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Novel synthesized SLC-0111 thiazole and thiadiazole analogues: Determination of their carbonic anhydrase inhibitory activity and molecular modeling studies

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ABSTRACT

In the presented work, we report the design and synthesis of novel SLC-0111 thiazole and thiadiazole analogues (**11a–d**, **12a–d**, **16a–c** and **17a–d**). A bioisosteric replacement approach was adopted to replace the 4-fluorophenyl tail of SLC-0111 with thiazole and thiadiazole ones, which were thereafter extended with lipophilic un/substituted phenyl moieties. All the newly synthesized SLC-0111 analogues were evaluated *in vitro* for their inhibitory activity towards a panel of the metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1) isoforms (hCA I, II, IX and XII), using a stopped-flow CO_2 hydrase assay. All the examined isoforms were inhibited by the primary sulfonamide derivatives (**11a–d** and **12a–d**) in variable degrees with the following K_I ranges: 162.6–7136 nM for hCA I, 9.0–833.6 nM for hCA II, 7.9–153.0 nM for hCA IX, and 9.4–94.0 nM for hCA XII. In particular, compounds **12b** and **12d** displayed 5.5-fold more potent inhibitory activity (K_Is = 8.3 and 7.9 nM, respectively) than SLC-0111 (K_I = 45 nM) towards hCA IX. Molecular docking study was carried out for **12d** within the hCA IX (PDB 3IAI) active site, to justify its inhibitory activity.

1. Introduction

Carbonic anhydrases (CAs, EC 4.2.1.1) are metalloenzymes acting as efficient catalysts for the reversible hydration of carbon dioxide to bicarbonate [1,2]. 16 different α-CA isoforms have been isolated in mammals, which differ in their subcellular localization and tissue distribution, and play crucial physiological roles in various processes such as lipogenesis, gluconeogenesis, ureagenesis and tumorigenicity [3–6]. Recently, different carbonic anhydrases become validated drug targets for various applications, such as CA VII and XIV for antiepileptic drugs, CA II, IV and XII for antiglaucoma drugs, CA II, IV, XII and XIV for diuretics and CA IX and XII isoforms for cancer therapies, especially in hypoxic tumors [3,4].

SLC-0111 (**I**, Fig. 1) is an ureido substituted benzenesulfonamide derivative that possesses selective inhibitory action towards CA isoforms IX and XII over the off-target ubiquitous hCA I and hCA II. SLC-0111 displayed significant anticancer/antimetastatic action in animal models with good pharmacokinetic profile. SLC-0111 is currently in Phase I/II clinical trials for the treatment of metastatic solid hypoxic tumors [7–9]. The ureido linker in SLC-0111 has an important role because it donates a great flexibility to the tail of the molecule which imparts the possibility for the inhibitor to adopt a diversity of orientations when bound within the enzyme active site. These orientations permit the specific interactions between the inhibitor tail and amino acid residues at the entrance of the active site cavity that represents the most variable region in several α-CA isoforms [8–13].

Recently, SLC-0111 has been adopted as a lead compound for designing other molecules with potential selective and potent CA IX inhibitory activity. Two studies have reported the design and synthesis of novel series of SLC-0111 hybrids *via* merging SLC-0111 and the pharmacophoric elements of isatin in a single chemical framework (compound **II**, Fig. 1). Also, the bioisosteric replacement strategy was

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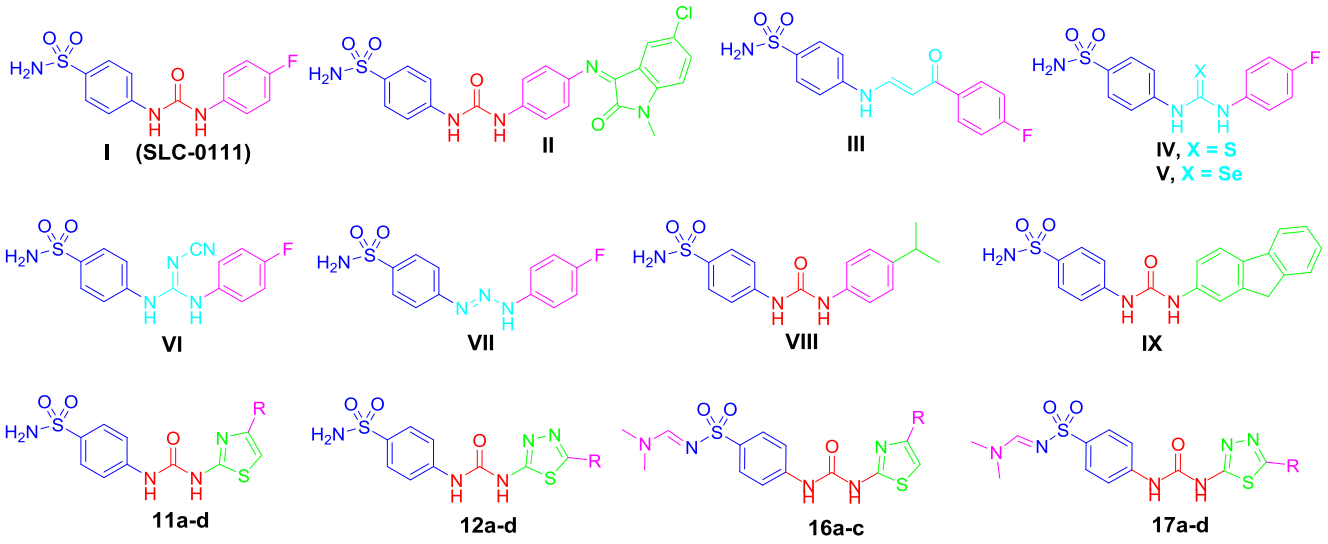


