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Synthesis 4-[2-(2-mercapto-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamides with subnanomolar carbonic anhydrase II and XII inhibitory properties

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Abstract. Condensation of substituted anthranilic acids with 4-isothiocyanatoethyl-benzenesulfonamide led to series of heterocyclic benzenesulfonamides incorporating 2-mercapto-quinazolin-4-one tails. These sulfonamides were investigated as inhibitors of the human carbonic anhydrase (hCA, EC 4.2.1.1) isoforms hCA I and II (cytosolic isozymes), as well as hCA XII (a transmembrane, tumor-associated enzyme also involved in glaucoma-genesis). The new sulfonamides acted as medium potency inhibitors of hCA I (K_Is of 28.5 – 2954 nM), being highly effective as hCA II (K_Is in the range of 0.62 – 12.4 nM) and XII (K_Is of 0.54 – 7.11 nM) inhibitors. All substitution patterns present in these compounds (e.g., halogens, methyl and methoxy moieties, in positions 6, 7 and/or 8 of the 2-mercapto-quinazolin-4-one ring) led to highly effective hCA II/XII inhibitors. These compounds should thus be of interest as preclinical candidates in pathologies in which the activity of these enzymes should be inhibited, such as glaucoma (CA II and XII as targets) or some tumors in which the activity of isoforms CA II and XII is dysregulated.

Keywords: carbonic anhydrase; sulfonamide; inhibitor; 2-mercapto-quinazolin-4-one; tail approach

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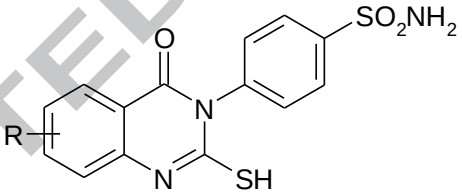
1. Introduction

In a previous work from our groups[1] we reported a series of benzenesulfonamides obtained from sulfanilamide, which was converted to the corresponding 4-isothiocyanato benzenesulfonamide, and reacted with anthranilic acid derivatives, leading thus to 2-mercapto-3H-quinazolin-4-ones. These sulfonamides possessed excellent inhibitory activity against the metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1). Several CA isoforms (of the 15 presently known in humans)[2,3] were inhibited in the low nanomolar range with some of these compounds, among which the human (h) hCA II and XII (involved in glaucoma)[2,3] as well as hCA IX and XII (transmembrane isoforms involved in tumorigenesis).[4-6] Although no crystal structures for adducts of 2-mercapto-3H-quinazolin-4-one-benzenesulfonamides (such as **A-C**, Fig. 1) with any CA isozymes are available to date, we hypothesized that the presence of the substituted-mercaptoquinazolinone tails in their scaffolds exerted a beneficial effect on the interaction with the enzyme active site, and thus decided to further investigate this type of tail for designing sulfonamide CA inhibitors (CAIs). Indeed, pharmacological agents belonging to this class are clinically used as diuretics,[7] antiglaucoma,[8,9] antiepileptic,[10,11] antiobesity,[12] and more recently antitumor agents,[4-6] with one such derivative, SLC-0111 in Phase Ib clinical trials for the treatment of metastatic hypoxic cancers.[13,14]



A: R = 8-Cl, K_I (hCA II) = 0.31 nM; K_I (hCA XII) = 5.5 nM

B: R = 6-Me, K_I (hCA II) = 0.25 nM; K_I (hCA XII) = 5.2 nM

C: R = 7,8-$(MeO)_2$, K_I (hCA II) = 0.61 nM; K_I (hCA XII) = 0.67 nM

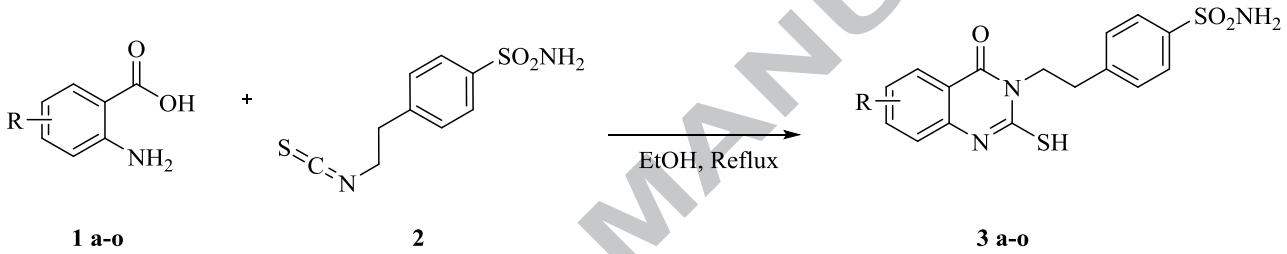
Fig. 1: Sulfonamides **A-C** incorporating 2-mercapto-3H-quinazolin-4-one functionalities and their hCA II and XII inhibitory properties.[1]

2. Results and discussion

2.1. Chemistry

The drug design strategy for obtaining novel sulfonamides used in this work is based on the tail approach developed by one of our groups earlier.[15] Substituted anthranilic acids **1a-o** and 4-isothiocyanatoethyl-benzenesulfonamide **2** have been employed for preparing the new sulfonamides **3a-o** (Scheme 1) by a chemistry already reported in the previous work, in which the derivatives

without the ethylene spacer, of types **A-C**, have been reported.[1,16] In fact we have prepared a large number of thioureas incorporating benzenesulfonamide functionalities, by reaction of aromatic sulfonamides incorporating isothiocyanato moieties with amines (aromatic, aliphatic or heterocyclic),[17b] amino acids,[17a] and oligopeptides.[17c] Many of the derivatives obtained by such procedures (incorporating the NH-CS-NH-C_6H_4-SO_2NH_2 functionality) showed excellent CA inhibitory properties against isoforms such as hCA II, IV, IX and XII,[17] involved in serious pathologies, such as glaucoma[2,3] and cancer.[4,5] Furthermore, some of these sulfonamides also showed good degrees of isoform selectivity inhibitory profiles.[17] Thus, in this work we extend our previous investigations[1,17] on the use of 4-isothiocyanatoethyl-benzenesulfonamide for obtaining CAIs incorporating novel heterocyclic scaffolds (Scheme 1).



Scheme 1. General synthetic procedure for compounds **3a-o**.

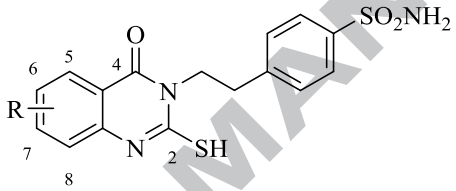
The reaction of the substituted anthranilic acid **1a-o** with the isothiocyanate **2** initially led to an *ortho*-carboxyphenyl-thiourea, which has not been isolated, as in the reaction conditions we used it underwent cyclisation with the formation of mercapto-quinazolinones **3a-o** (Scheme 1). The chemical diversity of the obtained series was achieved by using variously substituted-anthranilic acids which incorporated halogens (F, Cl, Br, I), methyl and methoxy moieties (one, two or three such moieties) in their molecules, similar with the sulfonamides reported in the previous work[1] (Table 1).

The structure of the new compounds was confirmed by means of NMR, MS and elemental analyses (see Experimental for details). The ^{1}H-NMR spectra showed D_2O exchangeable singlet peak corresponding to the SH group at around 13.0 ppm, D_2O exchangeable peaks due to the SO_2NH_2 protons at around 7.50 ppm. The aromatic protons were also detected at their expected respected regions. The ^{13}C-NMR spectra were also in agreement with the suggested structure of the target compounds (see Experimental for details).The mass spectra (MS) showed the exact mass ion for each compound and the corresponding isotopes when halogens were present in the new compounds (see Experimental for details).

2.2. Inhibition of CAs

We investigated the inhibition profile of sulfonamides **3a-3o** against the isoforms hCA I and II (cytosolic, off-target isoforms when considering the anti-cancer applications of CAIs, but target isoforms for glaucoma)[18] and hCA XII (predominantly present in tumors but also overexpressed in glaucomatous eyes).[8] For comparison reasons we also discuss the inhibition profiles of the standard, clinically used sulfonamide drug acetazolamide (5-acetamido-1,3,4-thiadiazole-2-sulfonamide, **AAZ**) against the same isoforms (Table 1).

