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Benzenesulfonamides incorporating bulky aromatic/heterocyclic tails with potent carbonic anhydrase inhibitory activity

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ABSTRACT

Three series of sulfonamides incorporating long, bulky tails were obtained by applying synthetic strategies in which substituted anthranilic acids, quinazolines and aromatic sulfonamides have been used as starting materials. They incorporate long, bulky diamide-, 4-oxoquinazoline-3-yl- or quinazoline-4-yl moieties in their molecules, and were investigated for the inhibition of four physiologically relevant carbonic anhydrase (CA, EC 4.2.1.1) isoforms, the cytosolic human (h) hCA I and II, as well as the transmembrane hCA IX and XII. Most of the new sulfonamides showed excellent inhibitory effects against the four isoforms, with K_Is of 7.6–322 nM against hCA I, of 0.06–85.4 nM against hCA II; of 6.7–152 nM against hCA IX and of 0.49–237 nM against hCA XII; respectively. However no relevant isoform-selective behavior has been observed for any of them, although hCA II and XII, isoforms involved in glaucoma-genesis were the most inhibited ones. The structure–activity relationship for inhibiting the four CAs with these derivatives is discussed in detail.

1. Introduction

The sulfonamides continue to be one of the important families of inhibitors of the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1), with many representatives in clinical use as diuretics, antiglaucoma, antiepileptic, antiobesity drugs, or agents useful for the treatment of some neurological disorders, such as idiopathic intracranial hypertension.[1–10] Recently, a sulfonamide CA inhibitor (CAI) developed by one of our groups by applying the tail approach, SLC-0111, entered Phase I clinical trials for the treatment of patients with advanced solid, metastatic tumors overexpressing CA IX/XII.[1–4]

The tail approach[1] reported by one of these groups more than 15 years ago allowed for the facile preparation of a large number of potent and isoform-selective classes of CA inhibitors (CAIs).[1–4] Indeed, an initial drug design strategy based on appending tails of different size, shape or nature to pharmacophores incorporating sulfonamides as zinc-binding group (ZBG),[4–10] as opposed to the ring approach[2] which explored various aromatic/heterocyclic ring systems on which the SO_2NH_2 moiety was bound, afforded CA inhibitors (CAIs) possessing both high affinity and desired pharmacologic properties.[4–10] This approach, based on an 'extension' of the aromatic/heterocyclic scaffolds through the anchoring tails has been thereafter explored for sulfamates, sulfamides, and dithiocarbamates (as alternative ZBGs to the sulfonamide) but also to CAIs incorporating scaffolds belonging to the aliphatic or glycosidic chemical species.[10–12] The advantage of the tail approach over other drug design strategies was thereafter explained at the molecular level, after the report of many X-ray crystallographic structures of adducts of various CA isoforms with such inhibitors. These studies demonstrated that the active site of most CA isoenzymes is a rather large conical cavity in which the Zn(II) ion is positioned at its bottom. The lining of the active site builds two adjacent very diverse halves, one entirely hydrophilic, the opposing one completely hydrophobic,[12] with the highest variability of amino acid residues between the different isoforms on the edge/entrance of the active site. This is exactly the region in which the tails of the inhibitors are usually accomodated,[12–16] explaining why these specific interactions between the inhibitor tail and amino acid residues at the entrance of the active site may lead to

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compounds showing selectivity for inhibiting isoforms with pharmacological applications.[1–4] In this way many sulfonamides/sulfamates/sulfamides/dithiocarbamates with excellent CA IX/XII over CA I and II selectivity were reported,[16] whereas the coumarins, which bind exclusively in this outward region of the active site, showed the highest isoform selectivity among all CAI classes reported to date.[17–24]

Here we report a new series of sulfonamide CAIs obtained by applying the tail approach. Our interest was to obtain compounds with even longer tails than the ones we reported earlier, in order to investigate whether prolonging the scaffold towards the outside part of the enzyme active cavity may have interesting consequences for the inhibition profiles of such sulfonamides against Ca isoforms with applications in pharmacology, such as CA I and II (cytosolic isoforms) as well as CA IX and XII (trans-membrane, tumor-associated isoforms).

2. Results and discussion

2.1. Chemistry

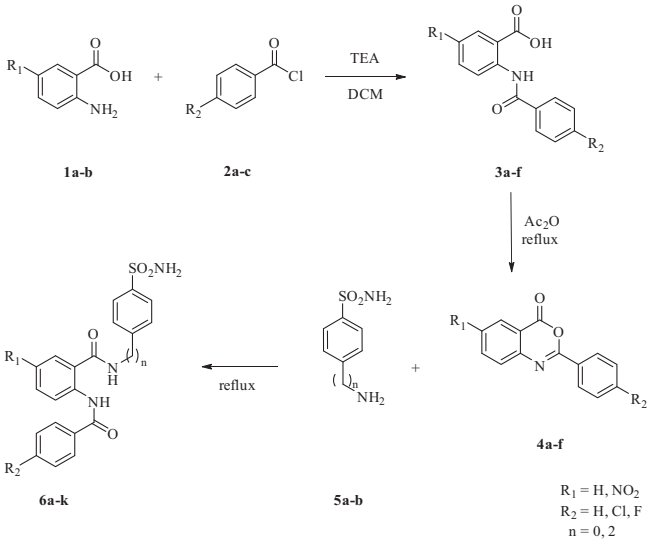
Aromatic benzene sulfonamides (sulfanilamide and 4-aminoethylbenzenesulfonamide) have been used in the present drug design as main scaffolds as they led to CA IX/XII-selective CAIs in several tail-approach studies.[15,24] Furthermore, the amino moiety present in these two compounds has a good reactivity and it is easily derivatizable by reaction with a variety of derivatives.

Several synthetic strategies have been thus employed for prolonging the scaffold of these aromatic sulfonamides in order to obtain long-tailed CAIs, as shown in Schemes 1–3.

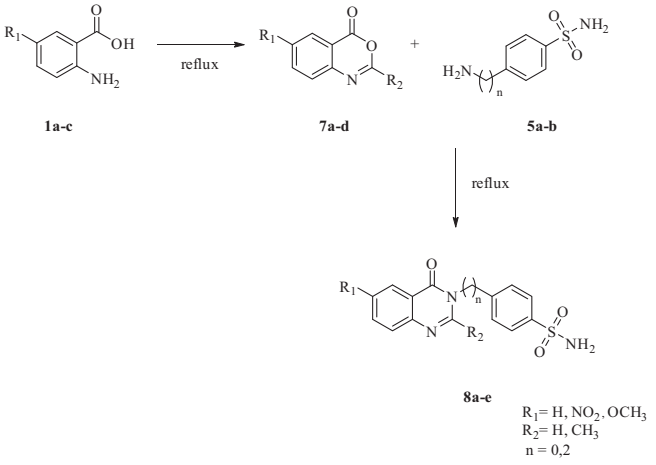
The anthranilic acid derivatives **1** were chosen for the first approach, due to the fact that the amino moiety in *ortho* to the carboxylic acid has a good reactivity with acyl halides, can participate in cyclization reactions and in addition, diamides with the geometry shown in Scheme 1 were not investigated earlier. Thus, 2-amino-5-substituted benzoic acids **1a**,**b** were reacted with 4-substituted-benzoyl chlorides **2a–c** leading to intermediates **3a–f** which were thereafter cyclized in the presence of acetic anhydrides to the key intermediates **4a–f**, which by reaction with sulfanilamide (**5a**) or 4-aminoethylbenzenesulfonamide (**5b**) led to a first group of long-tailed sulfonamides **6a–k** incorporating diamide functionalities (Scheme 1).

For the second approach, again anthranilic acid derivatives **1** were considered, but this time in such a way as to obtain cyclic compounds in which the amino and carboxylic moieties of the starting material are involved. Again such derivatives were not investigated earlier as CAIs. Condensation of 2-amino-5-substituted benzoic acids **1a–c** with acetic anhydride or triethylorthoformate afforded the cyclic intermediates **7a–d** which were subsequently reacted with the two sulfonamides mentioned above, **5a**,**b**, leading to the second group of sulfonamides reported here **8a–e**, incorporating 4-oxoquinazolin-3(4*H*)-yl tails in their molecules (Scheme 2).

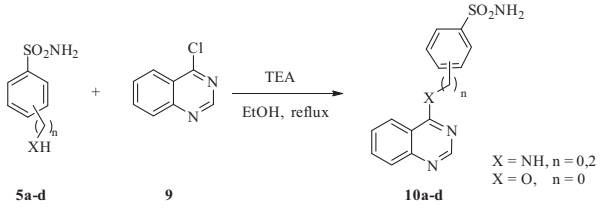
The last synthetic approach is shown in Scheme 3. In this case we used as model reaction the one between cyanuryl chloride and amino-sulfonamides,[15b] by which we have reported a large series of highly isoform-selective CAIs. Indeed, the chlorine atoms from cyanuryl chloride and the chlorine from **9** have a good reactivity with various nucleophiles, leading thus to chemical diversity by a facile synthetic approach. Thus, sulfanilamide, 4-aminoethyl-benzenesulfonamide or 4-hydroxybenzenesulfonamide were treated with 4-chloroquinazoline **9**, leading to the 4-substituted quinazolines **10a–d** (Scheme 3). Compounds **10a** and **10b** are also commercially available from Aurora Screening library, but we prepared them by the procedure illustrated above.



Scheme 1. Preparation of 2-benzamido-*N*-(4-sulfamoylphenyl)benzamides **6a–k**.



Scheme 2. Preparation of 4-(4-oxoquinazolin-3(4*H*)-yl)benzenesulfonamides **8a–e**.



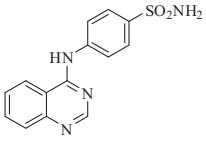
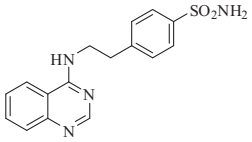
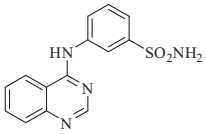
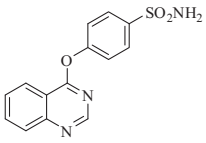
Scheme 3. Preparation of 4-substituted quinazolines **10a–d**.

The sulfonamides investigated here as CAIs were characterized by physico-chemical and spectroscopic methods which confirmed their structures (see Section 4).

