.p. 175-176 °C; ν_{max} (KBr) cm^{-1}, 3328, 3300, 1690, 1556; δ_H (400 MHz, DMSO-d_6) 1.23 (6H, d, *J* 6.2, 2 x 8'-H_3), 3.19 (1H, sept, *J* 6.2, 7'-H), 7.14 (2H, m, Ar-H), 7.35 (1H, d, *J* 7.2, Ar-H), 7.37 (2H, s, SO_2NH_2, exchange with D_2O), 7.59-

7.70 (4H, m, Ar-H), 8.00 (1H, s, N*H*, exchange with D_2O), 8.10 (1H, t, *J* 2.0, 2-H), 9.30 (1H, s, N*H*, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 153.9 (C=O), 145.6, 141.3, 140.7, 136.1, 130.3, 126.7, 126.2, 125.2, 124.7, 121.7, 119.5, 115.8, 27.8, 24.0; Elem. Anal. Calc. C, 57.64; H, 5.74; N, 12.60; Found C, 58.14; H, 5.73; N, 12.70; *m/z* (ESI^+) 334 $(M+H)^+$.

3-(3-(Perfluorophenyl)ureido)benzenesulfonamide **21**



3-(3-(Perfluorophenyl)ureido)benzenesulfonamide **21**: m.p. 224-227 °C; ν_{max} (KBr) cm^{-1}, 3390, 3287, 1785, 1560; δ_H (400 MHz, DMSO-d_6) 7.38 (2H, s, SO_2NH_2, exchange with D_2O), 7.50 (2H, m, Ar-H), 7.63 (1H, d, *J* 7.2, Ar-H), 8.09 (1H, s, 2-H), 8.63 (1H, s, ArN*H*CONH, exchange with D_2O), 9.47 ((1H, s, ArNHCON*H*, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 152.9 (C=O), 145.6, 144.1 (d, J^1_{C-F} 245), 140.7, 139.6 (d, J^1_{C-F} 253), 138.2 (d, J^1_{C-F} 245), 130.4, 122.3, 120.4, 116.4, 114.7 (t, J_{C-F} 12); δ_F (376 MHz, DMSO-d_6) –146.3 (2F, dd, *J* 19.2 4.8, 2 x 2'-F), -159.2 (1F, t, *J* 22.9, 4'-F), -164.0 (2F, dt, *J* 22.6 4.8, 2 x 3'-F); Elem. Anal. Calc. C, 40.95; H, 2.11; N, 11.02; Found C, 40.85; H, 1.89; N, 11.00; *m/z* (ESI^+) 382 $(M+H)^+$.

4.2. Carbonic anhydrase inhibition

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalysed CO_2 hydration activity.[28] 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 (pH 7.5) as buffer, and 20 mM Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10-100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5-10% of the reaction have been used for determining the initial velocity. The uncatalyzed 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-deionized water and dilutions up to 0.01 nM were done 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,[29] and represent the mean from at least three different determinations. All CA isofoms were recombinant ones obtained in-house as reported earlier. [30]

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Synthesis and carbonic anhydrase inhibition of a series of SLC-0111 analogs

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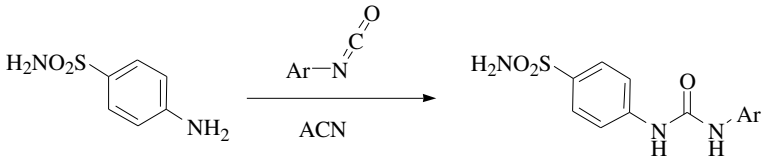

A **1-15**

1; Ar= 4-Chloro-2-fluorophenyl
2; Ar= 4-Bromo-2-fluorophenyl
3; Ar= 2-Fluoro-5-nitrophenyl
4; Ar= 2,4,5-Trifluorophenyl
5; Ar= 2-Fluoro-5-trifluorophenyl
6; Ar= 2-Fluoro-3-trifluorophenyl
7; Ar= 2,3,4-Trifluorophenyl
8; Ar= 2-Fluorophenyl
9; Ar= 2,4-Difluorophenyl
10; Ar= 3-Chlorophenyl
11; Ar= 2,5-Dichlorophenyl
12; Ar= 2-Chloro-5-nitrophenyl
13; Ar= 2-Chloro-4-trifluorophenyl
14; Ar= 2,6-Difluorophenyl
15; Ar=Perchlorophenyl