Table 1: Inhibition data of human CA isoforms hCA I, II and XII with sulfonamides **3a-o** reported here and the standard sulfonamide inhibitor acetazolamide (**AAZ**) by a stopped flow CO_2 hydrase assay.[18]



3a-o

Compound	R	K_I (nM) hCAI	hCA II	hCA XII
3a	H	31.5	0.62	0.59
3b	6-F	212	12.4	0.62
3c	7-F	39.9	9.1	0.64
3d	6-Cl	153	6.5	0.54
3e	8-Cl	41.4	0.78	0.66
3f	7-Br	104	0.91	1.45
3g	6-I	34.3	0.71	0.76
3h	6-CH_3	296	56.2	5.53
3i	8-CH_3	2954	1.2	0.61
3j	6,8-CH_3	1402	0.86	0.73
3k	6-OCH_3	150	1.0	0.54
3l	7-OCH_3	28.5	0.69	0.47
3m	8-OCH_3	42.3	31.9	5.74
3n	6,7-OCH_3	678	1.1	0.65

3o	6,7,8-OCH_3	268	10.7	7.11
AAZ	-	250	12	5.7

* Mean from 3 different assays, by a stopped flow technique (errors were in the range of ± 5-10 % of the reported values).

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

(i) hCA I, the slow, off-target cytosolic isoform was inhibited by all sulfonamides **3a-3o** with inhibition constants ranging between 28.5 and 2954 nM. Some derivatives showed good inhibitory properties, such as **3a, 3c, 3e, 3g, 3l** and **3m**, with K_Is ranging between 28.5 and 42.3 nM. They incorporated either H at the quinazolinone ring (**3a**) or 7-F, 8-Cl, 6-I, 7-MeO and 8-MeO substituents. Interestingly, small structural changes (such as the shift of the fluorine from the 7 to the 6 position of the heterocyclic ring, e.g., in **3b**), led to important differences of activity with **3b**, which resulted 5.3 fold weaker inhibitor of hCA I when compared to **3c** (Table 1). The same is true for the isomers **3d-3e**, **3k-3l**, etc. Some of these derivatives, such as **3b, 3d, 3f, 3h, 3k** and **3o** showed medium potency hCA I inhibition, in the same range as acetazolamide (K_I = 250 nM), with inhibition constants in the range of 104 – 268 nM. Three derivatives, **3i, 3j** and **3n** were even weaker inhibitors, with K_Is ranging between 678 and 2954 nM. Thus, the main SAR conclusion is that the type and, more importantly, the position of the R substituent in the quinazolinone ring have great influence the hCA I inhibitory properties.

(ii) SAR was even simpler for the inhibition of the physiologically dominant cytosolic isoform hCA II (Table 1). Thus, all the new sulfonamides **3a-3o** were highly effective inhibitors with two of them having K_Is in the range of 31.9 – 56.2 nM (compounds **3h** and **3m**) whereas the remaining ones acting as very string inhibitors with K_Is in the range of 0.62 – 12.4 nM (as effective as **AAZ** or one order of magnitude better inhibitors compared to this clinically used compound). Thus, basically all substitution patterns present in these sulfonamides led to effective hCA II inhibitors except the 6-Me and 8-MeO groups which led to slightly weaker inhibitors.

(iii) As hCA II, also the transmembrane isoform hCA XII was highly inhibited by all the new sulfonamides reported here with K_Is in the range of 0.54 – 7.11 nM. The SAR is thus very flat and all the substitution patterns from the new sulfonamides were highly beneficial for inducing high affinity for hCA II (Table 1).

3. Conclusions

Condensation of substituted anthranilic acids with 4-isothiocyanatoethyl-benzenesulfonamide led to series of heterocyclic benzenesulfonamides incorporating 2-mercapto-quinazolin-4-one tails. These sulfonamides were investigated as inhibitors of the following isoforms: hCA I and II (cytosolic isozymes), as well as hCA XII (a trans-membrane, tumor-associated enzyme also involved in glaucomagenesis). The new sulfonamides acted as medium potency inhibitors of hCA I (K_Is of 28.5 – 2954 nM), being highly effective as hCA II (K_Is in the range of 0.62 – 12.4 nM) and XII (K_Is of 0.54 – 7.11 nM) inhibitors. All substitution patterns present in these compounds (e.g., halogens, methyl and methoxy moieties, in positions 6, 7 and/or 8 of the 2-mercapto-quinazolin-4-one ring) led to highly effective hCA II/XII inhibitors. These compounds should thus be of interest as preclinical candidates in pathologies in which the activity of these enzymes should be inhibited, such as glaucoma (CA II and XII as targets) or some tumors in which the expression of isoforms CA II and XII is dysregulated.

4. Experimental protocols

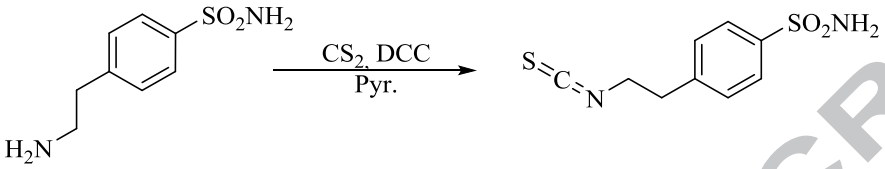
4.1. Chemistry. Anhydrous solvents and all reagents were purchased from Sigma-Aldrich, Alfa Aesar and TCI. All reactions involving air- or moisture-sensitive compounds were performed under a nitrogen atmosphere using dried glassware and syringes techniques to transfer solutions. Nuclear magnetic resonance (^{1}H-NMR, ^{13}C-NMR) 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; t, triplet; 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. Melting points (mp) were measured in open capillary tubes with a Gallenkamp MPD350.BM3.5 apparatus and are uncorrected.

General procedure for the synthesis of benzenesulfonamides **3a-o**.[1]

The commercially available 2-aminobenzoic acid derivatives **1a-o** (1.0 eq) and isothiocyanato-benzenesulfonamide **2** (1.0 eq) were suspended in EtOH, and the reaction was refluxed until starting materials were consumed (TLC monitoring). Then the mixture was cooled down to r.t. and the precipitate formed was collected by filtration to give a residue that was purified by silica gel column

chromatography eluting with the appropriate ethyl acetate/*n*-hexane solution or by trituration from diethyl ether to afford the desired compounds **3a-o**.

Synthesis of 4-(2-isothiocyanato-ethyl)-benzenesulfonamide **(2)**.[2]

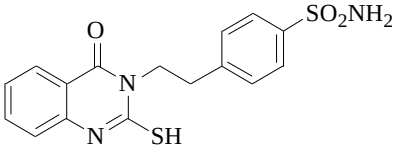


2

A suspension of ethylaminobenezene sulfonamide (1.0g, 1.0 eq) in dry pyridine (2.0 ml) was cooled to -10 °C and then treated with CS_2 (5.0 g, 13.2 eq) and DCC (1.0g, 1.0 eq). The reaction mixture was stirred at r.t. under a nitrogen atmosphere until consumption of the starting material (TLC monitoring). Then the solvent was removed under *vacuo* to give a pale yellow solid that was crystallized from acetone. The solution was recovered, concentrated under *vacuo* and the residue obtained was purified by silica gel column chromatography eluting with 5% MeOH/DCM to afford the titled compound as a pale yellow solid.