2.2. Carbonic anhydrase inhibition

The sulfonamides **6a–6k**, **8a–8e** and **10–10d** reported here were investigated for their enzyme inhibitory action against four physiologically relevant CA isoforms, the human (h) hCA I, II, IX and XII (Table 1). Acetazolamide (5-acetamido-1,3,4-thiadiazole-2-sulfonamide) was used as standard drug in the assay.[25]

Table 1
Inhibition of human (h) isoforms hCA I, II, IX and XII with the sulfonamides reported in the paper by a CO_2 hydrase stopped-flow assay[25]

No.	R1	R2	*n*	K_I^* (nM)			
				hCA I	hCA II	hCA IX	hCA XII
6a	H	H	0	9.7	2.6	21.3	0.85
6b	H	F	0	7.6	0.41	20.1	7.0
6c	NO_2	H	0	34.7	9.5	9.1	7.2
6d	NO_2	Cl	0	31.5	18.2	6.7	3.3
6e	NO_2	F	0	48.1	9.3	7.2	0.49
6f	H	H	2	62.6	7.2	8.0	26.3
6g	H	Cl	2	86.7	75.5	7.1	6.5
6h	H	F	2	85.2	32.3	25.0	5.4
6i	NO_2	H	2	261	85.4	79.1	18.2
6j	NO_2	Cl	2	67.1	65.5	79.5	42.3
6k	NO_2	F	2	64.3	7.3	41.4	25.8
8a	NO_2	CH_3	0	322	0.84	8.5	183
8b	H	CH_3	2	30.8	0.76	35.9	8.5
8c	NO_2	CH_3	2	84.4	4.3	8.0	237
8d	OCH_3	CH_3	2	40.8	3.4	79.3	67.8
8e	OCH_3	H	2	15.7	0.78	88.9	39.3
10a			–	9.5	0.06	8.1	1.3
10b			–	9.1	6.9	8.4	2.2
10c			–	55.5	0.08	152	0.96
10d			–	51.2	0.09	75.1	0.95
AAZ	–	–	–	250	12.1	25.3	5.7

* Mean from three different assays. Errors in the range of ±5–10% of the reported values (data not shown).

The following structure–activity relationship (SAR) can be drawn from the inhibition data shown in Table 1:

(i) The slow cytosolic isoform hCA I was inhibited by the new sulfonamides **6**, **8** and **10** reported here with K_Is ranging between 7.6 and 322 nM. Few compounds were low nanomolar inhibitors, such as **6a**, **6b**, **10a** and **10b** (K_Is of 7.6–9.5 nM). They usually incorporate sulfanilamide 'heads' (which led to better hCA I inhibitors compared to 4-aminoethylbenzenesulfonamide), except **10b** which has the latter derivative in its molecule, as well as diamide tails devoid of nitro moieties (in **6a** and **6b**) as well as quinazolin-4-yl moieties (in **10a** and **10b**). Two other derivatives, **6i** and **8a**, showed poor hCA I inhibitory power (similar to **AAZ**), with inhibition constants of 261–322 nM. They do not differ significantly from the remaining derivatives investigated here, which all of them showed a behavior of medium potency hCA I inhibitor, with K_Is ranging between 15.7 and 86.7 nM (Table 1), being more effective than the standard drug acetazolamide against this isoform. The most salient SAR features were that the nitro derivatives **6c–e** were less effective compared to the corresponding derivatives without this moiety (**6a**, **6b**) and that most of the time the sulfanilamide derivatives were more inhibitory compared to the corresponding 4-aminoethylbenzenesulfonamides. All three main chemotypes investigated here, **6**, **8** and **10**, showed rather comparable biological activities in inhibiting this isoform.

(ii) The physiologically dominant isoform hCA II (cytosolic like hCA I) was very effectively inhibited by most sulfonamides investigated here, with K_Is ranging between 0.06 and 85.4 nM. Four compounds were medium potency inhibitors, that is, **6g–6j**, with K_Is ranging between 32.3 and 85.4 nM, the remaining ones showing K_Is <20 nM (Table 1). All the less effective CAIs incorporate the diamide tail (with or without the NO_2 group) and are 4-aminoethylbenzenesulfonamide derivatives. It is interesting to note that the presence of the NO_2 moiety did not influence markedly the CA inhibitory properties of these derivatives, although we expected that its acidifying effects may enhance CA inhibition. In fact many times the nitro-derivatives were less effective compared to the corresponding compounds without this moiety (compare **6a** and **6c**, or **6b** and **6e**, etc). This is difficult to explain but probably the nitro moiety in that position

of the aromatic ring does not make good contacts with the enzyme active site. It should also be mentioned that several subnanomolar hCA II inhibitors were detected here, such as among others **6b**, **8b**, **8e**, **10a**, **10c** and **10d**. Especially the last three compounds, which are quite simple and all of them incorporate the quinazoline-4-yl moiety in their molecules, are among the most effective CAIs ever reported, with low picomolar activity against this isoform.

(iii) The transmembrane, tumor-associated isoform hCA IX was also effectively inhibited by most sulfonamides investigated here, which showed K_Is ranging between 6.7 and 152 nM (Table 1). Several low nanomolar hCA IX inhibitors were detected, such as **6c**, **6g**, **8a**, **8c**, **10a**, and **10b** (K_Is ranging between 6.7 and 9.1 nM), but unlike other classes of sulfonamides investigated earlier[15] these derivatives did not show any selectivity for inhibiting hCA IX over hCA II (or hCA I).

(iv) The second transmembrane, tumor-associated isoform hCA XII, was very effectively inhibited by sulfonamides **6**, **8** and **10** investigated here, with K_Is ranging between 0.49 and 237 nM. Few compounds (**8a**, **8c** and **8d**) showed weak inhibitory action, with K_Is ranging between 67.8 and 237 nM, whereas the vast majority of the remaining ones were highly effective inhibitors. Several subnanomolar hCA XII inhibitors were thus discovered, such as **6a**, **6e**, **10c** and **10d**. Thus, the simple scaffolds present in **10c** and **10d** as well as the least elaborated long tails from **6a** and **6e** may lead to effective, subnanomolar inhibitors of this isoform involved both in cancerogenesis as well as glaucoma.[3,19,20]

(v) No compounds with relevant isoform selectivity were detected in the study, probably because the tails were too long and they may adopt quite variable conformations when bound within the enzyme active site. For example in the case of **10c** and **10d**, where the position sulfonamide moieties (in *meta* and respectively *para* to the bulky scaffold) should force the molecules to adopt very different orientation, the activity of these compounds is rather similar against the various isoforms. This is probably due to the fact that, as shown by X-ray crystallography,[11–14] due to the fact that the active site is rather voluminous, the tail of the inhibitors may bind either in the hydrophobic or hydrophilic half of the active site with similar efficacy. The consequence is that the inhibitors may show similar potency although their binding modes are quite diverse.[11–14] Some of the compounds reported here were low nanomolar/subnanomolar inhibitors of two isoforms involved in glaucoma (hCA II and XII) and may be thus interesting candidates for such studies.

3. Conclusions

Three series of sulfonamides incorporating long, bulky tails were obtained by applying synthetic strategies in which substituted anthranilic acids, quinazolines and aromatic sulfonamides have been used as starting materials. They incorporate long, bulky diamide-, 4-oxoquinazoline-3-yl- or quinazoline-4-yl moieties in their molecules, and were investigated for the inhibition of four physiologically relevant CA isoforms, the cytosolic hCA I and II, as well as the transmembrane hCA IX and XII. Most of the new sulfonamides reported here showed excellent inhibitory effects against the four isoforms, with K_Is of 7.6–322 nM against hCA I, of 0.06–85.4 nM against hCA II; of 6.7–152 nM against hCA IX and of 0.49–237 nM against hCA XII; respectively. However no relevant isoform-selective behavior has been observed for any of them, although hCA II and XII, isoforms involved in glaucoma-genesis were the most inhibited ones.

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. Nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR, DEPT-135, DEPT-90, HSQC, HMBC) spectra were recorded using a Bruker Avance III 400 MHz spectrometer in DMSO-d_6. 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; br s, broad singlet; dd, double of doubles, appt, apparent triplet, appq, apparent quartet. 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. Melting points (mp) were carried out in open capillary tubes and are uncorrected.

(a) **General procedure for the synthesis of 2-benzamidobenzoic acids 3a–f:** Anthranilic acid **1a**,**b** (1.0 equiv) was treated with benzoyl chloride **2a**–**c** (1.1 equiv) in DCM followed by drop-wise addition of TEA (1.5 equiv) at 0 °C. The mixture was warmed-up to rt and stirred until the consumption of starting materials (TLC monitoring). The reaction was quenched with 1.0 M aqueous HCl solution, obtained precipitate was filtered, washed with *n*-hexane (3 × 10 ml) and dried under vacuum to obtain desired compounds **3a**–**f**.

(b) **General procedure for the synthesis of benzoxazin-4-ones 4a–f:** 2-Benzoylamino-benzoic acid **3a**–**f** (1.0 equiv) was refluxed in acetic anhydride (15.0 equiv) until the consumption of starting material (TLC monitoring). Excess of solvents were removed under vacuum and *n*-hexane (20 ml) was added to mixture and stirred vigorously for 30 min, filtered and washed with *n*-hexane (3 × 10 ml) and dried under vacuum to obtain desired compound **4a**–**f**.

(c) **General procedure for 2-benzamido-*N*-(4-sulfamoylphenyl)benzamides 6a–k:** Sulfonamide **5a**,**b** (1.0 equiv) was treated withbenzoxazin-4-ones **4a**–**f** (1.0 equiv) in a proper solvent and refluxed until the consumption of starting materials (TLC monitoring). The reaction was treated accordingly to obtain desired products **6a**–**k**.

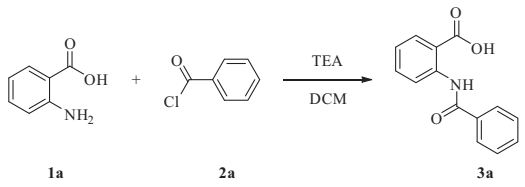
(d) **General procedure for 2-methyl-4*H*-1,3-benzoxazin-4-ones 7a–c:** Anthranilic acid **1a**–**c** (1.0 equiv) was refluxed in Ac_2O (10 equiv) until the consumption of starting material (TLC monitoring). The reaction treated accordingly to give the title compounds **7a**–**c**.

(e) **General procedure for 4-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)benzenesulfonamides 8a–e:** Compound **5a**–**b** was treated with 2-methyl-4*H*-1,3-benzoxazin-4-one **7a**–**c** in a proper dry solvent and the stirring mixture was heated (120–160 °C) until the consumption of starting materials (TLC monitoring). The reaction treated accordingly to obtain desired products **8a**–**e**.

(f) **General procedure for the synthesis of 4-substituted quinazolines 10a–d:** Sulfonamide **5a**–**d** (1.0 equiv) was treated with 4-chloroquinazoline **9** (1.0 equiv) in EtOH and followed by addition of TEA (1.2 equiv).The reaction refluxed until the consumption of starting materials (TLC monitoring). Reaction quenched with slush and acidified with 1 M

aqueous HCl solution to obtain a precipitate which was filtered and washed with Et_2O (3 × 10 ml) and dried under vacuum to obtain title product **10a–d**.

4.1.1. Synthesis of 2-benzamidobenzoic acid 3a

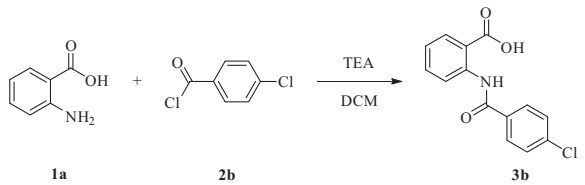


1a **2a** **3a**

Anthranilic acid **1a** (2.0 g, 1.0 equiv) was treated with benzoyl chloride **2a** (1.1 equiv) in DCM (30 ml) followed by drop-wise addition of TEA (1.5 equiv) at 0 °C. The reaction mixture was treated according to general procedure a, previously reported, to give the title compound **3a** as a white solid.