Fig. 1. Structures of SLC-0111 and its analogues **II-IX**, and structures of the target sulfonamides **11a–d, 12a–d, 16a–c** and **17a–d**.

adopted through substitution of the ureido spacer in SLC-0111 with different linkers [14–18] such as enaminone, thioureido, selenoureido, cyanoguanidine or 1,3-triazene linkers (compounds **III–VII**, respectively, Fig. 1). The inhibition profiles of these analogues displayed loss of selectivity against the tumor-associated hCA IX over the cytosolic hCA II isoform through significant improvement of the inhibitory activity against hCA II and/or with worsening of effectiveness towards hCA IX.

Furthermore, several SLC-0111 analogues were developed either *via* grafting different substituents, rather than *p*-fluoro, within the phenyl tail (as compound **VIII**, Fig. 1), or *via* replacement of 4-fluorophenyl tail with different polycyclic tails (as compound **IX**, Fig. 1) [9].

Taking the above into account, the presented work reports the design and synthesis of novel SLC-0111 thiazole and thiadiazole analogues (Fig. 1), with the prime aim of developing potent and selective hCA IX and hCA XII inhibitors. A bioisosteric replacement approach was adopted to replace the 4-fluorophenyl tail of SLC-0111 with thiazole and thiadiazole ones while keeping the ureido linker reported to induce selectivity towards hCA IX and XII. Further structure extension with un/substituted phenyl groups at 4- or 5-positions of thiazole and thiadiazole tails, respectively, was applied to ensure different lipophilic environments that might be harmonious with the hydrophobic nature of the CA IX active site, and to implement further elaboration of the target analogues to explore a valuable SAR. All the newly synthesized SLC-0111 analogues were evaluated *in vitro* for their inhibitory activity towards a panel of hCA isoforms (hCA I, hCA II, hCA IX and hCA XII), using the stopped-flow CO_2 hydrase assay. Finally, a molecular docking study was carried out for compound **12d** within the hCA IX (PDB 3IAI) active site, to justify its potent inhibitory activity.

2. Results and discussion

2.1. Chemistry

The synthesis of the novel sulfonamides in this study (**11a–d, 12a–d, 16a–c** and **17a–d**) is outlined in Schemes 1& 2. Chlorination of 4-sulfamoylbenzoic acid **1** to give 4-sulfamoylbenzoyl chloride **2** was achieved, in 55% yield, by heating with thionyl chloride under reflux temperature for 12 h. Then aroyl chloride **2** was reacted with sodium azide at 0 °C to afford aroyl azide **3** which subjected to Curtius rearrangement *via* heating in dry toluene to furnish the key intermediate 4-isocyanatobenzenesulfonamide (**4**).

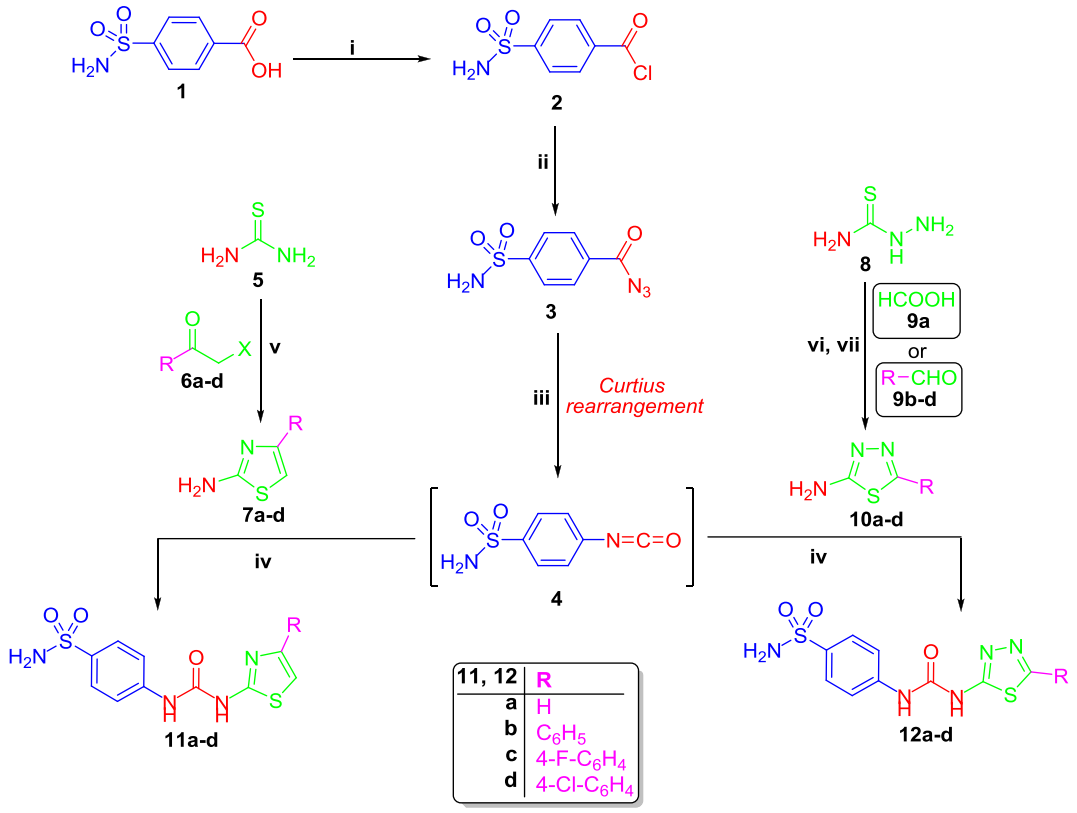
On the other hand, aminothiazole derivatives **7a–d** were prepared *via* the reaction of thiourea **5** with different α-halogenated carbonyl compounds **6a–d** in refluxing absolute ethanol. Whereas, thiosemicarbazide **8** was reacted with formic acid **9a** in HCl to furnish 2-amino-1,3,4-thiadiazole **10a**, or with aldehydes **9b–d** to afford 2-benzylidenehydrazine-1-carbothioamide intermediates which cyclized into 2-amino-5-aryl-1,3,4-thiadiazoles **10b–d** *via* refluxing in aqueous $FeCl_3$ solution. Finally, the key intermediates **4** was refluxed in dry toluene with aminothiazoles **7a–d** and aminothiadiazoles **10a–d** to furnish the primary sulfonamide-based ureides **11a–d** and **12a–d** (Scheme 1).