4-(2-Isothiocyanato-ethyl)-benzenesulfonamide **2**: 52% yield; silica gel TLC R_f 0.17 (MeOH/DCM 5 % *v/v*); m.p. 142°C (lit.[2] 146°C); δ_H (400 MHz, DMSO-d_6) 3.08 (2H, t, *J* 6.8, N-CH_2-CH_2-), 4.00 (2H, t, *J* 6.8, N-CH_2-CH_2-), 7.37 (2H, s, exchange with D_2O, SO_2NH_2), 7.52 (2H, d, *J* 8.4, Ar-H), 7.82 (1H, d, *J* 8.4, Ar-H); δ_C (100 MHz, DMSO-d_6) 35.8, 46.4, 126.7, 128.8 (-N=*C*=S), 130.3, 142.7, 143.6; Elemental analysis: calc: C 44.61, H 4.16, N 11.56, S 26.47; found: C 44.65, H 4.15, N 11.59, S 26.44; *m/z* (ESI positive) 243.02 $[M+H]^+$.
Experimental data are in agreement with reported data.[2]

Synthesis of 4-[2-(2-mercapto-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide (**3a).**



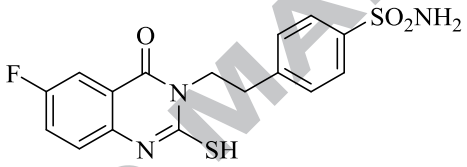
3a

2-Aminobenzoic acid **1a** (0.1g, 1.0 eq) and 4-(2-isothiocyanato-ethyl)-benzenesulfonamide **2** (0.17g, 1.0 eq) were treated in EtOH (10 ml) at reflux for 5h according to the general procedure

previously described. Then the solvent was removed under *vacuo* to give a residue that was triturated from diethyl ether to afford the title compound **3a** as a white solid

4-[2-(2-Mercapto-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide **3a**: 74% yield; silica gel TLC R_f 0.21 (Ethyl acetate/*n*-hexane 40 % *v/v*); m.p. 274°C sub.; δ_H (400 MHz, DMSO-d_6) 3.10 (2H, m, N-CH_2-CH_2-), 4.65 (2H, m, N-CH_2-CH_2-), 7.33 (2H, s, exchange with D_2O, SO_2NH_2), 7.39 (2H, m, Ar-H), 7.53 (2H, d, *J* 8.4, Ar-H), 7.78 (3H, m, Ar-H), 8.02 (1H, d, *J* 8.4, Ar-H), 13.05 (1H, s, exchange with D_2O, -S*H*); δ_C (100 MHz, DMSO-d_6) 33.0, 47.5, 116.4, 116.6, 125.5, 126.9, 128.2, 130.0, 136.4, 140.0, 143.3, 143.6, 160.1, 175.8; Elemental analysis: calc: C 53.17, H 4.18, N 11.63, S 17.7; found: C 53.20, H 4.15, N 11.62, S 17.9; *m/z* (ESI positive) 361.06 $[M+H]^+$.

Synthesis of 4-[2-(6-fluoro-2-mercapto-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide **(3b)**

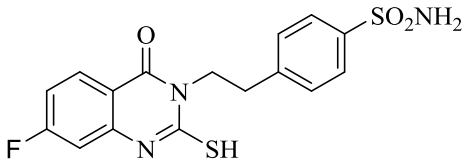


3b

2-Amino-5-fluorobenzoic acid **1b** (0.1g, 1.0 eq) and 4-(2-isothiocyanato-ethyl)-benzenesulfonamide **2** (0.16g, 1.0 eq) were treated in EtOH (10 ml) at reflux O.N. according to the general procedure previously described. The precipitate formed was collected by filtration and triturated from diethyl ether to afford the title compound **3b** as a white solid.

4-[2-(6-Fluoro-2-mercapto-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide **3b**: 80% yield; silica gel TLC R_f 0.19 (Ethyl acetate/*n*-hexane 50 % *v/v*); m.p. >310°C; δ_H (400 MHz, DMSO-d_6) 3.10 (2H, t, *J* 5.6, N-CH_2-CH_2-), 4.64 (2H, t, *J* 5.6, N-CH_2-CH_2-), 7.35 (2H, s, exchange with D_2O, SO_2NH_2), 7.49 (1H, m, Ar-H), 7.52 (2H, d, *J* 8.4, Ar-H), 7.70 (1H, m, Ar-H), 7.72 (1H, d, *J* 8.1, Ar-H), 7.82 (2H, d, *J* 8.4, Ar-H), 13.13 (1H, s, exchange with D_2O, -S*H*); δ_F (376 MHz, DMSO-d_6) -116.43 (1F, s); δ_C (100 MHz, DMSO-d_6) 32.9, 47.6, 113.1 (d, $^2J_{C\text{-}F}$ 24), 117.7 (d, $^3J_{C\text{-}F}$ 8), 119.3 (d, $^3J_{C\text{-}F}$ 8), 124.7 (d, $^2J_{C\text{-}F}$ 24), 126.9, 130.0, 136.9, 143.3, 143.5, 159.3 (d, $^1J_{C\text{-}F}$ 241), 159.5 (d, $^4J_{C\text{-}F}$ 3), 175.3; Elemental analysis: calc: C 50.65, H 3.72, N 11.07, S 16.90; found: C 50.66, H 3.70, N 11.10, S 16.78; *m/z* (ESI positive) 380.06 $[M+H]^+$.

Synthesis of 4-(2-(7-fluoro-2-mercapto-4-oxoquinazolin-3(4*H*)-yl)ethyl)benzenesulfonamide **(3c)**

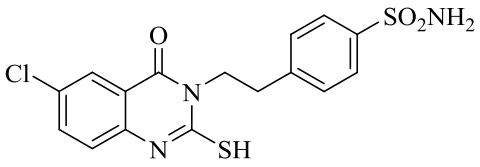


3c

2-Amino-4-fluorobenzoic acid **1c** (0.1g, 1.0 eq) and 4-(2-isothiocyanato-ethyl)-benzenesulfonamide **2** (0.16g, 1.0 eq) were treated in EtOH (10 ml) at reflux O.N. according to the general procedure previously described. The precipitate formed was collected by filtration and triturated from diethyl ether to afford the title compound **3c** as a white solid.

4-[2-(7-Fluoro-2-mercapto-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide **3c**: 78% yield; silica gel TLC R_f 0.17 (Ethyl acetate/*n*-hexane 40 % *v/v*); m.p. 246°C; δ_H (400 MHz, DMSO-d_6) 3.09 (2H, t, *J* 5.6, N-CH_2-CH_2-), 4.62 (2H, t, *J* 5.6, N-CH_2-CH_2-), 7.14 (1H, dd, *J* 8.1, 2.2, Ar-H), 7.23 (1H, m, Ar-H), 7.35 (2H, s, exchange with D_2O, SO_2NH_2), 7.52 (2H, d, *J* 8.4, Ar-H), 7.83 (2H, d, *J* 8.4, Ar-H), 8.08 (1H, m, Ar-H), 13.09 (1H, s, exchange with D_2O, -S*H*); δ_F (376 MHz, DMSO-d_6) -101.87 (1F, s); δ_C (100 MHz, DMSO-d_6) 33.0, 47.5, 102.7 (d, $J_{C\text{-}F}$ 27), 113.7 (d, $J_{C\text{-}F}$ 12), 113.9, 127.0, 130.1, 131.9, 141.8 (d, $J_{C\text{-}F}$ 13), 143.5 (d, $J_{C\text{-}F}$ 23), 159.5, 176.4; Elemental analysis: calc: C 50.65, H 3.72, N 11.07, S 16.90; found: C 50.68, H 3.69, N 11.11, S 16.85; *m/z* (ESI positive) 380.06 $[M+H]^+$.

Synthesis of 4-[2-(6-chloro-2-mercapto-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide (**3d)**

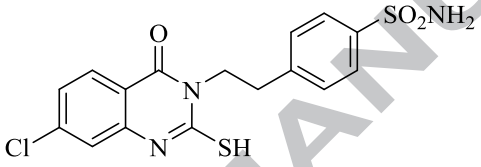


3d

2-Amino-5-chlorobenzoic acid **1d** (0.1g, 1.0 eq) and 4-(2-isothiocyanato-ethyl)-benzenesulfonamide **2** (0.14g, 1.0 eq) were treated in EtOH (10 ml) at reflux O.N. according to the general procedure previously described. The precipitate formed was collected by filtration and triturated from diethyl ether to afford the title compound **3d** as a light-brown solid.