2-Benzamidobenzoic acid **3a**: yield 95%; silica gel TLC R_f = 0.55 (Ethyl acetate/*n*-hexane 50% v/v); mp 176–177 °C; δ_H (400 MHz, DMSO-d_6) 7.26 (1H, t, *J* 8.0), 7.62–7.74 (4H, m), 8.01 (2H, d, *J* 8.0), 8.10 (1H, d, *J* 8.0), 8.75 (1H, d, *J* 8.0), 12.21 (1H, br s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 117.5, 120.8, 123.9, 127.9, 129.9, 132.2, 133.1, 135.2, 135.5, 142.0, 165.6, 170.9. Experimental in agreement with reported data.[26]

4.1.2. Synthesis of 2-(4-chlorobenzamido)benzoic acid 3b



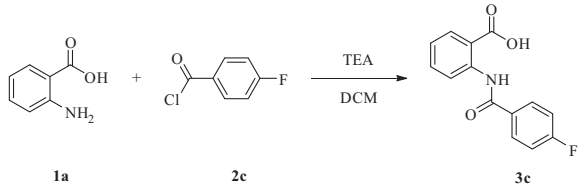
1a **2b** **3b**

Anthranilic acid **1a** (1.5 g, 1.0 equiv) was treated with 4-chlorobenzoyl chloride **2b** (1.1 equiv) in DCM (25 ml) followed by drop-wise addition of TEA (1.5 equiv) at 0 °C. The reaction mixture was treated according to general procedure a, previously reported, to give the title compound **3b** as a white solid.

2-(4-Chlorobenzamido)benzoic acid **3b**: yield 95%; silica gel TLC R_f = 0.57 (Ethyl acetate/*n*-hexane 20% v/v); mp 202–203 °C; δ_H (400 MHz, DMSO-d_6) 7.27 (1H, t, *J* 8.0), 7.70 (3H, m), 8.00 (2H, d, *J* 8.8), 8.10 (1H, d, *J* 8.0), 8.69 (1H, d, *J* 8.0), 12.19 (1H, s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 117.7, 120.9, 124.0, 129.7, 129.9, 132.1, 134.2, 135.0, 137.9, 141.7, 164.5, 170.7.

Experimental in agreement with reported data.[27]

4.1.3. Synthesis of 2-(4-fluorobenzamido)benzoic acid 3c

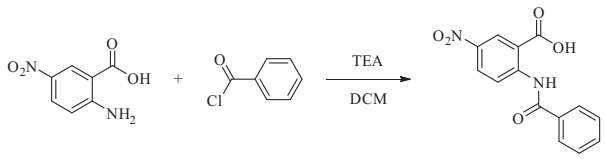


1a **2c** **3c**

Anthranilic acid **1a** (1.5 g, 1.0 equiv) was treated with 4-fluorobenzoyl chloride **2b** (1.1 equiv) in DCM (25 ml) followed by drop-wise addition of TEA (1.5 equiv) at 0 °C. The reaction mixture was treated according to general procedure a, previously reported, to give the title compound **3c** as a white solid.

2-(4-Fluorobenzamido)benzoic acid **3c**: yield 78%; silica gel TLC R_f = 0.7 (Ethyl acetate/*n*-hexane 40% v/v); mp 183–184 °C; δ_H (400 MHz, DMSO-d_6) 7.26 (1H, t, *J* 8.0), 7.48 (2H, m), 7.71 (1H, t, *J* 8.0), 8.07 (3H, m), 8.70 (1H, d, *J* 8.0), 12.17 (1H, s, exchange with D_2O, N*H*), 13.83 (1H, br s, exchange with D_2O, O*H*); δ_C (100 MHz, DMSO-d_6) 116.9 (d, $^2J_{C-F}$ 22), 117.6, 120.9, 123.9, 130.7 (d, $^3J_{C-F}$ 9), 132.0, 132.2, 135.2, 142.0, 164.5, 165.3 (d, $^1J_{C-F}$ 248), 171.0; δ_F (376 MHz, DMSO-d_6) −107.9 (1F, s).

4.1.4. Synthesis of 2-benzamido-5-nitrobenzoic acid 3d

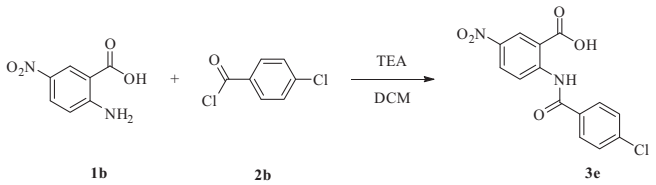


1b **2a** **3d**

5-Nitroanthranilic acid **1b** (1.5 g, 1.0 equiv) was treated with benzoyl chloride **2a** (1.1 equiv) in DCM (25 ml) followed by drop-wise addition of TEA (1.5 equiv) at 0 °C. The reaction mixture was treated according to general procedure a, previously reported, to give the title compound **3d** as a pale yellow solid.

2-Benzamido-5-nitrobenzoic acid **3d**: yield 85%; silica gel TLC R_f = 0.45 (Ethyl acetate/*n*-hexane 50% v/v); mp 175–176 °C; δ_H (400 MHz, DMSO-d_6) 7.66 (2H, t, *J* 7.2), 7.73 (1H, t, *J* 7.2), 8.02 (2H, d, *J* 7.2), 8.55 (1H, dd, *J* 2.8, 9.2), 8.20 (1H, d, *J* 2.8), 8.97 (1H, d, *J* 9.2), 12.54 (1H, s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 117.6, 121.0, 127.6, 128.2, 130.1, 133.8, 134.6, 142.3, 147.3, 147.3, 166.1, 169.5. Experimental in agreement with reported data.[28]

4.1.5. Synthesis of 2-(4-chlorobenzamido)-5-nitrobenzoic acid 3e

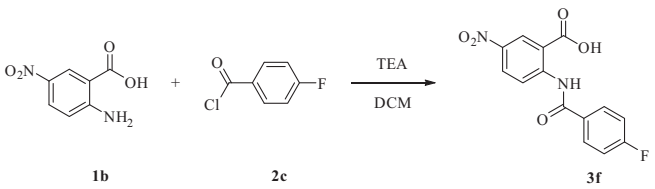


1b **2b** **3e**

5-Nitroanthranilic acid **1b** (1.5 g, 1.0 equiv) was treated with 4-chlorobenzoyl chloride **2b** (1.1 equiv) in DCM (25 ml) followed by drop-wise addition of TEA (1.5 equiv) at 0 °C. The reaction mixture was treated according to general procedure a, previously reported, to give the title compound **3e** as a pale brown solid.

2-(4-Chlorobenzamido)-5-nitrobenzoic acid **3e**: yield 80%; silica gel TLC R_f = 0.60 (Ethyl acetate/*n*-hexane 50% v/v); mp 192–193 °C; δ_H (400 MHz, DMSO-d_6) 7.75 (2H, d, *J* 8.8), 8.03 (2H, d, *J* 8.8), 8.56 (1H, dd, *J* 2.8, 9.2), 8.83 (1H, d, *J* 2.8), 8.94 (1H, d, *J* 9.2), 12.68 (1H, s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 121.3, 127.7, 130.1, 130.3, 130.3, 133.7, 138.7, 142.6, 147.2, 165.3, 169.5.

4.1.6. Synthesis of 2-(4-fluorobenzamido)benzoic acid 3f

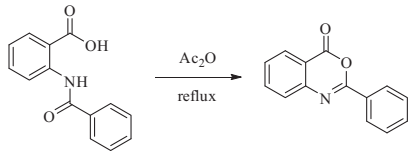


1b 2c 3f

5-Nitroanthranilic acid **1b** (1.5 g, 1.0 equiv) was treated with 4-fluorobenzoyl chloride **2c** (1.1 equiv) in DCM (25 ml) followed by drop-wise addition of TEA (1.5 equiv) at 0 °C. The reaction mixture was treated according to general procedure a, previously reported, to give the title compound **3f** as a pale yellow solid.

2-(4-Fluorobenzamido)benzoic acid **3f**: yield 83%; silica gel TLC R_f = 0.59 (Ethyl acetate/*n*-hexane 50% v/v); mp 198–199 °C; δ_H (400 MHz, DMSO-d_6) 7.50 (2H, m), 8.06 (2H, m), 8.53 (1H, dd, *J* 2.8, 9.2), 8.79 (1H, d, *J* 2.8), 8.91 (1H, d, *J* 9.2),12.49 (1H, s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 117.1 (d, $^2J_{C-F}$ 22), 117.4, 120.8, 127.5, 129.9, 130.9, 131.0 (d, $^3J_{C-F}$10), 142.1, 147.2, 164.8, 165.7 (d, $^1J_{C-F}$250), 169.5; δ_F (376 MHz, DMSO-d_6) −106.8 (1F, s).

4.1.7. Synthesis of 2-phenyl-4*H*-benzo[*d*][1,3]oxazin-4-one 4a

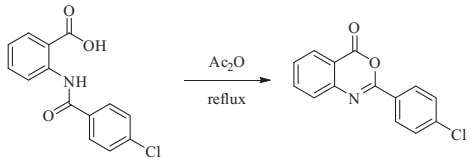


3a 4a

A suspension of 2-benzamidobenzoic acid **3a** (0.32 g, 1.0 equiv) was refluxed in Ac_2O (15.0 equiv) and treated accordingly to general procedure b, previously reported, to obtain title compound **4a** as a white solid.

2-Phenyl-4*H*-benzo[*d*][1,3]oxazin-4-one **4a**: yield 40%; silica gel TLC R_f = 0.80 (Ethyl acetate/*n*-hexane 50% v/v); mp 124–125 °C; δ_H (400 MHz, DMSO-d_6) 7.70 (4H, m), 7.78 (1H, d, *J* 8.0), 8.00 (1H, t, *J* 8.0), 8.21 (1H, d, *J* 8.0), 8.25 (2H, d, *J* 8.0); δ_C (100 MHz, DMSO-d_6) 117.8, 127.8, 128.7, 129.0, 129.5, 129.9, 130.9, 133.6, 137.7, 147.2, 157.3, 159.8. Experimental in agreement with reported data.[26]

4.1.8. Synthesis of 2-(4-chlorophenyl)-4*H*-benzo[*d*][1,3]oxazin-4-one 4b

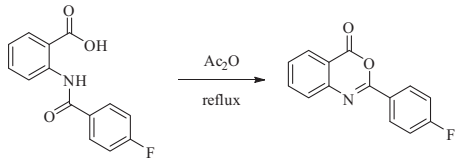


3b 4b

A suspension of 2-(4-chlorobenzamido)benzoic acid **3b** (2.5 g, 1.0 equiv) was refluxed in Ac_2O (15.0 equiv) and treated accordingly to general procedure b, previously reported, to obtain title compound **4b** as a white solid.