In our attempt to improve the yield of chlorination reaction for 4-sulfamoylbenzoic acid **1** and shorten the reaction time, we used DMF as a catalyst. Interestingly, upon its addition to the chlorination reaction mixture, DMF condensed with the amino function of 4-sulfamoylbenzoic acid **1** to furnish 4-(*N*-((dimethylamino)methylene)sulfamoyl)benzoyl chloride **13** in 70% yield, which subsequently converted to the key intermediate *N′*-((4-isocyanatophenyl)sulfonyl)-*N,N*-dimethylformimidamide (**15**) as described above in Scheme 1. The tertiary sulfonamide-based ureides **16a–c** and **17a–d** were prepared through reaction of the key intermediate **15** with aminothiazoles **7c–e** and aminothiadiazoles **10a–d** in refluxing toluene (Scheme 2).

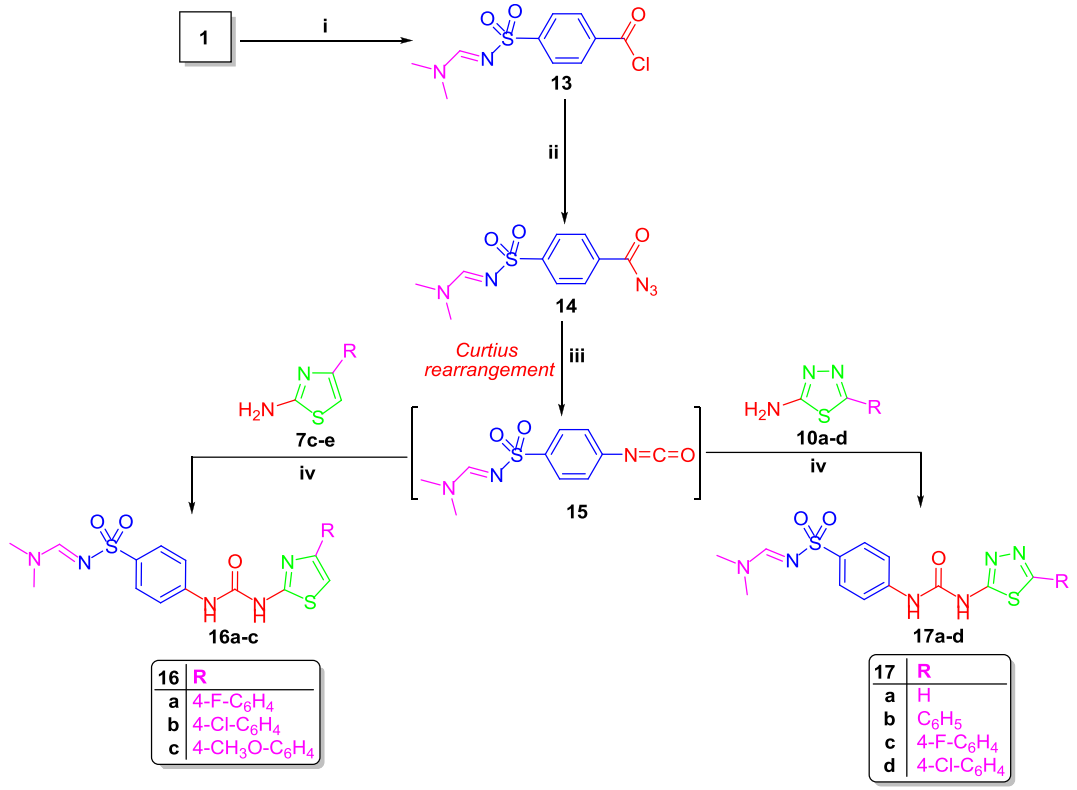
The elemental and spectral data supported the structures of the newly synthesized ureas **11a–d**, **12a–d**, **16a–c** and **17a–d**. IR spectra of the latter products showed the absorption bands due to the (C═O) group in the region 1701–1720 cm^{-1}, in addition to two bands of (SO_2) at 1053–1140 and 1327–1338 cm^{-1}.