4-[2-(6-Chloro-2-mercapto-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide **3d**: 68% yield; silica gel TLC R_f 0.21 (Ethyl acetate/*n*-hexane 50 % *v/v*); m.p. >310°C; δ_H (400 MHz, DMSO-d_6) 3.09 (2H, t, *J* 5.6, N-CH_2-CH_2-), 4.63 (2H, t, *J* 5.6, N-CH_2-CH_2-), 7.35 (2H, s, exchange with D_2O, SO_2NH_2), 7.45 (1H, d, *J* 8.1, Ar-H), 7.52 (2H, d, *J* 8.4, Ar-H), 7.83 (2H, d, *J* 8.4, Ar-H), 7.84 (1H, d, *J* 8.1, Ar-H), 7.95 (1H, s, Ar-H), 13.16 (1H, s, exchange with D_2O, -S*H*); δ_C (100 MHz, DMSO-d_6) 32.9, 47.7, 117.9, 119.0, 127.0, 127.2, 129.4, 130.1, 136.5, 138.9, 143.4,. 143.6, 159.3, 175.8; Elemental analysis: calc: C 48.54, H 3.56, N 10.61, S 16.20; found: C 48.56, H 3.58, N 10.57, S 16.17; *m/z* (ESI positive) 396.02 $[M+H]^+$.

Synthesis of 4-[2-(7-chloro-2-mercapto-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide (**3e)**

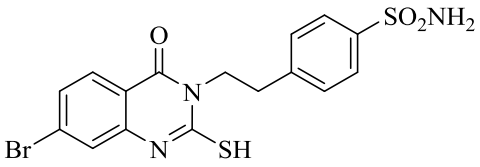


3e

2-Amino-4-chlorobenzoic acid **1e** (0.1g, 1.0 eq) and 4-(2-isothiocyanato-ethyl)-benzenesulfonamide **2** (0.14g, 1.0 eq) were treated in EtOH (10 ml) at reflux O.N. according to the general procedure previously described. The precipitate formed was collected by filtration and triturated from diethyl ether to afford the title compound **3e** as a white solid.

4-[2-(7-Chloro-2-mercapto-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide **3e**: 65% yield; silica gel TLC R_f 0.25 (Ethyl acetate/*n*-hexane 50 % *v/v*); m.p. >310°C; δ_H (400 MHz, DMSO-d_6) 3.09 (2H, t, *J* 5.6, N-CH_2-CH_2-), 4.63 (2H, t, *J* 5.6, N-CH_2-CH_2-), 7.35 (2H, s, exchange with D_2O, SO_2NH_2), 7.42 (2H, m, Ar-H), 7.52 (2H, d, *J* 8.4, Ar-H), 7.80 (2H, d, *J* 8.4, Ar-H), 8.00 (1H, s, Ar-H), 13.08 (1H, s, exchange with D_2O, -S*H*); δ_C (100 MHz, DMSO-d_6) 33.6, 48.2, 116.1, 116.6, 126.4, 127.6, 130.7, 131.2, 141.6, 141.6, 144.0, 144.3, 160.3, 177.0; Elemental analysis: calc: C 48.54, H 3.56, N 10.61, S 16.20; found: C 48.59, H 3.60, N 10.58, S 16.16; *m/z* (ESI positive) 396.02 $[M+H]^+$.

Synthesis of 4-[2-(7-Bromo-2-mercapto-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide **(3f)**

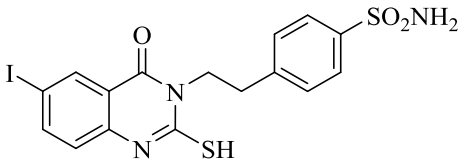


3f

2-Amino-4-bromobenzoic acid **1f** (0.1g, 1.0 eq) and 4-(2-isothiocyanato-ethyl)-benzenesulfonamide **2** (0.11g, 1.0 eq) were treated in EtOH (10 ml) at reflux O.N. according to the general procedure previously described. The precipitate formed was collected by filtration and triturated from diethyl ether to afford the title compound **3f** as a white solid.

4-[2-(7-Bromo-2-mercapto-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide **3f**: 55% yield; silica gel TLC R_f 0.40 (Ethyl acetate/*n*-hexane 50 % *v/v*); m.p. 307°C; δ_H (400 MHz, DMSO-d_6) 3.07 (2H, t, *J* 5.6, N-CH_2-CH_2-), 4.62 (2H, t, *J* 5.6, N-CH_2-CH_2-), 7.33 (2H, s, exchange with D_2O, SO_2NH_2), 7.54 (3H, m, Ar-H), 7.60 (1H, s, Ar-H), 7.79 (2H, d, *J* 8.4, Ar-H), 7.90 (1H, d, *J* 8.1, Ar-H), 13.07 (1H, s, exchange with D_2O, -S*H*); δ_C (100 MHz, DMSO-d_6) 34.7, 49.5, 119.0, 125.0, 126.4, 128.0, 128.3, 130.0, 131.0, 141.0, 143.0, 149.1, 159.3, 177.1. Elemental analysis: calc: C 43.64, H 3.20, N 9.54, S 14.56; found: C 43.66, H 3.25, N 9.50, S 14.52; *m/z* (ESI positive) 441.34 $[M+H]^+$.

Synthesis of 4-[2-(6-iodo-2-mercapto-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide **(3g)**



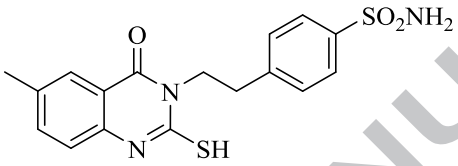
3g

2-Amino-5-iodobenzoic acid **1g** (0.1g, 1.0 eq) and 4-(2-isothiocyanato-ethyl)-benzenesulfonamide **2** (0.09g, 1.0 eq) were treated in EtOH (10 ml) at reflux O.N. according to the general procedure previously described. The precipitate formed was collected by filtration and triturated from diethyl ether to afford the title compound **3g** as a white solid.

4-[2-(6-Iodo-2-mercapto-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide **3g**: 68% yield; silica gel TLC R_f 0.12 (Ethyl acetate/*n*-hexane 40 % *v/v*); m.p. 230°C with dec; δ_H (400 MHz,

DMSO-d_6) 3.10 (2H, t, *J* 5.6, N-CH_2-CH_2-), 4.63 (2H, t, *J* 5.6, N-CH_2-CH_2-), 7.40 (5H, m, SO_2NH_2, Ar-H), 7.79 (2H, d, *J* 8.4, Ar-H), 8.10 (1H, brs, Ar-H), 8.25 (1H, m, Ar-H), 13.11 (1H, s, exchange with D_2O, -S*H*); δ_C (100 MHz, DMSO-d_6) 33.0, 47.5, 98.4, 120.0, 126.4, 127.1, 129.0, 130.2, 136.1, 137.2, 140.7, 143.6, 160.1, 177.0. Elemental analysis: calc: C 39.43, H 2.90, N 8.62, S 13.16; found: C 39.40, H 2.87, N 8.59, S 13.12; *m/z* (ESI positive) 487.94 $[M+H]^+$.

Synthesis of 4-[2-(2-mercapto-6-methyl-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide **(3h)**

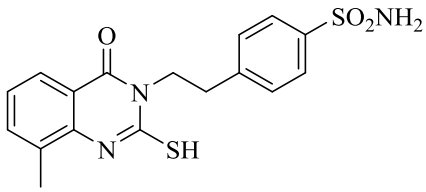


3h

2-Amino-5-methylbenzoic acid **1h** (0.1g, 1.0 eq) and 4-(2-isothiocyanato-ethyl)-benzenesulfonamide **2** (0.16g, 1.0 eq) were treated in EtOH (10 ml) at reflux for 5h according to the general procedure previously described. The precipitate formed was collected by filtration and was triturated from diethyl ether to afford the title compound **3h** as a light-brown solid

4-[2-(2-Mercapto-6-methyl-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide **3h**: 32% yield; silica gel TLC R_f 0.25 (Ethyl acetate/*n*-hexane 40 % *v/v*); m.p. >310°C; δ_H (400 MHz, DMSO-d_6) 2.41 (3H, s, CH_3), 3.10 (2H, t, *J* 5.6, N-CH_2-CH_2-), 4.66 (2H, t, *J* 5.6, N-CH_2-CH_2-), 7.35 (2H, s, exchange with D_2O, SO_2NH_2), 7.37 (1H, d, *J* 8.1, Ar-H), 7.52 (2H, d, *J* 8.4, Ar-H), 7.63 (1H, dd, *J* 8.1, 2.2, Ar-H), 7.82 (2H, d, *J* 8.4, Ar-H), 7.83 (1H, d, *J* 2.2, Ar-H), 13.12 (1H, s, exchange with D_2O, -S*H*); δ_C (100 MHz, DMSO-d_6) 22.0, 33.4, 48.0, 121.0, 126.8, 127.1, 129.0, 130.2, 136.2, 137.0, 137.1, 140.7, 143.6, 160.1, 176.4. Elemental analysis: calc: C 54.38, H 4.56, N 11.19, S 17.08; found: C 54.40, H 4.60, N 11.17, S 17.12; *m/z* (ESI positive) 376.09 $[M+H]^+$.