2-(4-Chlorophenyl)-4*H*-benzo[*d*][1,3]oxazin-4-one **4b**: yield 60%; silica gel TLC R_f = 0.80 (Ethyl acetate/*n*-hexane 50% v/v); mp 189–190 °C; δ_H (400 MHz, DMSO-d_6) 7.70 (3H, m), 7.77 (1H, d, *J* 8.0), 8.00 (1H, t, *J* 8.0), 8.20 (1H, d, *J* 8.0), 8.23 (2H, d, *J* 8.8); δ_C (100 MHz, DMSO-d_6) 117.6, 127.7, 128.8, 129.5, 129.7, 129.9, 130.3, 137.6, 138.4, 146.9, 156.4, 159.3. Experimental in agreement with reported data.[29]

4.1.9. Synthesis of 2-(4-fluorophenyl)-4*H*-benzo[*d*][1,3]oxazin-4-one 4c



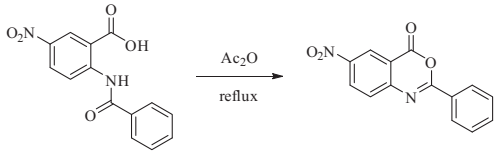
3c 4c

A suspension of 2-(4-fluorobenzamido)benzoic acid **3c** (2.75 g, 1.0 equiv) was refluxed in Ac_2O (15.0) and treated accordingly to general procedure **b**, previously reported, to obtain title compound **4c** as a white solid.

2-(4-Fluorophenyl)-4*H*-benzo[*d*][1,3]oxazin-4-one **4c**: yield 83%; silica gel TLC R_f = 0.7 (Ethyl acetate/*n*-hexane 50% v/v); mp 174–175 °C; δ_H (400 MHz, DMSO-d_6) 7.48 (2H, m), 7.67 (1H, t, *J* 8.0), 7.76 (1H, d, *J* 8.0), 8.00 (1H, t, *J* 8.0), 8.20 (1H, d; *J* 8.0),8.30 (2H, m); δ_C (100 MHz, DMSO-d_6) 117.1 (d, $^2J_{C-F}$ 22), 117.8, 127.5 (d, $^4J_{C-F}$ 3), 127.8, 129.0, 129.5, 131.4 (d, $^3J_{C-F}$ 9), 137.8, 147.1, 156.5, 159.7, 165.7 (d, $^1J_{C-F}$ 250); δ_F (376 MHz, DMSO-d_6) −106.7 (1F, s).

Experimental in agreement with reported data.[30]

4.1.10. Synthesis of 6-nitro-2-phenyl-4*H*-benzo[*d*][1,3]oxazin-4-one 4d



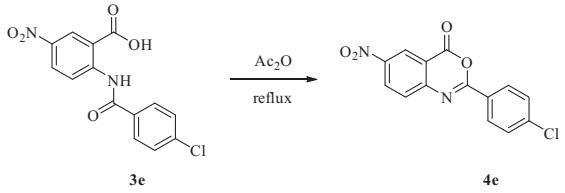
3d 4d

A suspension of 2-benzamido-5-nitrobenzoic acid **3d** (1.6 g, 1 equiv) was refluxed in Ac_2O (15.0 equiv) and treated accordingly to general procedure b, previously reported, to obtain title compound **4d** as a pale yellow solid.

6-Nitro-2-phenyl-4*H*-benzo[*d*][1,3]oxazin-4-one **4d**: yield 83%; silica gel TLC R_f = 0.72 (Ethyl acetate/*n*-hexane 50% v/v); mp 167–168 °C; δ_H (400 MHz, DMSO-d_6) 7.68 (2H, t, *J* 8.0), 7.77 (1H, t, *J* 8.0), 7.96 (1H, d, *J* 8.8), 8.29 (2H, d, *J* 8.0), 8.70 (1H, dd, *J* 2.8, 8.8), 8.81 (1H, d, *J* 2.8); δ_C (100 MHz, DMSO-d_6) 118.8, 124.3, 129.3, 129.5, 130.1, 130.3, 131.6, 134.6, 146.8, 151.6, 158.7, 160.0.

Experimental in agreement with reported data.[31]

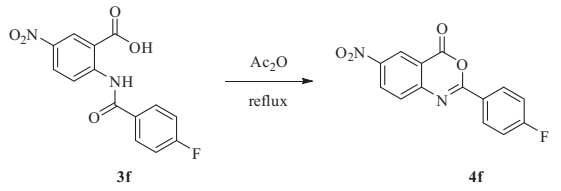
4.1.11. Synthesis of 2-(4-chlorophenyl)-6-nitro-4*H*-benzo[*d*][1,3]oxazin-4-one 4e



A suspension of 2-(4-chlorobenzamido)-5-nitrobenzoic acid **3e** (1.0 g, 1.0 equiv) was refluxed in Ac_2O (15.0 equiv) and treated accordingly to general procedure b, previously reported, to obtain title compound **4e** as pale yellow solid.

2-(4-Chlorophenyl)-4*H*-benzo[*d*][1,3]oxazin-4-one **4e**: yield 35%; silica gel TLC R_f = 0.75 (Ethyl acetate/*n*-hexane 50% v/v); mp 239–240 °C; δ_H (400 MHz, DMSO-d_6) 7.76 (2H, d, *J* 8.4), 7.97 (1H, d, *J* 8.8), 8.29 (2H, d, *J* 8.4), 8.72 (1H, dd, *J* 2.8, 8.8), 8.82 (1H, d, *J* 2.8); δ_C (100 MHz, DMSO-d_6) 118.9, 124.4, 129.4, 129.5, 130.3, 131.1, 131.7, 139.5, 147.0, 151.5, 158.6, 159.2.

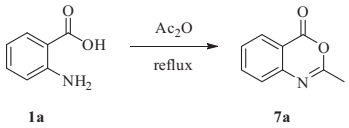
4.1.12. Synthesis of 2-(4-fluorophenyl)-6-nitro-4*H*-benzo[*d*][1,3]oxazin-4-one 4f



A suspension of 2-(4-fluorobenzamido)-5-nitrobenzoic acid **3f** (1.9 g, 1.0 equiv) was refluxed in Ac_2O (15.0 equiv) and treated accordingly to general procedure b, previously reported, to obtain title compound **4f** as a pale yellow solid.

2-(4-Fluorophenyl)-6-nitro-4*H*-benzo[*d*][1,3]oxazin-4-one **4f**: yield 57%; silica gel TLC R_f = 0.70 (Ethyl acetate/*n*-hexane 50% v/v); mp 181–182 °C; δ_H (400 MHz, DMSO-d_6) 7.52 (2H, m), 7.95 (1H, d, *J* 8.8), 8.35 (2H, m), 8.71 (1H, dd, *J* 2.8, 8.8), 8.81 (1H, d, *J* 2.8); δ_C (100 MHz, DMSO-d_6) 117.3 (d, $^2J_{C-F}$ 22), 118.6, 124.3, 126.8, 129.5, 131.7, 132.1 (d, $^3J_{C-F}$ 10), 146.8, 151.6, 158.5, 159.1, 166.1 (d, $^1J_{C-F}$ 251); δ_F (376 MHz, DMSO-d_6) −105.0 (1F, s).

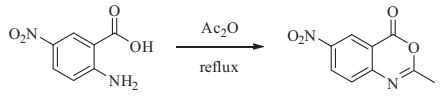
4.1.13. Synthesis of 2-methyl-4*H*-benzo[*d*][1,3]oxazin-4-one 7a[32]



Anthranilic acid **1a** (3.75 g, 1.0 equiv) was refluxed in Ac_2O (10.0 equiv) until the consumption of starting material (TLC monitoring). The solvent was evaporated under vacuum to obtain the residue that was crystallized with EtOAc/*n*-hexane mixture to obtain title compound **7a** as a white solid.

2-Methyl-4*H*-benzo[*d*][1,3]oxazin-4-one **7a**: yield 60%; silica gel TLC R_f = 0.16 (Ethyl acetate/*n*-hexane 80% v/v); mp 120–121 °C; δ_H (400 MHz, DMSO-d_6) 2.16 (3H, s), 7.16 (1H, t, *J* 7.6), 7.60 (1H, t, *J* 7.6), 8.00 (1H, d, *J* 7.6), 8.49 (1H, d, *J* 7.6); δ_C (100 MHz, DMSO-d_6) 25.7, 117.4, 120.8, 123.3, 131.8, 134.7, 141.7, 169.2, 170.2.

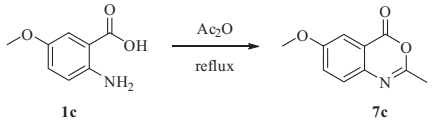
4.1.14. Synthesis of 2-methyl-6-nitro-4*H*-benzo[*d*][1,3]oxazin-4-one 7b



2-Amino-5-nitrobenzoic acid **1b** (5.0 g, 1.0 equiv) was refluxed in Ac_2O (10.0 equiv) until the consumption of starting materials (TLC monitoring). The excess of solvents were evaporated under vacuum to obtain the residue that washed with *n*-hexane (3 × 10 ml), filtered and dried under vacuum to obtain title compound **7b** as a brown solid.

2-Methyl-6-nitro-4*H*-benzo[*d*][1,3]oxazin-4-one **7b**: yield 85%; silica gel TLC R_f = 0.55 (Ethyl acetate/*n*-hexane 50% v/v); mp 220–221 °C; δ_H (400 MHz, DMSO-d_6) 2.51 (3H, s), 7.82 (1H, d, *J* 8.8), 8.66 (1H, dd, *J* 2.8, 8.8), 8.77 (1H, d, *J* 2.8); δ_C (100 MHz, DMSO-d_6) 22.2, 118.3, 124.2, 128.8, 131.6, 146.8, 151.4, 159.0, 164.5; *m*/*z* (ESI negative) 205.08 $[M-H]^-$. Experimental in agreement with reported data.[33]

4.1.15. Synthesis of 6-methoxy-2-methyl-4*H*-benzo[*d*][1,3]oxazin-4-one 7c

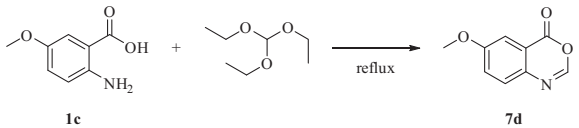


5-Methoxyanthranilic acid **1c** (1.5 g, 1.0 equiv) was refluxed in acetic anhydrate (10.0 equiv) until the consumption of starting materials (TLC monitoring). The excess of solvents were evaporated under vacuum to obtain the residue that washed with *n*-hexane, filtered and dried under vacuum to obtain title compound **7c** as a pale yellow solid.

6-Methoxy-2-methyl-4*H*-benzo[*d*][1,3]oxazin-4-one **7c**: yield 91%; silica gel TLC R_f = 0.25 (Ethyl acetate/*n*-hexane 20% v/v); mp 157–158 °C; δ_H (400 MHz, DMSO-d_6) 2.41 (3H, s), 3.92 (3H, s), 7.55 (3H, m); δ_C (100 MHz, DMSO-d_6) 21.6, 56.8, 109.5, 118.1, 126.1, 128.7, 141.1, 158.8, 159.4, 160.2.