Furthermore, ^{1}H NMR spectra of compounds **11a–d**, **12a–d**, **16a–c** and **17a–d** revealed the presence of two D_2O exchangeable singlet signals assigned to the NH groups of urea function at δ 8.45–9.67 and 9.47–11.96 *ppm*. While, ^{1}H NMR spectra of primary sulfonamides **11a–d** and **12a–d** displayed an additional D_2O exchangeable singlet signal at δ 7.26–7.29 *ppm* attributable for (NH_2) of sulfamoyl group, the ^{1}H NMR spectra of tertiary sulfonamides **16a–c** and **17a–d** revealed the absence of such signal and the presence of a non-D_2O exchangeable singlet signal at δ 7.41–8.27 *ppm* assigned to the (–N═CH–) olefinic proton. Moreover, ^{1}H NMR spectra of **16a–c** and **17a–d** showed two characteristic signals resonating in the aliphatic range δ 2.90–2.97 and 3.14–3.20 *ppm* attributable for the two methyl groups of *N*–($\underline{CH_3}$)$_2$.

On the other hand, the ^{13}C NMR spectra of **11a–d**, **12a–d**, **16a–c** and **17a–d** revealed the presence of the signal of the carbonyl carbon (C═O) in the range δ 159.21–166.94 *ppm*, whereas the signals due to the carbon of the two methyl groups of *N*–($\underline{CH_3}$)$_2$ in compounds **16a–c** and **17a–d** appeared at δ 35.40–35.51 and 41.29–42.09 *ppm*.

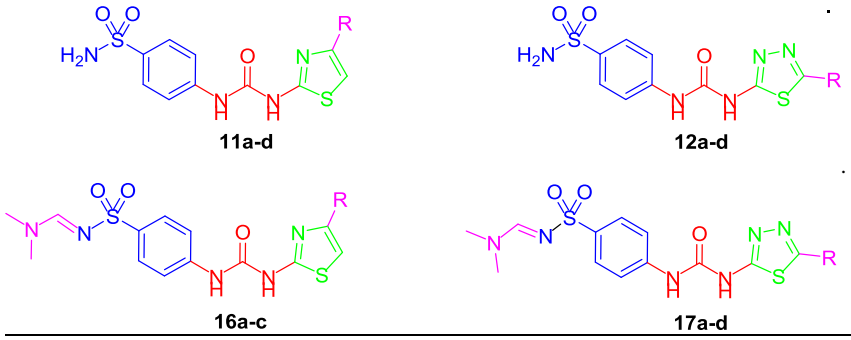


Scheme 1. Synthesis of aminothiazoles **7a–d** and aminothiadiazoles **10a–d** and target SLC-0111 analogues **11a–d** and **12a–d**; *Reagents and conditions*: (**i**) $SOCl_2$, reflux 12 h, (**ii**) NaN_3/Ice bath/stirring 2 h, (**iii**) Dry toluene/reflux 1 h, (**iv**) Dry toluene/reflux 4 h, (**v**) Absolute ethanol/reflux 8 h, (**vi**) HCl/reflux 8 h (for **10a**), **or** Glacial acetic acid/reflux 3 h (for **10b–d**), (**vii**) $FeCl_3$ solution/reflux 5 h.



Scheme 2. Synthesis of the tertiary sulfonamide-based ureides **16a–c** and **17a–d**; *Reagents and conditions*: (**i**) $SOCl_2$/ DMF/reflux 5 h (**ii**) NaN_3/Ice bath/stirring 2 h, (**iii**) Dry toluene/reflux 1 h, (**iv**) Dry toluene/reflux 4 h.

Table 1
Inhibition data of hCA isoforms I, II, IX and XII with ureides (**11a–d**, **12a–d**, **16a–c** and **17a–d**), determined by stopped-flow CO_2 hydrase assay, using SLC-0111 and AAZ as a standard inhibitors.



Comp.	R	K_I (nM)			
		hCA I	hCA II	hCA IX	hCA XII
11a	H	1005.1	833.6	153.0	94.0
11b	C_6H_5	270.1	17.2	64.7	71.3
11c	4-FC_6H_4	191.4	9.2	13.8	27.6
11d	4-ClC_6H_4	282.0	24.1	53.9	57.4
12a	H	7136.0	671.7	143.4	86.9
12b	C_6H_5	162.6	26.7	8.3	9.4
12c	4-FC_6H_4	397.2	9.0	12.4	26.2
12d	4-ClC_6H_4	623.7	45.0	7.9	9.9
16a	4-FC_6H_4	> 10,000	> 10,000	> 10,000	> 10,000
16b	4-ClC_6H_4	> 10,000	> 10,000	> 10,000	> 10,000
16c	4-$OCH_3C_6H_4$	> 10,000	> 10,000	> 10,000	> 10,000
17a	H	> 10,000	6815.2	> 10,000	> 10,000
17b	C_6H_5	> 10,000	> 10,000	> 10,000	> 10,000
17c	4-FC_6H_4	> 10,000	> 10,000	> 10,000	> 10,000
17d	4-ClC_6H_4	> 10,000	> 10,000	> 10,000	> 10,000
SLC-0111	–	5080.0	960.0	45.0	4.5
AAZ	–	250.0	12.5	25.0	5.7

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

2.2. Biological evaluation

2.2.1. Carbonic anhydrase inhibition

All the newly prepared ureas (**11a–d**, **12a–d**, **16a–c** and **17a–d**) were evaluated for their ability to inhibit the physiologically relevant hCA isoforms, hCA I and II (cytosolic) as well as hCA IX and XII (trans membrane, tumor associated isoforms) *via* a stopped flow CO_2 hydrase assay [19] using SLC-0111 and acetazolamide (**AAZ**) as standard inhibitors. The following structure–activity relationship (SAR) can be deduced from the inhibition data displayed in Table 1:

(**i**) All the tertiary sulfonamides reported here (**16a–c** and **17a–d**) failed to inhibit all the tested hCAs up to 10 µM, except compound **17a** displayed weak inhibitory activity towards hCA II (K_I = 6.815 µM).