Synthesis of 4-[2-(2-mercapto-8-methyl-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide **(3i)**

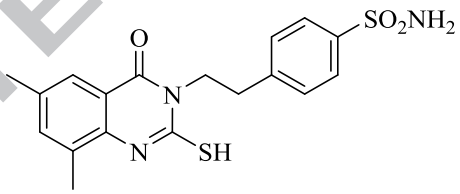


3i

2-Amino-3-methylbenzoic acid **1i** (0.1g, 1.0 eq) and 4-(2-isothiocyanato-ethyl)-benzenesulfonamide **2** (0.16g, 1.0 eq) were treated in EtOH (10 ml) at reflux for 5h according to the general procedure previously described. The precipitate formed was collected by filtration and was triturated from diethyl ether to afford the title compound **3i** as a light-brown solid.

4-[2-(2-Mercapto-8-methyl-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide **3i**: 20% yield; silica gel TLC R_f 0.22 (Ethyl acetate/*n*-hexane 40 % *v/v*); m.p. 286 °C; δ_H (400 MHz, DMSO-d_6) 2.53 (3H, s, C*H*$_3$), 3.10 (2H, t, *J* 5.6, N-CH$_2$-C*H*$_2$-), 4.66 (2H, t, *J* 5.6, N-C*H*$_2$-CH$_2$-), 7.31 (1H, t, *J* 7.6, Ar-H), 7.35 (2H, s, exchange with D_2O, SO_2NH_2), 7.54 (2H, d, *J* 8.4, Ar-H), 7.63 (1H, dd, *J* 8.1, 2.2, Ar-H), 7.82 (2H, d, *J* 8.4, Ar-H), 7.89 (1H, d, *J* 7.6, Ar-H), 13.00 (1H, s, exchange with D_2O, -S*H*); δ_C (100 MHz, DMSO-d_6) 18.2, 33.0, 47.8, 116.8, 125.5, 125.5, 126.2, 127.0, 130.1, 137.8, 138.5, 143.4, 143.7, 160.3, 176.2. Elemental analysis: calc: C 54.38, H 4.56, N 11.19, S 17.08; found: C 54.42, H 4.58, N 11.20, S 17.15; *m/z* (ESI positive) 376.09 $[M+H]^+$.

Synthesis of 4-[2-(2-mercapto-6,8-dimethyl-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide **(3j)**



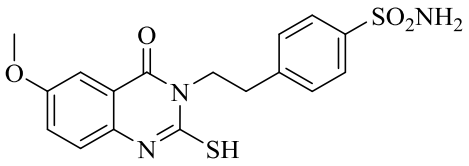
3j

2-Amino-3,5-dimethylbenzoic acid **1j** (0.1 g, 1.0 eq) and 4-(2-isothiocyanato-ethyl)-benzenesulfonamide **2** (0.15g, 1.0 eq) were treated in EtOH (10 ml) at reflux for 5h according to the general procedure previously described. The precipitate formed was collected by filtration and was triturated from diethyl ether to afford the title compound **3j** as a light-brown solid.

4-[2-(2-Mercapto-6,8-dimethyl-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide **3j**: 22% yield; silica gel TLC R_f 0.30 (Ethyl acetate/*n*-hexane 40 % *v/v*); m.p. 275°C; δ_H (400 MHz, DMSO-d_6) 2.37 (3H, s, C*H*$_3$), 2.49 (3H, s, C*H*$_3$), 3.11 (2H, t, *J* 5.6, N-CH$_2$-C*H*$_2$-), 4.68 (2H, t, *J* 5.6, N-C*H*$_2$-CH$_2$-), 7.35 (2H, s, exchange with D_2O, SO_2NH_2), 7.47 (1H, s, Ar-H), 7.53 (2H, d, *J* 8.4, Ar-H), 7.70 (1H, s, Ar-H), 7.82 (2H, d, *J* 8.4, Ar-H), 11.80 (1H, s, exchange with D_2O, -S*H*); δ_C (100 MHz, DMSO-d_6) 18.4, 20.0, 33.4, 48.1, 116.9, 124.4, 125.5, 126.0, 126.8, 130.2, 136.6, 138.5, 143.4,

143.6, 160.2, 176.2. Elemental analysis: calc: C 55.51, H 4.92, N 10.79, S 16.47; found: C 54.53, H 4.89, N 10.82, S 16.50; *m/z* (ESI positive) 390.50 $[M+H]^+$.

Synthesis of 4-[2-(2-mercapto-6-methoxy-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide **(3k)**

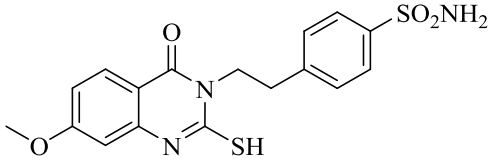


3k

2-Amino-5-methoxy-benzoic acid **1k** (0.2g, 1.0 eq) and 4-(2-isothiocyanato-ethyl)-benzenesulfonamide **2** (0.29g, 1.0 eq) were treated in EtOH (10 ml) at reflux O.N. according to the general procedure previously described. The precipitate formed was collected by filtration and was triturated from diethyl ether to afford the title compound **3k** as a light-brown solid.

4-[2-(2-Mercapto-6-methoxy-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide **3k**: 28% yield; silica gel TLC R_f 0.11 (MeOH/CDM 5 % *v/v*); m.p. 273°C; δ_H (400 MHz, DMSO-d_6) 3.09 (2H, t, *J* 5.6, N-CH_2-CH_2-), 3.87 (3H, s, OCH_3), 4.66 (2H, t, *J* 5.6, N-CH_2-CH_2-), 7.35 (2H, s, exchange with D_2O, SO_2NH_2), 7.42 (3H, m, Ar-H), 7.52 (2H, d, *J* 8.4, Ar-H), 7.82 (2H, d, *J* 8.4, Ar-H), 13.05 (1H, s, exchange with D_2O, -S*H*); δ_C (100 MHz, DMSO-d_6) 33.6, 48.2, 57.3, 109.4, 117.8, 119,2, 126.4, 127.5, 130.8, 135.0, 143.5, 144.5, 157.8, 161.0, 174.9; Elemental analysis: calc: C 52.16, H 4.38, N 10.73, S 16.38; found: C 52.20, H 4.35, N 10.75, S 16.40; *m/z* (ESI positive) 392.09 $[M+H]^+$.

Synthesis of 4 4-[2-(2-mercapto-7-methoxy-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide **(3l)**



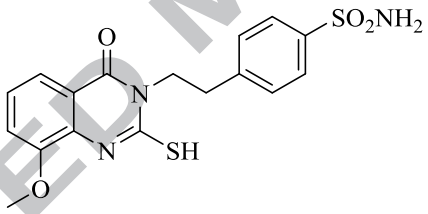
3l

2-Amino-4-methoxybenzoic acid **1l** (0.1g, 1.0 eq) and 4-(2-isothiocyanato-ethyl)-benzenesulfonamide **2** (0.14g, 1.0 eq) were treated in EtOH (10 ml) at reflux O.N. according to the

general procedure previously described. The solvent was removed under vacuo to give a residue that was triturated from diethyl ether and then purified by silica gel column chromatography eluting with 50% ethyl acetate/*n*-hexane to afford the title compound **3l** as a light-brown solid.