Experimental in agreement with reported data.[33]

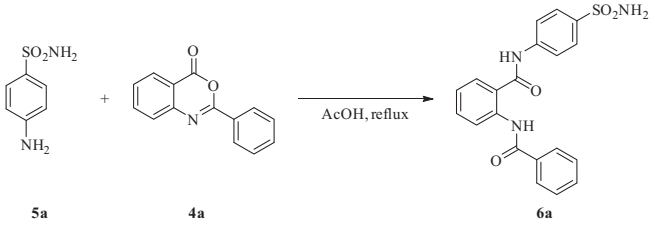
4.1.16. Synthesis of 6-methoxy-4*H*-benzo[*d*][1,3]oxazin-4-one 7d



5-Methoxyanthranilic acid **1c** (3.0 g, 1.0 equiv) was refluxed in triethylorthoformate (5.0 equiv) and the mixture was stirred until the consumption of starting materials (TLC monitoring). The excess of solvents were evaporated under vacuum to obtain the residue that was washed with *n*-hexane, filtered and dried under vacuum to obtain title compound **7d** as a pale yellow solid.

6-Methoxy-4*H*-benzo[*d*][1,3]oxazin-4-one **7d**: yield 89%; silica gel TLC R_f = 0.63 (Ethyl acetate/*n*-hexane 50% v/v); mp 180–181 °C (decomp.); δ_H (400 MHz, DMSO-d_6) 3.93 (3H, s), 7.55 (2H, m), 7.64 (1H, m), 8.14 (1H, s); δ_C (100 MHz, DMSO-d_6) 56.9, 109.7, 120.4, 126.0, 129.3, 140.0, 149.7, 159.4, 160.1.

4.1.17. Synthesis of 2-benzamido-*N*-(4-sulfamoylphenyl) benzamide 6a



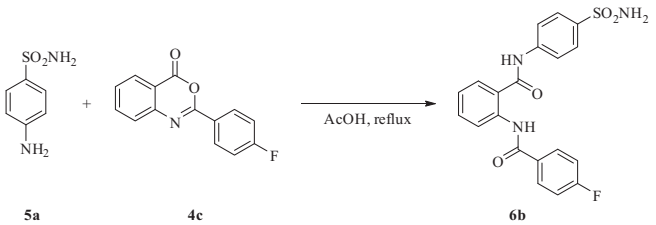
5a **4a** **6a**

Sulfanilamide **5a** (0.15 g, 1.0 equiv) was treated with **4a** (1.0 equiv) in glacial acetic acid (3.0 ml) according to the general procedure, previously reported. The reaction was quenched with slush, obtained precipitate was filtered, washed with Et_2O (3 × 10 ml) and dried under vacuum to obtain the title compound **6a** as a white solid.

2-Benzamido-*N*-(4-sulfamoylphenyl)benzamide **6a**: yield 45%; silica gel TLC R_f = 0.16 (Ethyl acetate/*n*-hexane 50% v/v); mp 270–271 °C; δ_H (400 MHz, DMSO-d_6) 7.33 (2H, s, exchange with D_2O, SO_2NH_2), 7.36 (1H, t, *J* 8.0), 7.58–7.70 (4H, m), 7.84 (2H, d, *J* 8.9), 7.93 (5H, m), 8.40 (1H, d, *J* 8.0), 10.84 (1H, s, exchange with D_2O, N*H*), 11.45 (1H, s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 121.4, 122.8, 124.4, 124.6, 127.4, 128.0, 129.8, 130.0, 132.9, 133.3, 135.4, 139.3, 140.1, 142.6, 165.7, 168.5; *m*/*z* (ESI negative) 394.1 $[M-H]^-$.

Experimental in agreement with reported data.[34a]

4.1.18. Synthesis of 2-(4-fluorobenzamido)-*N*-(4-sulfamoyl-phenyl)benzamide 6b

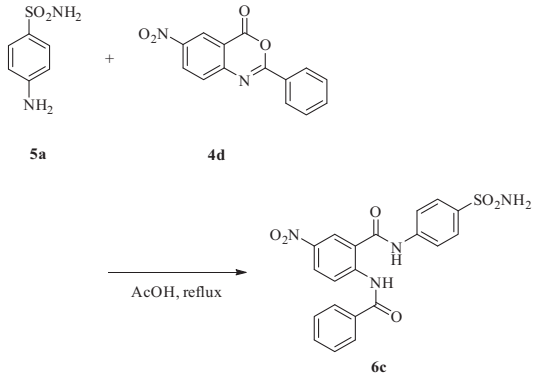


5a **4c** **6b**

Sulfanilamide **5a** (0.18 g, 1.0 equiv) was treated with **4c** (1.0 equiv) in glacial acetic acid (3.0 ml) according to the general procedure c, previously reported. The reaction was quenched with slush and obtained precipitate was filtered, washed with Et_2O (3 × 10 ml) and dried under vacuum to obtain the title compound **6b** as a white solid.

2-(4-Fluorobenzamido)-*N*-(4-sulfamoylphenyl)benzamide **6b**: yield 50%; silica gel TLC R_f = 0.17 (Ethyl acetate/*n*-hexane 50% v/v); mp 272–273 °C; δ_H (400 MHz, DMSO-d_6) 7.32 (2H, s, exchange with D_2O, SO_2NH_2), 7.36 (1H, t, *J* 8.0), 7.44 (2H, m), 7.67 (1H, t, *J* 8.0), 7.84 (2H, d, *J* 8.8), 7.92 (3H, m), 8.01 (2H, m), 8.33 (1H, d, *J* 8.0), 10.82 (1H, s, exchange with D_2O, N*H*), 11.38 (1H, s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 116.8 (d, $^2J_{C-F}$ 22), 121.3, 123.0, 124.6, 125.1, 127.4, 130.0, 130.8 (d, $^3J_{C-F}$ 9), 131.9 (d, $^4J_{C-F}$ 3), 133.2, 139.0, 140.0, 142.6, 164.7, 165.2 (d, $^1J_{C-F}$ 248), 168.5; δ_F (376 MHz, DMSO-d_6) −108.1 (1F, s); *m*/*z* (ESI negative) 412.1 $[M-H]^-$.

4.1.19. Synthesis of 2-benzamido-5-nitro-*N*-(4-sulfamoyl-phenyl)benzamide 6c

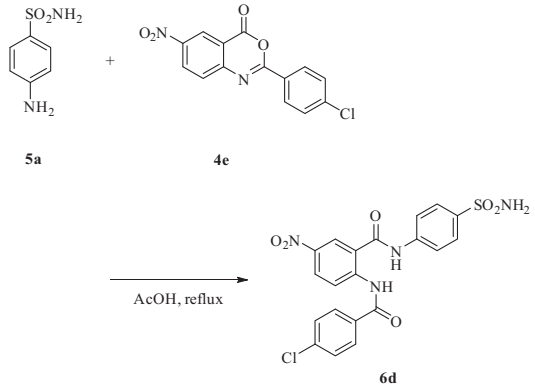


5a **4d** **6c**

Sulfanilamide **5a** (0.1 g, 1.0 equiv) was treated with **4d** (1.0 equiv) in glacial acetic acid (3.0 ml) according to the general procedure c, previously reported. The reaction was quenched with slush and obtained precipitate was filtered, washed with Et_2O (3 × 10 ml) and dried under vacuum to obtain the title compound **6c** as a white solid.

2-Benzamido-5-nitro-*N*-(4-sulfamoylphenyl)benzamide **6c**: yield 52%; silica gel TLC R_f = 0.25 (Ethyl acetate/*n*-hexane 50% v/v); mp >300 °C; δ_H (400 MHz, DMSO-d_6) 7.35 (2H, s, exchange with D_2O, SO_2NH_2),7.64 (2H, t, *J* 7.2), 7.70 (1H, t, *J* 7.2), 7.90 (4H, m), 7.98 (2H, d, *J* 7.2), 8.55 (1H, dd, *J* 2.4, 9.2), 8.71 (1H, d, *J* 9.2), 8.38 (1H, d, *J* 2.4), 11.16 (1H, s, exchange with D_2O, N*H*), 11.89 (1H, s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 121.8, 122.5, 123.7, 125.8, 127.4, 128.3, 128.5, 129.9, 133.5, 134.7, 140.6, 142.1, 142.7, 145.2, 166.1, 166.9; *m*/*z* (ESI negative) 439.0 $[M-H]^-$.

4.1.20. Synthesis of 2-(4-chlorobenzamido)-5-nitro-*N*-(4-sulfa-moylphenyl)benzamide 6d

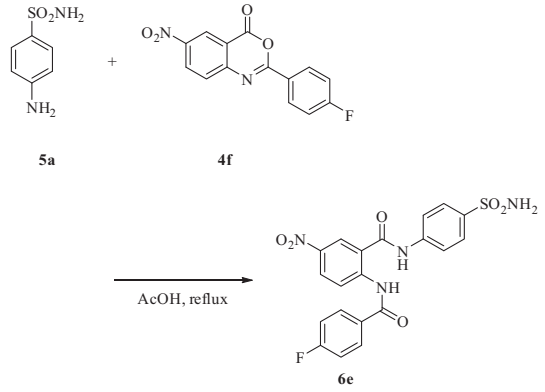


5a **4e** **6d**

Sulfanilamide **5a** (0.1 g, 1.0 equiv) was treated with **4e** (1.0 equiv) in glacial acetic acid (3 ml) according to the general procedure c, previously reported. Reaction was quenched with slush and obtained precipitate was filtered and washed with Et_2O (3 × 10 ml), and dried under vacuum to obtain the title compound **6d** as a white solid.

2-(4-Chlorobenzamido)-5-nitro-*N*-(4-sulfamoylphenyl)benzamide **6d**: yield 60%; silica gel TLC R_f = 0.35 (Ethyl acetate/*n*-hexane 50% v/v); mp 299–300 °C (decomp.); δ_H (400 MHz, DMSO-d_6) 7.36 (2H, s, exchange with D_2O, SO_2NH_2), 7.70 (2H, d, *J* 8.4), 7.90 (4H, m), 7.97 (2H, d, *J* 8.4), 8.53 (1H, dd, *J* 2.8, 9.2), 8.63 (1H, d, *J* 9.2), 8.81 (1H, d, *J* 2.8),11.15 (1H, s, exchange with D_2O, N*H*), 11.85 (1H, s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 121.8, 122.8, 124.3, 125.8, 127.5, 128.4, 130.0, 130.2, 133.5, 138.4, 140.5, 142.1, 142.9, 144.9, 165.2, 166.8; *m*/*z* (ESI negative) 473.0 $[M-H]^-$.