(**ii**) The ubiquitous cytosolic isoform hCA I was moderately inhibited by the prepared primary sulfonamides (**11a–d** and **12a–d**) with K_I values ranging from 162.6 to 1005.1 nM, apart from sulfonamide **12a** which exhibited a lower inhibitory efficacy (K_I = 7136.0 nM). Noteworthy, grafting un/substituted phenyl groups at thiazole (**11b–d**) and thiadiazole (**12b–d**) rings improved the activity against hCA I.

(**iii**) The *in vitro* kinetic data displayed in Table 1 showed that the physiologically dominant isoform hCA II was efficiently inhibited by the primary sulfonamides (**11b–d** and **12b–d**) endowed with un/substituted phenyl groups with inhibition constants ranging in the low nanomolar range, in detail, between 9.0 and 45.0 nM. In particular, the 4-fluorophenyl-bearing thiazolo (**11c**) and thiadiazolo (**12c**) derivatives emerged as the most potent counterparts against hCA II with single-digit nanomolar inhibitory activities (K_Is = 9.2 and 9.0 nM, respectively). Contrariwise, unsubstituted thiazolo (**11a**) and thiadiazolo (**12a**) derivatives elicited weak activity towards this isoform with K_I values of 833.6 and 671.7 nM, respectively.

(**iv**) The tumor-associated isoform hCA IX was effectively inhibited by all the prepared primary sulfonamides (**11a–d** and **12a–d**) with K_Is spanning in the nanomolar range: 7.9–153 nM. Superiorly, the phenyl and 4-chlorophenyl thiadiazole derivatives **12b** and **12d** emerged as a single-digit nanomolar hCA IX inhibitors with K_I values of 8.3 and 7.9 nM, respectively, which are 5.5-fold more potent than SLC-0111 (K_I = 45.0 nM against hCA IX) and 3-fold more potent than the standard drug **AAZ** (K_I = 25.0 nM against hCA IX). Also, compounds **11c** (K_I = 13.8 nM) and **12c** (K_I = 12.4 nM) were 3.6 times more potent than SLC-0111 and 2 times more active than AAZ.

Noteworthy, the SAR outcomes highlighted that incorporation of thiadiazole moiety (compounds **12a–d**; K_Is: 7.9–143.4 nM) is more advantageous for activity towards hCA IX than the thiazole moiety

Table 2
Selectivity indexes for the inhibition of hCA IX and XII over hCA I and II for ureides (**11a–d** and **12a–d**), SLC-0111 and acetazolamide.

Cmpd	I/IX	II/IX	I/XII	II/XII
11a	6.6	5.4	10.7	8.9
11b	4.2	0.3	3.8	0.2
11c	13.9	0.7	6.9	0.3
11d	5.2	0.4	4.9	0.4
12a	49.8	4.7	82.1	7.7
12b	19.6	3.2	17.3	2.8
12c	32.0	0.7	15.2	0.3
12d	78.9	5.7	63.0	4.5
SLC-0111	112.9	21.3	1128.9	213.3
AAZ	10.0	0.5	43.9	2.2