4-[2-(2-Mercapto-7-methoxy-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide **3l**: 21% yield; silica gel TLC R_f 0.10 (MeOH/DCM 5 % *v/v*); m.p. 235°C; δ_H (400 MHz, DMSO-d_6) 3.07 (2H, t, *J* 5.6, N-CH_2-CH_2-), 3.90 (3H, s, OCH_3), 4.70 (2H, t, *J* 5.6, N-CH_2-CH_2-), 7.34 (2H, s, exchange with D_2O, SO_2NH_2), 7.48 (3H, m, Ar-H), 7.50 (2H, d, *J* 8.4, Ar-H), 7.90 (2H, d, *J* 8.4, Ar-H), 12.90 (1H, s, exchange with D_2O, -S*H*); δ_C (100 MHz, DMSO-d_6) 34.0, 48.1, 56.1, 118.9, 119.4, 126.2, 128.0, 131.1, 135.4, 143.3, 144.0, 153.0, 156.1, 161.1, 177.6. Elemental analysis: calc: C 52.16, H 4.38, N 10.73, S 16.38; found: C 52.18, H 4.36, N 10.76, S 16.35; *m/z* (ESI positive) 392.09 $[M+H]^+$.

Synthesis of 4-[2-(2-mercapto-8-methoxy-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide **(3m)**

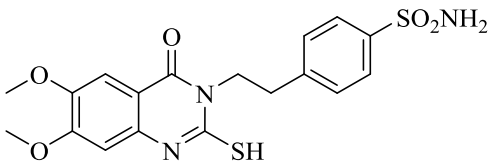


3m

2-Amino-3-methoxybenzoic acid **1m** (0.1g, 1.0 eq) and 4-(2-isothiocyanato-ethyl)-benzenesulfonamide **2** (0.14g, 1.0 eq) were treated in EtOH (10 ml) at reflux O.N. according to the general procedure previously described. The solvent was removed under vacuo to give a residue that was triturated from diethyl ether to afford the title compound **3m** as a light-brown solid.

4-[2-(2-Mercapto-8-methoxy-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide **3m**: 33% yield; silica gel TLC R_f 0.15 (MeOH/DCM 5 % *v/v*); m.p. 295°C; δ_H (400 MHz, DMSO-d_6) 3.10 (2H, t, *J* 5.6, N-CH_2-CH_2-), 4.00 (3H, s, OCH_3), 4.67 (2H, t, *J* 5.6, N-CH_2-CH_2-), 7.36 (3H, m, exchange with D_2O, SO_2NH_2, Ar-H), 7.44 (1H, m, Ar-H), 7.62 (2H, d, *J* 8.4, Ar-H), 7.68 (1H, m, Ar-H), 7.75 (2H, d, *J* 8.4, Ar-H), 11.80 (1H, brs, exchange with D_2O, -S*H*); δ_C (100 MHz, DMSO-d_6) 33.6, 48.2, 56.0, 116.0, 118.8, 119.1, 126.0, 127.6, 130.7, 135.1, 143.5, 144.4, 152.9, 161.1, 176.9. Elemental analysis: calc: C 52.16, H 4.38, N 10.73, S 16.38; found: C 52.22, H 4.39, N 10.75, S 16.40; *m/z* (ESI positive) 392.09 $[M+H]^+$.

Synthesis of 4-[2-(2-mercapto-6,7-dimethoxy-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide **(3n)**

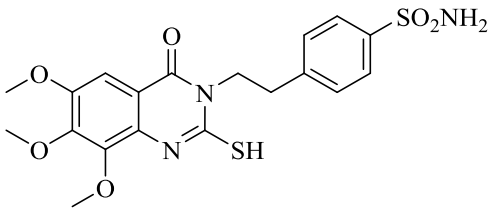


3n

2-Amino-4,5-dimethoxybenzoic acid **1n** (0.1g, 1.0 eq) and 4-(2-isothiocyanato-ethyl)-benzenesulfonamide **2** (0.12g, 1.0 eq) were treated in EtOH (10 ml) at reflux O.N. according to the general procedure previously described. The precipitate formed was collected by filtration and triturated from diethyl ether to afford the title compound **3n** as a white solid.

4-[2-(2-Mercapto-6,7-dimethoxy-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide **3n**: 80% yield; silica gel TLC R_f 0.20 (MeOH/DCM 5 % *v/v*); m.p. 302°C; δ_H (400 MHz, DMSO-d_6) 3.09 (2H, t, *J* 5.6, N-CH_2-CH_2-), 3.88 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 4.66 (2H, t, *J* 5.6, N-CH_2-CH_2-), 6.98 (1H, s, Ar-H), 7.35 (3H, s, exchange with D_2O, SO_2NH_2, Ar-H), 7.52 (2H, d, *J* 8.4, Ar-H), 7.82 (2H, d, *J* 8.4, Ar-H), 12.86 (1H, brs, exchange with D_2O, -S*H*); δ_C (100 MHz, DMSO-d_6) 33.2, 47.4, 56.8, 57.0, 107.8, 109.1, 126.9, 130.1, 130.2, 136.1, 143.3, 143.7, 147.8, 156.4, 159.7, 174.7; Elemental analysis: calc: C 51.29, H 4.54, N 9.97, S 15.22; found: C 51.30, H 4.56, N 10.01, S 15.25; *m/z* (ESI positive) 422.09 $[M+H]^+$.

Synthesis of 4-[2-(2-mercapto-6,7,8-trimethoxy-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide **(3o)**



3o

2-Amino-3,4,5-trimethoxybenzoic acid **1o** (0.1g, 1.0 eq) and 4-(2-isothiocyanato-ethyl)-benzenesulfonamide **2** (0.11g, 1.0 eq) were treated in EtOH (10 ml) at reflux O.N. according to the

general procedure previously described. The precipitate formed was collected by filtration and triturated from diethyl ether to afford the title compound **3o** as a white solid.

4-[2-(2-Mercapto-6,7,8-trimethoxy-4-oxo-4*H*-quinazolin-3-yl)-ethyl]-benzenesulfonamide **3o**: 75% yield; silica gel TLC R_f 0.27 (Ethyl acetate/*n*-hexane 50 % *v/v*); m.p. 260°C; δ_H (400 MHz, DMSO-d_6) 3.09 (2H, t, *J* 5.6, N-CH_2-CH_2-), 3.92 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 4.67 (2H, t, *J* 5.6, N-CH_2-CH_2-), 7.27 (1H, s, Ar-H), 7.35 (2H, s, exchange with D_2O, SO_2NH_2), 7.53 (2H, d, *J* 8.4, Ar-H), 7.82 (2H, d, *J* 8.4, Ar-H), 12.18 (1H, s, exchange with D_2O, -S*H*); δ_C (100 MHz, DMSO-d_6) 33.0, 48.2, 56.8, 56.9, 59.2, 117.2, 119.1, 127.0, 131.0, 131.2, 136.2, 143.0, 146.0, 148.1, 156.4, 160.4, 170.0. Elemental analysis: calc: C 50.54, H 4.69, N 9.31, S 14.20; found: C 50.57, H 4.71, N 9.27, S 14.16; *m/z* (ESI positive) 453.00 $[M+H]^+$.

4.2. CA inhibition

An Applied Photophysics stopped-flow instrument has been used for assaying the CA-catalyzed CO_2 hydration activity.[18] Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes buffer (pH 7.5) and 20 mM $NaClO_4$ for maintaining constant ionic strength, following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10-100 s, at 20 °C. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor, at least six traces of the initial 5-10% of the reaction have been used to determine the initial velocity. The uncatalyzed rates were measured in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (1 mM) were prepared in distilled-deionized water and dilutions down to 0.01 nM were made thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E-I complex. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3 and the Cheng-Prusoff equation as reported earlier,[19-21] and represent the mean from at least three different determinations. All CA isoforms were recombinant ones obtained in huse as reported earlier,[19-21] and their concentrations in the assay system were: 12.3 nM for hCA I, 8.5 nM for hCA II and 10.2 nM for hCA XII, respectively.

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References

1. Alafeefy, A.M.; Carta, F.; Ceruso, M.; Al-Tamimi, A.M.; Al-Kahtani, A.A.; Supuran. C.T. *Bioorg. Med. Chem*. **2016**, *24*, 1402.

2. a) Krishnamurthy, V. M.; Kaufman, G. K.; Urbach, A. M.; Gitlin, I.; Gudiksen, K. L.; Weibel, D. B.; Whitesides, G. M. *Chem. Rev.* **2008**, *108*, 946; b) Supuran, C. T. *Nat. Rev. Drug. Discovery* **2008**, *7*, 168; c) Supuran, C.T. *J. Enzyme Inhib. Med. Chem*. **2012**, *27*, 759; d) Supuran, C.T. *J. Enzyme Inhib. Med. Chem*. **2016**, *31*, 345.