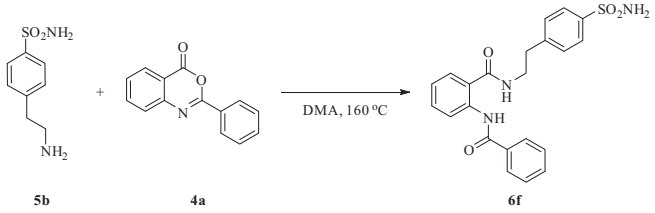
4.1.21. Synthesis of 2-(4-fluorobenzamido)-5-nitro-*N*-(4-sulfamoylphenyl)benzamide 6e



Sulfanilamide **5a** (0.1 g, 1.0 equiv) was treated with **4f** (1.0 equiv) in glacial acetic acid (3 ml) according to the general procedure c, previously reported. Reaction was quenched with slush and obtained precipitate was filtered and washed with Et_2O (3 × 10 ml), and dried under vacuum to obtain the title compound **6e** as a white solid.

2-(4-Fluorobenzamido)-5-nitro-*N*-(4-sulfamoylphenyl)benzamide **6e**: yield 73%; silica gel TLC R_f = 0.35 (Ethyl acetate/*n*-hexane 50% v/v); mp >300 °C; δ_H (400 MHz, DMSO-d_6) 7.36 (2H, s, exchange with D_2O, SO_2NH_2), 7.48 (2H, m), 7.89 (4H, m), 8.05 (2H, m), 8.55 (1H, dd, *J* 2.4, 9.2), 8.63 (1H, d, *J* 9.2), 8.81 (1H, d, *J* 2.4),11.15 (1H, s, exchange with D_2O, N*H*), 11.82 (1H, s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 116.9 (d, $^2J_{C-F}$ 22), 121.8, 122.6, 124.0, 125.8, 127.4, 128.4, 131.1 (d, $^3J_{C-F}$ 9), 140.6, 142.1, 142.8, 145.1, 165.1, 165.5 (d, $^1J_{C-F}$ 248), 166.8; δ_F (376 MHz, DMSO-d_6) −107.0 (1F, s); *m*/*z* (ESI negative) 457.0 $[M-H]^-$.

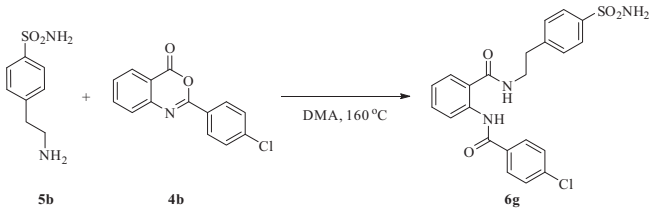
4.1.22. Synthesis of 2-benzamido-*N*-(4-sulfamoylphenethyl) benzamide 6f



4(2-Aminoethyl)benzenesulfonamide **5b** (0.2 g, 1.0 equiv) was treated with **4a** (1.0 equiv)in dry DMA (10 ml) according to the general procedure c, previously reported. Reaction was quenched with slush and obtained precipitate was filtered and washed with Et_2O (3 × 10 ml) and dried under vacuum to obtain the title compound **6f** as a white solid.

2-Benzamido-*N*-(4-sulfamoylphenethyl)benzamide **6f**: yield 47%, silica gel TLC R_f = 0.11 (Ethyl acetate/*n*-hexane 50% v/v); mp 258–259 °C; δ_H (400 MHz, DMSO-d_6) 3.00 (2H, t, *J* 6.8), 3.62 (2H, q, *J* 6.8), 7.23 (1H, t, *J* 8.0), 7.33 (2H, s, exchange with D_2O, SO_2NH_2), 7.49 (2H, d, *J* 8.4), 7.59–7.70 (4H, m), 7.78 (3H, m),7.97 (2H, m), 8.67 (1H, d, *J* 8.0), 9.02 (1H, t, *J* 6.8, exchange with D_2O, N*H*), 12.55 (1H, s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 35.3, 41.3, 121.2, 121.2, 123.8, 126.6, 127.9, 129.0, 129.9, 130.1, 133.0, 133.2, 135.5, 140.3, 143.1, 144.5, 165.3, 169.6; *m*/*z* (ESI negative)422.17 $[M-H]^-$.

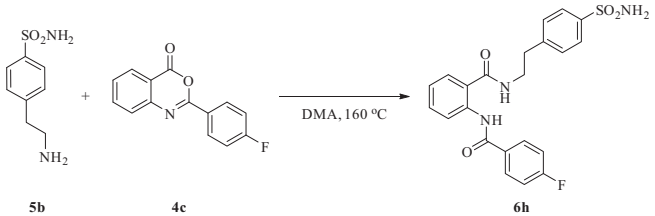
4.1.23. Synthesis of 2-(4-chlorobenzamido)-*N*-(4-sulfamoylphenethyl)benzamide 6g



4(2-Aminoethyl)benzenesulfonamide **5b** (0.2 g, 1.0 equiv) was treated with **4b** (1.0 equiv) in dry DMA (10 ml) according to the general procedure c, previously reported. Reaction was quenched with slush and obtained precipitate was filtered and washed with Et_2O (3 × 10 ml), and dried under vacuum to obtain the title compound **6g** as a white solid.

2-(4-Chlorobenzamido)-*N*-(4-sulfamoylphenethyl)benzamide **6g**: yield 75%, silica gel TLC R_f = 0.63 (Ethyl acetate/*n*-hexane 80% v/v); mp 272–273 °C (decomp.); δ_H (400 MHz, DMSO-d_6) 3.00 (2H, t, *J* 6.8), 3.61 (2H, q, *J* 6.8), 7.25 (1H, t, *J* 8.0), 7.32 (2H, s, exchange with D_2O, SO_2NH_2), 7.49 (2H, d, *J* 8.4), 7.61 (1H, t, *J* 8.0), 7.72 (2H, d, *J* 8.4), 7.80 (3H, m), 7.97 (2H, d, *J* 8.4), 8.64 (1H, d, *J* 8.0), 9.04 (1H, t, *J* 6.8, exchange with D_2O, N*H*), 12.59 (1H, s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 35.3, 41.3, 121.3, 121.3, 124.0, 126.6, 129.0, 129.8, 130.0, 130.1, 133.2, 134.2, 137.8, 140.1, 143.1, 144.5, 164.3, 169.5; *m*/*z* (ESI negative) 456.17 $[M-H]^-$.

4.1.24. Synthesis of 2-(4-fluorobenzamido)-*N*-(4-sulfamoylphenethyl)benzamide 6h

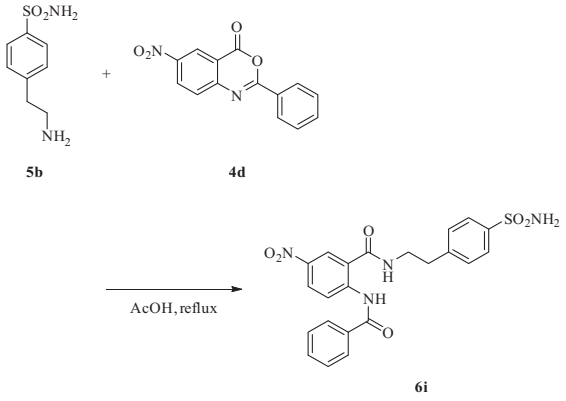


4(2-Aminoethyl)benzenesulfonamide **5b** (0.4 g, 1.0 equiv) was treated with **4c** (1.0 equiv) in dry DMA (20 ml) according to the general procedure c, previously reported. Reaction was quenched with slush and extracted with ethyl acetate (3 × 20 ml) and the combined organic layers were washed with H_2O (3 × 20 ml), dried over Na_2SO_4, filtered and concentrated in vacuum to give the title compound **6h** as a white solid.

2-(4-Fluorobenzamido)-*N*-(4-sulfamoylphenethyl)benzamide **6h**: yield 94%; silica gel TLC R_f = 0.37 (Ethyl acetate/*n*-hexane 50% v/v); mp 242–243 °C; δ_H (400 MHz, DMSO-d_6) 3.00 (2H, t, *J* 6.8), 3.61 (2H, q, *J* 6.8), 7.24 (1H, t, *J* 7.8), 7.32 (2H, s, exchange with D_2O, SO_2NH_2), 7.48 (4H, m), 7.60 (1H, d, *J* 7.8), 7.78 (3H, m), 8.00

(2H, m), 8.64 (1H, d, *J* 7.8), 9.03 (1H, t, *J* 6.8, exchange with D_2O, N*H*), 12.56 (1H, s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 35.2, 41.2, 116.8 (d, $^2J_{C-F}$ 22), 121.2, 123.7, 126.5, 128.9, 129.9, 130.4 (d, $^3J_{C-F}$ 9), 131.9 (d, $^4J_{C-F}$ 3), 132.0 133.0, 140.2, 143.0, 144.4, 164.2 165.1 (d, $^1J_{C-F}$ 248), 169.5; δ_F (376 MHz, DMSO-d_6) −108.1 (1F, s); *m*/*z* (ESI negative) 440.2 $[M-H]^-$.

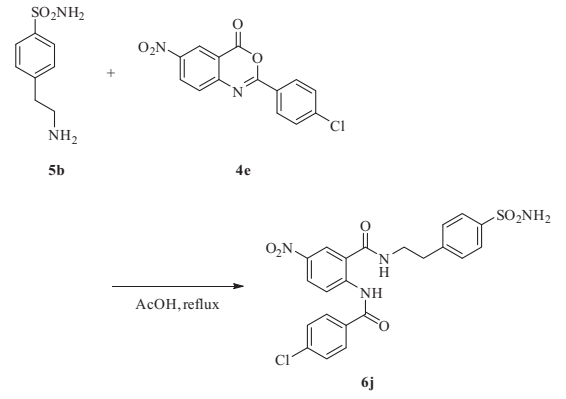
4.1.25. Synthesis of 2-benzamido-5-nitro-*N*-(4-sulfamoylphenethyl)benzamide 6i



4-(2-Aminoethyl)benzenesulfonamide **5b** (0.1 g, 1.0 equiv) was treated with **4d** (1.0 equiv) in glacial acetic acid (3 ml) according to the general procedure c, previously reported. Reaction was quenched with slush and obtained precipitate was filtered and washed with Et_2O (3 × 10 ml), and dried under vacuum to obtain the title compound **6i** as a white solid.

2-Benzamido-5-nitro-*N*-(4-sulfamoylphenethyl)benzamide **6i**: yield 60%; silica gel TLC R_f = 0.23 (Ethyl acetate/*n*-hexane 50% v/v); mp >300 °C; δ_H (400 MHz, DMSO-d_6) 3.04 (2H, t, *J* 7.2), 3.65 (2H, q, *J* 7.2), 7.33 (2H, s, exchange with D_2O, SO_2NH_2), 7.51 (2H, d, *J* 8.4), 7.70 (3H, m), 7.80 (2H, d, *J* 8.4), 8.00 (2H, d, *J* 8.4), 8.50 (1H, dd, *J* 2.4, 9.2), 8.78 (1H, d, *J* 2.4), 8.95 (1H, d, *J* 9.2), 9.49 (1H, t, *J* 7.2), 13.01 (1H, s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 35.2, 41.6, 120.6, 121.3, 124.9, 126.7, 128.1, 128.5, 130.1, 130.1, 133.6, 134.7, 142.4, 143.1, 144.4, 146.2, 165.9, 168.0; *m*/*z* (ESI negative) 467.0 $[M-H]^-$.