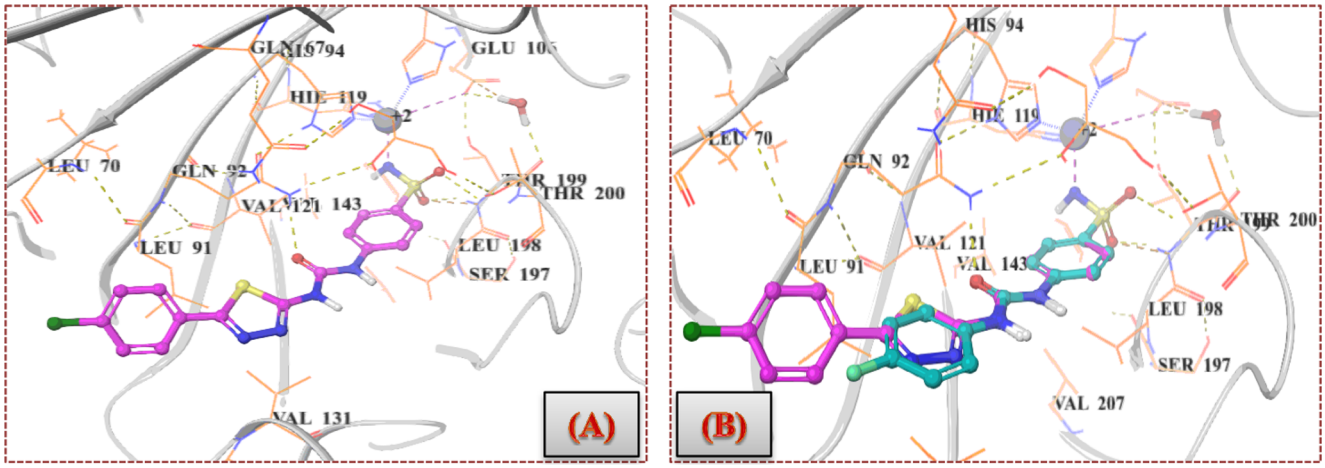


Fig. 2. (A) Docking pose of compound **12d** within the active site of hCA IX (PDB 3IAI) showing the favorable interactions with key amino acids and Zn metal. **(B)** Structural superposition of both compounds **12d** and **SLC-0111** within the active site of hCA IX (PDB 3IAI) illustrating the extra hydrophobic interactions of (4-chlorophenyl) ring. Protein unit was illustrated as grey ribbon except the amino acids of active site were drawn as lines with orange carbon atoms. Hydrogen bonds were shown as yellow dots, metallic interaction were shown as violet dots and polar hydrogens only were visible for clarity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(compounds **11a–d**; K_Is: 13.8–153.0 nM). In addition, grafting 4-aryl or 5-aryl substituents in the first (**11b–d**; K_Is: 13.8–64.7 nM) and second (**12b–d**; K_Is: 7.9–12.4 nM) series resulted in about 11–2.3 and 18–11.5 fold efficacy enhancement in comparison to their unsubstituted analogues **11a** (K_I = 153.0 nM) and **12a** (K_I = 143.4 nM), respectively. Regarding, the impact of substitution with aryl moieties within the first series **11**; the inhibitory activities were decreased in the order of 4-F-C_6H_4 > 4-Cl-C_6H_4 > C_6H_5, whereas the decreasing order for the second series **12** was 4-Cl-C_6H_4 > C_6H_5 > 4-F-C_6H_4.

(**v**) The second tumor-associated isoform examined here was hCA XII [20]. It was apparent from the obtained results, Table 1, that the newly prepared sulfonamides **11a–d** and **12a–d** exhibited high to excellent inhibitory activities towards hCA XII with K_Is in the range of 94–9.4 nM. In particular, thiadiazolo derivatives **12b** and **12d** were found to be the most potent hCA XII inhibitors with K_I values equal to 9.4 and 9.9 nM, respectively. Additionally, compounds **11c** and **12c** showed high inhibitory activity with K_I values of 27.6 and 26.2 nM, respectively. Similar to the SAR for hCA IX inhibition; both incorporation of thiadiazole moiety rather than thiazole moiety, and grafting an aryl substituent within the first and second series are more beneficial for inhibitory activity against hCA XII.

(**vi**) As a result of the inhibitory profiles of the prepared ureides listed in Table 1, the selectivity index (SI) for each of the primary sulfonamides (**11a–d** and **12a–d**) herein reported was calculated and displayed in Table 2. Regarding selectivity towards hCA IX and XII over hCA I, all the examined primary sulfonamides exhibited good SIs spanning in the range 4.2–78.9 and 3.8–82.1, respectively. Meanwhile, only compounds **11a**, **12a**, **12b** and **12d** were found to be selective towards hCA IX and XII over hCA II with SIs in the range of 3.2–5.7 and 2.8–8.9, respectively.

In summary, the adopted bioisosteric replacement approach in this study to replace the 4-fluorophenyl tail of SLC-0111 with different thiazole and thiadiazole tails, successfully improved the inhibitory activity towards hCA IX, such as ureas **12b** and **12d** that possessed 5.5-fold more potent activity than SLC-0111 towards hCA IX. Unfortunately, such improved activity against hCA IX was accompanied with an enhanced activity towards hCA II, too. This trend resulted in a decreasing of the hCA IX/II selectivity for both compounds **12b** and **12d** by 6.6- and 3.7-fold in comparison to SLC-0111, respectively.

In terms of both hCA IX inhibitory activity and selectivity, compound **12d** would be considered as a promising lead molecule for the development of further effective CA inhibitors candidates targeting the tumor-associated isoforms hCA IX.

3. Molecular modeling studies

Molecular docking simulations were performed in order to justify the inhibitory activity of compound **12d** by investigating its interactions with the key amino acids within the active site of hCA IX isoform (PDB 3IAI). As shown in Fig. 2, the interaction of **12d** was found to be analogous to SLC-0111 in many points. The primary sulfonamide moiety make its usual role as zinc-binding group with the additional hydrogen bonds with Thr199 (2.63 Å) and Thr200 (3.14 Å). Also, the carbonyl moiety of the ureido group of both **12d** and SLC-0111 can interact with Gln92 by forming a hydrogen bond (2.86 and 2.88 Å, respectively) in the middle of the pocket. Moreover, the thiadiazole tail possessed hydrophobic interactions with Val131, Gln92 and Leu91 which is similar to 4-fluourophenyl in SLC-0111. Pose analysis for the alignment of both **12d** and SLC-0111 (as shown in Fig. 2**B**), unveiled the ability of **12d** to establish an extra hydrophobic interaction between the terminal *p*-chlorophenyl moiety and Leu91, that could account for the enhancement of its hCA IX inhibition (Table 1).