2. a) Alterio, V.; Di Fiore, A.; D'Ambrosio, K.; Supuran, C. T.; De Simone, G. *Chem. Rev.* **2012**, *112*, 4421; b) Marini, A.M.; Maresca, A.; Aggarwal, M.; Orlandini, E.; Nencetti, S.; Da Settimo, F.; Salerno, S.; Simorini, F.; La Motta, C.; Taliani, S.; Nuti, E.; Scozzafava, A.; McKenna, R.; Rossello, A.; Supuran C.T., *J.Med. Chem*. **2012**, *55*, 9619; c) Supuran, C.T. *Bioorg. Med. Chem.* **2013**, *21*, 1377.

3. a) Scozzafava, A.; Menabuoni, L.; Mincione, F.; Mincione, G.; Supuran, C.T. *Bioorg. Med. Chem. Lett*. **2001**, *11*, 575; b) Borras, J.; Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C.T. *Bioorg. Med. Chem*. **1999**, *7*, 2397; c) Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C.T. *J. Med. Chem*. **1999**, *42*, 2641; d) Scozzafava, A.; Briganti, F.; Mincione, G.; Menabuoni, L.; Mincione, F.; Supuran, C.T. *J. Med. Chem*. **1999**, *42*, 3690.

4. a) Di Fiore, A.; Pedone, C.; D'Ambrosio, K.; Scozzafava, A.; De Simone, G.; Supuran, C. T. *Bioorg. Med. Chem. Lett*. **2006**, *16*, 437; b) Lock, F.E.; McDonald, P.C.; Lou, Y.; Serrano, I.; Chafe, S.C.; Ostlund, C.; Aparicio, S.; Winum, J.Y.; Supuran, C.T.; Dedhar, S. *Oncogene* **2013**, *32*, 5210; c) Temperini, C.; Cecchi, A.; Scozzafava, A.; Supuran, C.T. *Org. Biomol. Chem*. **2008**, *6*, 2499; d) Winum, J.Y.; Maresca, A.; Carta, F.; Scozzafava, A.; Supuran, C.T. *Chem. Commun.* **2012**, *48*, 8177.

5. a) Neri, D.; Supuran, C. T. *Nat. Rev. Drug Discovery* **2011**, *10*, 767; b) Supuran, C.T. *J. Enzyme Inhib. Med. Chem*. **2013**, *28*, 229; c) Aggarwal, M.; Boone, C.D.; Kondeti, B.; McKenna, R. *J. Enzyme Inhib. Med. Chem*. **2013**, *28*, 267; d) Scozzafava, A.; Menabuoni, L.; Mincione, F.; Supuran, C.T. *J. Med. Chem*. **2002**, *45*, 1466.

6. a) Dogné, J. M.; Hanson, J.; Supuran, C. T.; Pratico, D. *Curr. Pharm. Des*. **2006**, *12*, 971; b) Dogne', J. M.; Thiry, A.; Pratico, D.; Masereel, B.; Supuran, C.T. *Curr. Top. Med. Chem*. **2007**, *7*, 885; c) Dogné, J. M.; Supuran, C. T.; Pratico, D. *J. Med. Chem.* **2005**, *48*, 2251; d) Supuran, C.T. *Expert Rev. Neurother*. **2015**, *15*, 851; d) Touisni, N.; Maresca, A.; McDonald, P.C.; Lou, Y.; Scozzafava, A.; Dedhar, S.; Winum, J.Y.; Supuran, C.T. *J. Med. Chem*. **2011**, *54*, 8271.

7. a) De Simone, G.; Alterio, V.; Supuran, C. T. *Expert Opin. Drug Disc.* **2013,** *8,* 793; b) Aggarwal, M.; Kondeti, B.; McKenna, R. *Expert Opin. Ther. Pat.* **2013,** *23,* 717; c) Supuran, C.T. *Bioorg. Med. Chem. Lett.* **2010,** *20,* 3467; d) Dubois, L.; Lieuwes, N. G.; Maresca, A.; Thiry, A.; Supuran, C. T.; Scozzafava, A.; Wouters, B. G.; Lambin, P. *Radiother. Oncol.* **2009,** *92,* 423; e) Tanpure, R.; Ren, B.; Peat, T.S.; Bornaghi, L.F.; Vullo, D.; Supuran, C.T.; Poulsen, S.A. *J. Med. Chem*. **2015**, *58*, 1494.

8. a) Pacchiano, F.; Carta, F.; McDonald, P.C.; Lou, Y.; Vullo, D.; Scozzafava, A.; Dedhar, S.; Supuran, C.T. *J. Med. Chem.* **2011**, *54*, 1896; b) Krall, N.; Pretto, F.; Decurtins, W.; Bernardes, G. J. L.; Supuran, C. T.; Neri, D., *Angew. Chem. Int. Ed. Engl.* **2014**, *53*, 4231; c) Dubois, L.; Douma, K.; Supuran, C.T.; Chiu, R.K.; van Zandwoort, M.A.; Pastorekova, S.; Scozzafava, A.; Wouters, B.G.; Lambin, P. *Radiother. Oncol*. **2007**, *83*, 367; d) De Simone, G.; Supuran, C.T. *Biochim. Biophys. Acta* **2010**, *1804*, 404.

9. a) Supuran, C. T.; Casini, A.; Mastrolorenzo, A.; Scozzafava, A. *Mini. Rev. Med. Chem.* **2004**, *4*, 625; b) Capasso, C.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem*. **2014**, *29*, 379; c) De Simone, G.; Di Fiore, A.; Supuran, C.T. *Curr. Pharm. Des*. **2008**, *14*, 655; d) Ferraroni, M.; Del Prete, S.; Vullo, D.; Capasso, C.; Supuran, C.T. *Acta Crystallogr.D Biol. Crystallogr.* **2015**, *71*, 2449; e) Capasso, C.; Supuran, C.T. *Expert Opin. Ther. Targets* **2015**, *19*, 1689; f) Supuran, C.T.; Capasso, C. *Expert Opin. Ther. Targets* **2015**, *19*, 551; g) Del Prete, S.; Vullo, D.; Fisher, G. M.; Andrews, K. T.; Poulsen S.A.; Capasso, C.; Supuran C.T. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 4389.

10. a) Smith, K. S.; Jakubzick, C.; Whittam, T. S.; Ferry, J. G. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 15184; b) Zimmerman, S. A.; Ferry, J. G.; Supuran, C. T. *Curr. Top. Med. Chem.* **2007**, *7*, 901; c) Del Prete, S.; De Luca, V.; Scozzafava, A.; Carginale, V.; Supuran, C.T.; Capasso, C. *J. Enzyme Inhib. Med. Chem*. **2014**, *29*, 23; d) Del Prete, S.; De Luca, V.; Vullo, D.; Scozzafava, A.; Carginale, V.; Supuran, C.T.; Capasso, C. *J. Enzyme Inhib. Med. Chem.* **2014**, *29*, 532; e) Chohan, Z.H.; Scozzafava, A.; Supuran, C.T. *J. Enzyme Inhib*. *Med. Chem*. **2003**, *18*, 259.

11. a) Biswas, S.; Aggarwal, M.; Guzel, O.; Scozzafava, A.; McKenna, R.; Supuran, C.T. *Bioorg. Med. Chem*. **2011**, *19*, 3732; b) Biswas, S.; Carta,F.; Scozzafava, A.; McKenna, R.; Supuran, C.T. *Bioorg. Med. Chem*. **2013**, *21*, 2314; c) Bozdag, M.; Pinard, M.; Carta, F.; Masini, E.; Scozzafava, A.; McKenna, R.; Supuran, C.T. *J. Med. Chem*. **2014**, *57*, 9673; d) Carta, F.; Di Cesare Mannelli, L.; Pinard, M.; Ghelardini, C.; Scozzafava, A.; McKenna, R.; Supuran, C.T. *Bioorg. Med. Chem*. **2015**, *23*, 1828; e) Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C.T. *J. Med. Chem*. **2000**, *43*, 4542; f) Bayram, E.; Senturk, M.; Kufrevioglu, O.I.; Supuran, C.T. *Bioorg. Med. Chem.* **2008**, *16*, 9101.