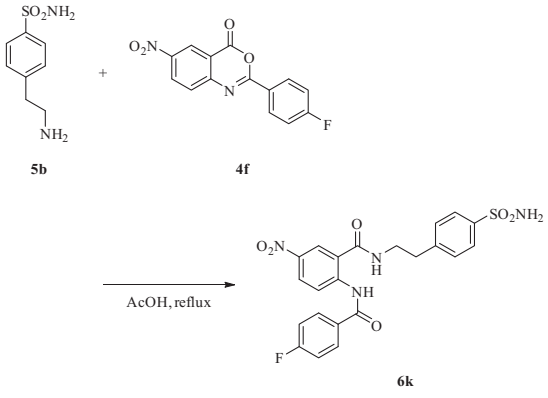
4.1.26. Synthesis of 2-(4-chlorobenzamido)-5-nitro-*N*-(4-sulfamoylphenethyl)benzamide 6j



4-(2-Aminoethyl)benzenesulfonamide **5b** (0.15 g, 1.0 equiv) was treated with **4e** (1.0 equiv)in glacial acetic acid (3 ml) according to the general procedure c, previously reported. Reaction was quenched with slush and obtained precipitate was filtered and washed with Et_2O (3 × 10 ml), and dried under vacuum to obtain the title compound **6j** as a white solid.

2-(4-Chlorobenzamido)-5-nitro-*N*-(4-sulfamoylphenethyl)benzamide **6j**: yield 65%; silica gel TLC R_f = 0.60 (Ethyl acetate/*n*-hexane 50% v/v); mp >300 °C; δ_H (400 MHz, DMSO-d_6) 3.03 (2H, t, *J* 6.8), 3.65 (2H, q, *J* 6.8), 7.33 (2H, s, exchange with D_2O, SO_2NH_2), 7.51 (2H, d, *J* 8.4), 7.76 (4H, m), 8.01 (2H, d, *J* 8.4), 8.50 (1H, dd, *J* 2.8, 9.2), 8.78 (1H, d, *J* 2.8), 8.91 (1H, d, *J* 9.2), 9.5 (1H, t, *J* 6.8, exchange with D_2O, N*H*), 13.03 (1H, s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 120.7, 121.3, 124.9, 126.7, 128.5, 130.0, 130.1, 130.2, 133.5, 138.5, 142.5, 143.1, 144.3, 145.9, 164.9, 167.9; *m*/*z* (ESI negative) 501.0 $[M-H]^-$.

4.1.27. Synthesis of 2-(4-fluorobenzamido)-5-nitro-*N*-(4-sulfamoylphenethyl)benzamide 6k



4-(2-Aminoethyl)benzenesulfonamide **5b** (0.15 g, 1.0 equiv) was treated with **4f** (1.0 equiv) in glacial acetic acid (3 ml) according to the general procedure c, previously reported. Reaction was quenched with slush and obtained precipitate was filtered and washed with Et_2O (3 × 10 ml), and dried under vacuum to obtain the title compound **6k** as a white solid.

2-(4-Fluorobenzamido)-5-nitro-*N*-(4-sulfamoylphenethyl)benzamide **6k**: yield 69%; silica gel TLC R_f = 0.24 (Ethyl acetate/*n*-hexane 50% v/v); mp >300 °C; δ_H (400 MHz, DMSO-d_6) 3.03 (2H, t, *J* 6.8), 3.65 (2H, q, *J* 6.8), 7.33 (2H, s, exchange with D_2O, SO_2NH_2), 7.52 (4H, m), 7.80 (2H, d, *J* 8.4), 8.06 (2H, m), 8.50 (1H, dd, *J* 2.8, 9.2), 8.77 (1H, d, *J* 2.8), 8.91 (1H, d, *J* 9.2), 9.49 (1H, t, *J* 6.8, exchange with D_2O, N*H*), 13.00 (1H, s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 117.1 (d, $^2J_{C-F}$ 22), 120.7, 121.3, 124.9, 126.6, 126.7, 128.5, 130.0, 130.1, 130.9 (d, $^3J_{C-F}$ 9), 131.2, 142.5, 143.1, 144.3, 146.1, 164.9, 165.6 (d, $^1J_{C-F}$ 249), 168.0; δ_F (376 MHz, DMSO-d_6) −106.9 (1F, s); *m*/*z* (ESI negative) 485.0 $[M-H]^-$.

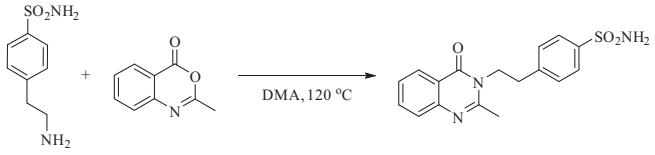
4.1.28. Synthesis of 4-(2-methyl-6-nitro-4-oxoquinazolin-3(4*H*)-yl)benzenesulfonamide 8a

Sulfanilamide **5a** (0.4 g, 1.0 equiv) was treated with **7b** (1.0 equiv) in dry DMA (25 ml) according to general procedure e, previously reported. Reaction quenched with 10 ml of 1 M aqueous HCl, obtained precipitate was filtered and washed with Et_2O

(3 × 10 ml) and dried under vacuum to obtain the title compound **8a** as an orange solid.

4-(2-Methyl-6-nitro-4-oxoquinazolin-3(4*H*)-yl)benzenesulfonamide **8a**: yield 63%, silica gel TLC R_f = 0.12 (Ethyl acetate/*n*-hexane 80% v/v); mp 280–281 °C (decomp.); δ_H (400 MHz, DMSO-d_6) 2.25 (3H, s), 7.60 (2H, s, exchange with D_2O, SO_2NH_2), 7.78 (2H, d, *J* 8.8), 7.93 (1H, d, *J* 8.4), 8.07 (2H, d, *J* 8.8), 8.64 (1H, dd, *J* 2.8, 8.4), 8.84 (1H, d, *J* 2.8); δ_C (100 MHz, DMSO-d_6) 25.3, 121.5, 123.3, 128.1, 129.5, 129.6, 130.1, 140.9, 145.7, 145.8, 152.4, 158.9, 161.5; *m*/*z* (ESI negative) 359.17 $[M-H]^-$.

4.1.29. Synthesis of 4-(2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl) ethyl)benzenesulfonamide 8b

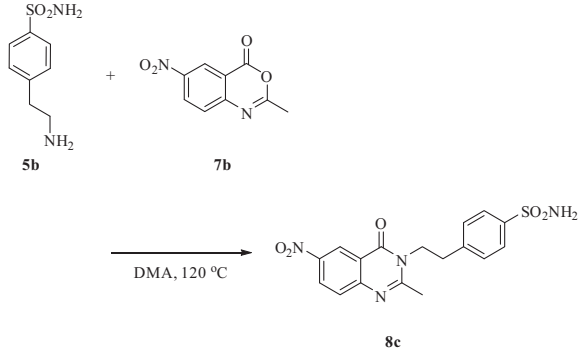


5b **7a** **8b**

4-(2-Aminoethyl)benzenesulfonamide **5b** (0.4 g, 1.0 equiv) was treated with **7a** (1.0 equiv) in dry DMA (20 ml) according to general procedure e, previously reported Reaction quenched with slush, obtained precipitate was filtered and washed with H_2O (3 × 10 ml) and dried under vacuum to obtain the title compound **8b** as a white solid.

4-(2-(2-Methyl-4-oxoquinazolin-3(4*H*)-yl)ethyl)benzenesulfonamide **8b**: yield 45%; silica gel TLC R_f = 0.2 (Ethyl acetate/*n*-hexane 30% v/v); mp 265–266 °C; δ_H (400 MHz, DMSO-d_6) 2.58 (3H, s), 3.11 (2H, t, *J* 8.0), 4.30 (2H, t, *J* 8.0), 7.37 (2H, s, exchange with D_2O, SO_2NH_2), 7.53 (3H, m), 7.63 (1H, d, *J* 8.0), 7.83 (3H, m), 8.17 (1H, d, *J* 8.0); δ_C (100 MHz, DMSO-d_6) 23.7, 34.3, 46.1, 120.9, 126.9, 127.1, 127.2, 127.5, 130.2, 135.3, 143.4, 143.5, 147.9, 155.8, 162.0; *m*/*z* (ESI positive) 344.17 $[M+H]^+$.

4.1.30. Synthesis of 4-(2-(2-methyl-6-nitro-4-oxoquinazolin-3 (4*H*)-yl)ethyl)benzenesulfonamide 8c

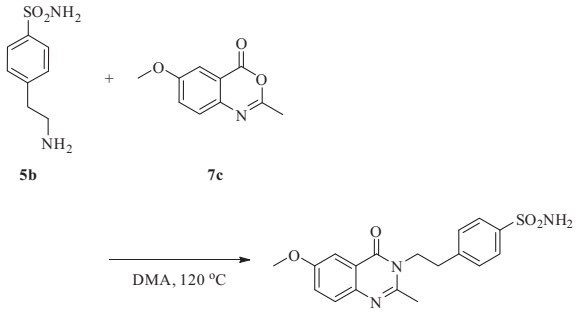


5b **7b** **8c**

4-(2-Aminoethyl)benzenesulfonamide **5b** (0.1 g, 1.0 equiv) was treated with **7b** (1.0 equiv) in dry DMA (6 ml) according to general procedure e, previously reported Reaction was quenched with slush, extracted with ethyl acetate (3 × 20 ml) and the combined organic layers were washed with H_2O (3 × 20 ml), dried over Na_2SO_4, filtered and concentrated in vacuum to give desired compound **8c** as a yellow solid.

4-(2-(2-Methyl-6-nitro-4-oxoquinazolin-3(4*H*)-yl)ethyl)benzenesulfonamide **8c**: yield 65%, silica gel TLC R_f = 0.13 (Ethyl acetate/*n*-hexane 80% v/v); 263–264 °C (decomp.); δ_H (400 MHz, DMSO-d_6) 2.64 (3H, s), 3.13 (2H, t. *J* 8.0), 4.33 (2H, t, *J* 8.0), 7.37 (2H, s, exchange with D_2O, SO_2NH_2), 7.53 (2H, d, *J* 8.4), 7.82 (3H, m), 8.57 (1H, dd, *J* 2.8, 9.2), 8.87 (1H, d, *J* 2.8); δ_C (100 MHz, DMSO-d_6) 24.0, 33.9, 46.5, 120.8, 123.3, 126.9, 129.2, 129.3, 130.3, 143.1, 143.6, 145.6, 151.9, 160.0, 161.3; *m*/*z* (ESI positive) 389.17 $[M+H]^+$.