4. Conclusion

In summary, we report here the synthesis of novel series of SLC-0111 analogues *via* replacement of 4-fluorophenyl tail of SLC-0111 with different un/substituted thiazole and thiadiazole tails. The structure of the novel analogues was confirmed by the different spectral and elemental analyses methods. The inhibition profiles of the newly synthetized compounds were determined against hCA I, II, IX and XII using the stopped-flow CO_2 hydrase assay. All the tertiary sulfonamides (**16a–c** and **17a–d**) failed to inhibit all the tested hCAs up to 10 μM, except **17a** that displayed weak inhibitory activity towards hCA II (K_I = 6.815 μM). Whereas, the tested isoforms were inhibited by the synthesized primary sulfonamides (**11a–d** and **12a–d**) in variable degrees with K_Is range: 162.6–7136 nM for hCA I, 9.0–833.6 nM for hCA II, 7.9–153.0 nM for hCA XI, and 9.4–94.0 nM for hCA XII. In particular, compounds **11c** and **12b–d** showed superior inhibitory activity against hCA IX with 3.3–5.7-fold enhanced potency than SLC-0111. The weightiness of incorporation of thiadiazole tail, rather than a thiazole one, and grafting an aryl substituent within the first and second series for the inhibitory activity against hCA IX and XII, was explored *via* the SAR study. Moreover, the molecular docking for **12d** unveiled its ability to establish an extra hydrophobic interaction between the terminal *p*-chlorophenyl moiety and Leu91 that could account for the enhancement of its hCA IX inhibition.

5. Experimental

5.1. Chemistry

5.1.1. General

Melting points were measured with a Stuart melting point apparatus and were uncorrected. Infrared (IR) Spectra were recorded as KBr disks using Schimadzu FT-IR 8400S spectrophotometer. Mass spectral data are given by GCMS-QP1000 EX spectrometer at 70 e.V. NMR Spectra were recorded on Bruker spectrometer. ^{1}H spectrum was run at 400 MHz and ^{13}C spectrum was run at 100 MHz in deuterated dimethylsulfoxide (DMSO-d_6). Chemical shifts are expressed in values (*ppm*) using the solvent peak as internal standard. All coupling constant (*J*) values are given in hertz. The abbreviations used are as follows: s, singlet; d, doublet; m, multiplet. Elemental analyses were carried out at the Regional Center for Microbiology and Biotechnology, Al-Azhar University, Cairo, Egypt. High-resolution mass spectra were recorded using a Bruker MicroTOF spectrometer. Analytical thin layer chromatography (TLC) on silica gel plates containing UV indicator was employed routinely to follow the course of reactions and to check the purity of products.

5.1.2. Synthesis of compounds 7a–e and 10a–d

Compounds **7a–e** and **10a–d** were prepared according to the literature procedures [21-23].

5.1.3. Synthesis of isocyanate derivatives 4,15

A stirred suspension of 4-sulfamoylbenzoic acid **1** (1 gm, 5 mmol) in $SOCl_2$ (30 mL) was refluxed for 12 h in the absence of DMF, or refluxed for 5 h in the presence of DMF (0.73 gm, 10 mmol). The excess of thionyl chloride was removed by repeated evaporation with dry benzene *in vacuo*. The obtained crude products of aroyl chlorides **2** and **13**, respectively, are used in the next step without further purification. Then, an aqueous solution of sodium azide (0.65 gm, 10 mmole) was added to the solution of compounds **2** and **13** (5 mmol) in dry acetone (10 mL) at 0 °C. The reaction mixture was stirred for 2 h at room temperature. The obtained solid was filtered off and washed with cold water then petroleum ether to give aroyl azides **3** and **14**.

5.1.4. Synthesis of target compounds 11a–d, 12a–d, 16a–c and 17a–d

Both of azides **3** and **14** (1 mmol) are subjected to Curtius Rearrangement to afford the isocyanate derivatives **4** and **15**, respectively, *via* heating under reflux temperature in dry toluene (10 mL) for 1 h. To this hot solution of **4** and **15** in toluene, the appropriate aminothiazole (**7a–e**) and aminothiadiazole (**10a–d**) derivatives (1 mmol) were added. This mixture was further heated under reflux for 4 h. The formed precipitate was filtered off while hot and washed with ethanol and petroleum ether, then recrystallized from DMF/ethanol to obtain ureides **11a–d**, **12a–d**, **16a–c** and **17a–d**.

5.1.4.1. 4-(3-(Thiazol-2-yl)ureido)benzenesulfonamide ***11a***. Yellow crystal (yield 55%), m.p. 228–230 °C; IR (KBr, ν cm^{-1}): 3313, 3332, 3375 (NH_2, 2NH), 1712 (C═O) and 1149, 1315 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 7.14 (d, 1H, H-5 of thiazole ring, J = 8.0 Hz), 7.26 (s, 2H, NH_2, D_2O exchangeable), 7.39 (d, 1H, H-4 of thiazole ring, J = 8.0 Hz), 7.65 (d, 2H, H-3 and H-5 of $-C_6H_4-SO_2NH_2$, J = 8.0 Hz), 7.75 (d, 2H, H-2 and H-6 of $-C_6H_4-SO_2NH_2$, J = 8.0 Hz), 9.34 (s, 1H, NH urea, D_2O exchangeable), 10.05 (s, 1H, NH urea, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz) δ *ppm*: 118.36, 126.14, 127.35, 128.14, 138.11, 142.36, 146.64, 159.21 (C═O); MS *m/z* [%]: 298.80 [M$^+$, 13.95], 48.26 [100]; Anal. Calcd. for $C_{10}H_{10}N_4O_3S_2$ (298.02): C, 40.26; H, 3.38; N, 18.78; found C, 40.01; H, 3.41; N, 18.81.