12. a) Nishimori, I.; Vullo, D.; Innocenti, A.; Scozzafava, A.; Mastrolorenzo, A.; Supuran, C.T. *J. Med. Chem.* **2005**, *48*, 7860; b) Durdagi, S.; Scozzafava, G.; Vullo, D.; Sahin, H.; Kolayli, S.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2014**, *29*, 469; c) Abbate, F.; Supuran, C.T.; Scozzafava, A.; Orioli, P.; Stubbs, M.T.; Klebe, G. *J. Med. Chem*. **2002**, *45*, 3583; d) Briganti, F.; Pierattelli, R.; Scozzafava, A.; Supuran, C.T. *Eur. J. Med. Chem.* **1996**, *31*, 1001; e) Abbate, F.; Winum, J.Y.; Potter, B.V.; Casini, A.; Montero, J.L.; Scozzafava, A.; Supuran, C.T. *Bioorg. Med. Chem.Lett.***2004**, *14*, 231.

13. a) Masini, E.; Carta, F.; Scozzafava, A.; Supuran, C.T. *Expert Opin. Ther. Pat*. **2013**, *23*, 705; b) Supuran, C. T. *Future Med. Chem.* **2011**, *3*, 1165; c) Supuran, C.T.; Clare, B.W. *Eur. J. Med. Chem*. **1999**, *34*, 41; d) Supuran, C.T.; Scozzafava, A.; Mastrolorenzo, A. *Expert Opin. Ther. Pat.***2001**, *11*, 221; e) Garaj, V.; Puccetti, L.; Fasolis, G.; Winum, J.Y.; Montero, J.L.; Scozzafava, A.; Vullo, D.; Innocenti, A.; Supuran, C.T. *Bioorg. Med. Chem. Lett*. **2005**, *15*, 3102.

14. a) Monti, S.M.; Supuran, C.T.; De Simone, G. *Expert Opin. Ther. Pat*. **2013**, *23*, 737; b) Ward, C.; Langdon, S.P.; Mullen, P.; Harris, A.L.; Harrison, D.J.; Supuran, C.T.; Kunkler, I. *Cancer Treatm. Rev*. **2013**, *39*, 171; c) Alafeefy, A. M.; Isik, S.; Abdel-Aziz, H. A.; Ashour, A. E.; Vullo, D.; Al-Jaber, N.A.; Supuran, C. T. *Bioorg. Med. Chem.* **2013**, *21*, 1396; d) Supuran, C.T.; Nicolae, A.; Popescu, A. *Eur. J. Med. Chem*. **1996**, 31, 431

15. a) Scozzafava, A.; Supuran, C.T.; Carta, F. *Expert Opin. Ther. Pat*. **2013**, *23*, 725; b) Arechederra, R.L.; Waheed, A.; Sly, W.S.; Supuran, C.T.; Minteer, S.D. *Bioorg. Med. Chem.* **2013**, *21*, 1544; c) Thiry, A.; Dognè, J.M.; Supuran, C.T.; Masereel, B. *Curr. Pharm. Des*. **2008**, *14*, 661; b) Thiry, A.; Dognè, J.M.; Masereel, B.; Supuran, C.T. *Curr. Top. Med. Chem*. **2007**, *7*, 855.

16. a) Carta, F.; Supuran, C.T. *Expert Opin. Ther. Pat*. **2013**, *23*, 681; b) Supuran, C.T. *Front. Pharmacol*. **2011**, *2*, 34; b) Capasso, C.; Supuran, C.T. *Expert Opin. Ther. Pat*. **2013**, *23*, 693; c) Capasso, C.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2014**, *29*, 379.

17. a) Innocenti, A.; Casini, A.; Alcaro, M.C.; Papini, A.M.; Scozzafava, A.; Supuran, C.T. *J. Med. Chem*. **2004**, *47*, 5224; b) Casini, A.; Scozzafava, A.; Mincione, F.; Menabuoni, L.; Ilies, M.A.; Supuran, C.T. *J. Med. Chem.* **2000**, *43*, 4884; c) Mincione, F.; Starnotti, M.; Masini, E.; Bacciottini, L.; Scrivanti, C.; Casini, A.; Vullo, D.; Scozzafava, A.; Supuran, C.T. *Bioorg. Med. Chem. Lett*. **2005**, *15*, 3821; d) Korkmaz, N.; Obaidi, O.A.; Senturk, M.; Astley, D.; Ekinci, D.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem*. **2015**, *30*, 75.

18. Khalifah, R.J. *J. Biol. Chem.* **1971,** *246*, 2561.

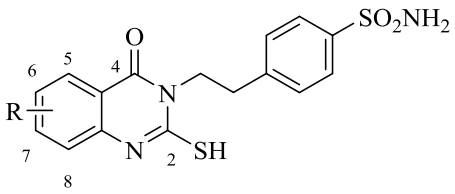
19. a) Ceruso, M.; Vullo, D.; Scozzafava, A.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem*. **2014**, *29*, 686; b) Korkmaz, N.; Obaidi, O.A.; Senturk, M.; Astley, D.; Ekinci, D.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem*. **2015**, *30*, 75; c) Abdel-Aziz, A.A.M.; El-Azab; A.S., Ekinci, D.; Şentürk, M.;

Supuran, C.T. *J. Enzyme Inhib. Med. Chem*. **2015**, *30*, 81; d) Abdel-Aziz, A.A.M.; El-Azab, A.S.; Ekinci, D.; Şentürk, M.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2015**, *30*, 81; e) Akdemir, A; De Monte, C.; Carradori, S.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2015**, *30*, 114.

20. a) Akbaba, Y.; Akincioglu, A.; Göçer, H.; Göksu, S.; Gülçin, I.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2014**, *29*, 35; b) Sharma, A.; Tiwari, M.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2014**, *29*, 292; c) Sahin, A.; Isik, S.; Arslan, O.; Supuran, C.T.; Ozensoy Guler, O. *J. Enzyme Inhib. Med. Chem*. **2015**, *30*, 224; d) Bozdag, M.; Isik, S.; Beyaztas, S.; Arslan, O.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem*. **2015**, *30*, 240; e) De Luca, V.; Del Prete, S.; Supuran, C.T.; Capasso, C. *J. Enzyme Inhib. Med. Chem*. **2015**, *30*, 277; f) Vullo, D.; Milos, M.; Galic, B.; Scozzafava, A.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem*. **2015**, *30*, 341; g) Del Prete, S.; Vullo, D.; De Luca, V.; AlOthman, Z.; Supuran, C.T.; Capasso, C. *J. Enzyme Inhib. Med. Chem*. **2015**, *30*, 366; h) Riafrecha, L.E.; Vullo, D.; Supuran, C.T.; Colinas, P.A. *J. Enzyme Inhib. Med. Chem*. **2015**, *30*, 857.

21. a) Migliardini, F.; De Luca, V.; Carginale, V.; Rossi, M.; Corbo, P.; Supuran, C. T.; Capasso, C. *J. Enzyme Inhib. Med. Chem.* **2014**, *29*, 146; b) Ceruso, M.; Bragagni, M.; AlOthman, Z.; Osman, S.M.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem*. **2015**, *30*, 430; c) Zolfaghari Emameh, R.; Syrjänen, L.; Barker, H.; Supuran, C.T.; Parkkila, S. *J. Enzyme Inhib. Med. Chem*. **2015**, *30*, 505; d) Alafeefy, A.M.; Ceruso, M.; Al-Tamimi, A.M.S.; Del Prete, S.; Supuran, C.T.; C. Capasso, *J. Enzyme Inhib. Med. Chem*. **2015**, *30*, 592; e) Scozzafava, A.; Passaponti, M.; Supuran, C.T.; Gülçin, I. *J. Enzyme. Med. Chem*. **2015**, *30*, 586; f) Le Darz, A.; Mingot, A.; Bouazza, F.; Castelli, U.; Tanc Karam, O.M.; Supuran, C.T.; Thibaudeau, S. *J. Enzyme Inhib. Med. Chem*. **2015**, *30*, 737..

Graphical abstract



3a-o

K_I (hCA I) = 28.5 – 2954 nM
K_I (hCA II) = 0.625 – 12.4 nM
K_I (hCA XII) = 0.54 – 7.11 nM