4.1.31. Synthesis of 4-(2-(6-methoxy-2-methyl-4-oxoquinazolin -3(4*H*)-yl)ethyl)benzenesulfonamide 8d

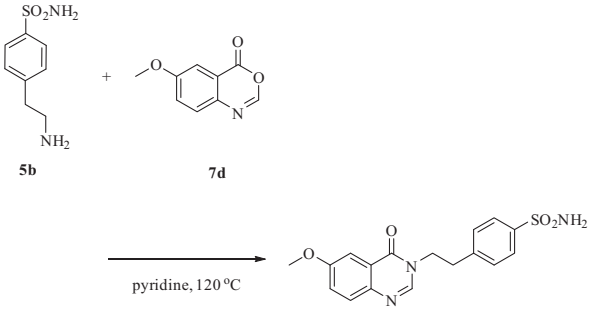


5b **7c** **8d**

4-(2-Aminoethyl)benzenesulfonamide **5b** (0.5 g, 1.0 equiv) was treated with **7c** (1.0 equiv) in dry DMA (20 ml) according to general procedure e, previously reported Reaction quenched with slush and acidified with 1 M aqueous HCl, obtained precipitate was filtered and washed with Et_2O (3 × 10 ml) and then filtered and dried under vacuum to obtain title compound **8d** as a beige solid.

4-(2-(6-Methoxy-2-methyl-4-oxoquinazolin-3(4*H*)-yl)ethyl) benzenesulfonamide **8d**: yield 40%, silica gel TLC R_f = 0.2 (Ethyl acetate/*n*-hexane 80% v/v); mp 245–246 °C; δ_H (400 MHz, DMSO-d_6) 2.55 (3H, s), 3.10 (2H, t, *J* 8.0), 3.91 (3H, s), 4.30 (2H, t, *J* 8.0), 7.37 (2H, s, exchange with D_2O, SO_2NH_2), 7.45 (1H, dd, *J* 2.8, 8.8), 7.51 (2H, d, *J* 8.0), 7.54 (1H, d, *J* 2.8), 7.58 (1H, d, *J* 8.8), 7.81 (2H, d, *J* 8.0); δ_C (100 MHz, DMSO-d_6) 23.4, 34.3, 46.2, 56.5, 106.9, 121.6, 124.9, 126.8, 129.2, 130.2, 142.5, 143.4, 143.5, 153.3, 158.3, 161.8; *m*/*z* (ESI positive) 374.25 $[M+H]^+$.

4.1.32. Synthesis of 4-(2-(6-methoxy-4-oxoquinazolin-3(4*H*)-yl) ethyl)benzenesulfonamide 8e



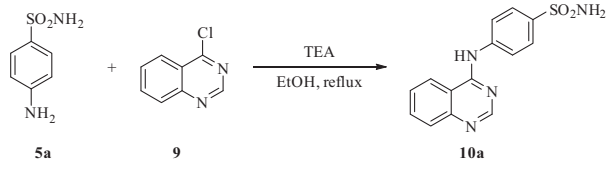
5b **7d** **8e**

4-(2-Aminoethyl)benzenesulfonamide **5b** (0.3 g, 1.0 equiv) was treated with **7d** (1.0 equiv) in dry pyridine (10 ml) according to general procedure e. Reaction quenched with slush and neutralized

with 1 M aqueous HCl solution, extracted with ethyl acetate (3 × 20 ml) and the combined organic layers were washed with H_2O (3 × 20 ml), dried over Na_2SO_4, filtered and concentrated in vacuum to give title compound **8e** as a beige solid.

4-(2-(6-Methoxy-4-oxoquinazolin-3(4*H*)-yl)ethyl)benzenesulfonamide **8e**: yield 60%, silica gel TLC R_f = 0.11 (Ethyl acetate/*n*-hexane 80% v/v); mp 244–245 °C; δ_H (400 MHz, DMSO-d_6) 3.15 (2H, t, *J* 7.6), 3.92 (3H, s), 4.29 (2H, t, *J* 7.6), 7.35 (2H, s, exchange with D_2O, SO_2NH_2), 7.46 (3H, m), 7.59 (1H, d, *J* 3.2), 7.64 (1H, d, *J* 8.8), 7.78 (2H, d, *J* 8.4), 8.13 (1H, s); δ_C (100 MHz, DMSO-d_6) 34.9, 47.8, 56.6, 106.9, 123.3, 124.7, 126.8, 129.8, 130.2, 143.0, 143.2, 143.4, 146.5, 158.9, 160.8; *m*/*z* (ESI positive) 360.2 $[M+H]^+$.

4.1.33. Synthesis of 4-(quinazolin-4-ylamino)benzenesulfonamide 10a



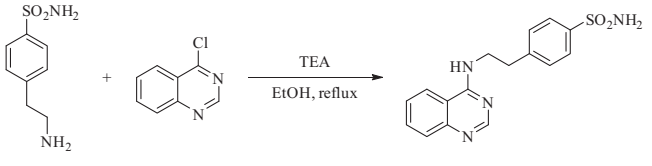
5a 9 10a

A solution of sulfanilamide **5a** (0.2 g, 1.0 equiv) in EtOH (10 ml) was treated with 4-chloroquinazoline **9** (1.0 equiv), followed by the addition of TEA (1.2 equiv). The reaction mixture was treated according to the general procedure f, to obtain the title compound **10a** as a white solid.

4-(Quinazolin-4-ylamino)benzenesulfonamide **10a**: yield 63%, silica gel TLC R_f = 0.18 (Ethyl acetate/*n*-hexane 30% v/v); mp 195–196 °C; δ_H (400 MHz, DMSO-d_6) 7.48 (2H, s, exchange with D_2O, SO_2NH_2), 7.96 (6H, m), 8.16 (1H, t, *J* 8.0), 8.88 (1H, d, *J* 8.0), 9.02 (1H, s), 11.59 (1H, br s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 115.1, 121.6, 126.0, 126.8, 128.4, 131.2, 138.7, 140.3, 141.3, 142.9, 152.6, 161.9; *m*/*z* (ESI positive) 301.1 $[M+H]^+$.

Experimental in agreement with reported data.[34b]

4.1.34. Synthesis of 4-(2-(quinazolin-4-ylamino)ethyl)benzenesulfonamide 10b

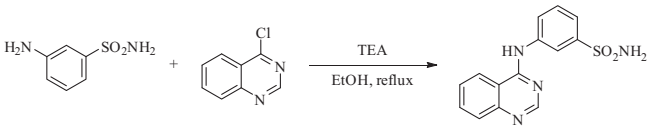


5b 9 10b

A solution of 4-(2-aminoethyl)benzenesulfonamide **5b** (0.2 g, 1.0 equiv) in EtOH (10 ml) was treated with 4-chloroquinazoline **9** (1.0 equiv), followed by the addition of TEA (1.2 equiv). The reaction mixture was treated according to the general procedure f, to obtain the title compound **10b** as a white solid.

4-(2-(Quinazolin-4-ylamino)ethyl)benzenesulfonamide **10b**: yield 63%; silica gel TLC R_f = 0.1 (Ethyl acetate/*n*-hexane 80% v/v); mp 215–216 °C (decomp.); δ_H (400 MHz, DMSO-d_6) 3.17 (2H, t, *J* 8.0), 4.02 (2H, q, *J* 8.0), 7.35 (2H, s, exchange with D_2O, SO_2NH_2),7.51 (2H, d, *J* 8.4), 7.78 (2H, d, *J* 8.4), 7.82 (1H, t, *J* 8.0), 7.92 (1H, d, *J* 8.0), 8.08 (1H, t, *J* 8.0), 8.63 (1H, d, *J* 8.0), 8.95 (1H, s), 10.59 (1H, t, *J* 8.0, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 34.5, 43.6, 113.9, 120.1, 125.6, 126.7, 129.3, 130.1, 136.8, 138.3, 143.3, 143.8, 151.7, 161.4; *m*/*z* (ESI positive) 329.0 $[M+H]^+$.

4.1.35. Synthesis of 3-(quinazolin-4-ylamino)benzenesulfonamide 10c

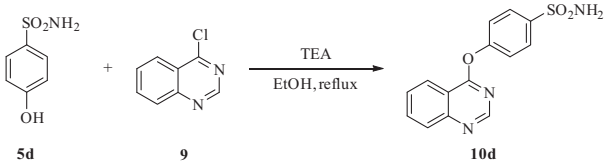


5c 9 10c

A solution of 3-aminobenzenesulfonamide **5c** (0.2 g, 1 equiv) was treated with 4-chloroquinazoline **9** (1.0 equiv) in EtOH (10 ml), followed by the addition of TEA (1.2 equiv).The reaction mixture was treated according to the general procedure f, previously reported, to give the title compound **10c** as a white solid.

3-(Quinazolin-4-ylamino)benzenesulfonamide **10c**: yield 63%; mp >300 °C; δ_H (400 MHz, DMSO-d_6) 7.44 (2H, s, exchange with D_2O, SO_2NH_2), 7.64 (2H, m), 7.72 (1H, t, *J* 8.0), 7.87 (1H, d, *J* 8.0), 7.94 (1H, d, *J* 8.0), 8.22 (1H, m), 8.44 (1H, s), 8.64 (1H, d, *J* 8.0), 8.70 (1H, s,), 10.10 (1H, s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 116.6, 121.0, 122.8, 124.5, 127.5, 128.8, 129.1, 131.3, 135.6, 141.0, 145.5, 150.8, 155.9, 159.6; *m*/*z* (ESI positive) 301.1 $[M+H]^+$.

4.1.36. Synthesis of 4-(quinazolin-4-yloxy)benzenesulfonamide 10d



5d 9 10d

A solution of 4-hydroxybenzenesulfonamide **5d** (0.2 g, 1 equiv) was treated with 4-chloroquinazoline **9** (1.0 equiv) in EtOH (10 ml), followed by the addition of TEA (1.2 equiv).The reaction mixture was treated according to the general procedure f, to give the title compound **10d** as a white solid.

4-(Quinazolin-4-yloxy)benzenesulfonamide **10d**: yield 34%;silica gel TLC R_f = 0.12 (Ethyl acetate/*n*-hexane 50% v/v); mp 212–213 °C; δ_H (400 MHz, DMSO-d_6) 7.49 (2H, s, exchange with D_2O, SO_2NH_2), 7.62 (2H, d, *J* 8.4), 7.86 (1H, t, *J* 7.6), 7.99 (2H, d, *J* 8.4), 8.10 (2H, m), 8.45 (1H, d, *J* 7.6), 8.79 (1H, s); δ_C (100 MHz, DMSO-d_6) 117.1, 124.5, 125.1, 128.9, 129.4, 130.3, 136.9, 142.7, 152.5, 155.3, 156.3, 167.9; *m*/*z* (ESI positive) 302.1 $[M+H]^+$.

4.2. CA inhibition

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity.[25] 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,[35–37] and represent the mean from at least three different determinations. All CA isoforms were recombinant ones obtained in-house as reported earlier.[35–37] The enzyme concentrations in the assay system were: 8.3 nM for hCA I; 4.8 nM for hCA II; 5.1 nM for hCA IX and 10.4 nM for hCA XII. When highly potent inhibitors were detected, the enzyme concentrations were lowered up to the pM range and the time to register the reaction increased. This allowed the calculation of inhibition constants in the picomolar range.

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