5.1.4.2. 4-(3-(4-Phenylthiazol-2-yl)ureido)benzenesulfonamide ***11b***. Buff crystal (yield 51%), m.p. 270–271 °C; IR (KBr, ν cm^{-1}): 3259, 3348, 3498 (NH_2, 2NH), 1701 (C═O) and 1153, 1319 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 7.29 (s, 2H, NH_2, D_2O exchangeable), 7.42–7.45 (m, 3H, H-3, H-4 and H-5 of $-C_6H_5$), 7.59 (s, 1H, H-5 of thiazole ring), 7.67 (d, 2H, H-3 and H-5 of $-C_6H_4-SO_2NH_2$, J = 8.0 Hz), 7.78 (d, 2H, H-2 and H-6 of $-C_6H_4-SO_2NH_2$, J = 8.0 Hz), 7.89 (d, 2H, H-2 and H-6 of $-C_6H_5$, J = 8.0 Hz), 9.35 (s, 1H, NH urea, D_2O exchangeable), 10.91 (s, 1H, NH urea, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz) δ *ppm*: 107.91, 118.51, 126.08, 127.39, 128.22, 129.19, 134.67, 138.30, 142.14, 149.23, 151.93, 159.25 (C═O); Anal. Calcd. for $C_{16}H_{14}N_4O_3S_2$ (374.05): C, 51.32; H, 3.77; N, 14.96; found C, 51.22; H, 3.75; N, 14.92.

5.1.4.3. 4-(3-(4-(4-Fluorophenyl)thiazol-2-yl)ureido)benzenesulfonamide ***11c***. Buff crystal (yield 60%), m.p. 240–242 °C; IR (KBr, ν cm^{-1}): 3390, 3417, 3444 (NH_2, 2NH), 1712 (C═O) and 1145, 1327 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 7.25–7.29 (m, 4H; 2H of SO_2NH_2, H-3 and H-5 of 4-F-C_6H_4), 7.56 (s, 1H, H-5 of thiazole ring), 7.68 (d, 2H, H-3 and H-5 of $-C_6H_4-SO_2NH_2$, J = 8.0 Hz), 7.77 (d, 2H, H-2 and H-6 of $-C_6H_4-SO_2NH_2$, J = 8.0 Hz), 7.92 (t, 2H, H-2 and H-6 of 4-F-C_6H_4, J = 8.0 Hz), 9.67 (s, 1H, NH urea, D_2O exchangeable), 11.10 (s, 1H, NH urea, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz) δ *ppm*: 107.62, 115.91, 116.12, 118.45, 120.33, 126.12, 127.36, 128.05, 128.13, 131.37, 138.21, 142.29, 152.10, 159.49 (C═O); Anal. Calcd. for $C_{16}H_{13}FN_4O_3S_2$ (392.04): C, 48.97; H, 3.34; N, 14.28; found C, 48.90; H, 3.38; N, 14.22.

5.1.4.4. 4-(3-(4-(4-Chlorophenyl)thiazol-2-yl)ureido)benzenesulfonamide ***11d***. Red crystal (yield 49%), m.p. 239–240 °C; IR (KBr, ν cm^{-1}): 3375, 3387, 3390 (NH_2, 2NH), 1712 (C═O) and 1145, 1327 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 7.28 (s, 2H, NH_2, D_2O exchangeable), 7.48–7.51 (m, 3H; H-5 of thiazole ring, H-3 and H-5 of 4-Cl-C_6H_4), 765 (d, 2H, H-3 and H-5 of $-C_6H_4-SO_2NH_2$, J = 8.0 Hz), 7.78 (d, 2H, H-2 and H-6 of $-C_6H_4-SO_2NH_2$, J = 8.0 Hz), 7.91 (d, 2H, H-2 and H-6 of 4-Cl-C_6H_4, J = 8.0 Hz), 9.39 (s, H, NH urea, D_2O exchangeable), 10.95 (s, 2H, NH urea, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz) δ *ppm*: 108.71, 117.95, 118.52, 126.12, 127.39, 127.79, 128.11, 129.21, 132.63, 133.55, 138.33, 142.10, 146.58, 147.98, 151.93, 159.44 (C═O); HRMS (ESI) for $C_{16}H_{14}ClN_4O_3S_2$, calcd 409.01904, found 409.01901 [M + H]$^+$; Anal. Calcd. for $C_{16}H_{13}ClN_4O_3S_2$ (408.01): C, 47.00; H, 3.20; N, 13.70; found C, 47.07; H, 3.15; N, 13.68.

5.1.4.5. 4-(3-(1,3,4-Thiadiazol-2-yl)ureido)benzenesulfonamide ***12a***. White crystal (yield 62%), m.p. 240–242 °C; IR (KBr, ν cm^{-1}): 3345, 3380, 3391 (NH_2, 2NH), 1711 (C═O) and 1149, 1315 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 7.27 (s, 2H, NH_2, D_2O exchangeable), 7.67 (d, 2H, H-3 and H-5 of $-C_6H_4-SO_2NH_2$, J = 8.0 Hz), 7.76 (d, 3H; H-5 of thiadiazole ring, H-2 and H-6 of $-C_6H_4-SO_2NH_2$, J = 8.0 Hz), 9.13 (s, 1H, NH urea, D_2O exchangeable), 9.47 (s, 1H, NH urea, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz) δ *ppm*: 118.65, 127.33, 138.45, 140.48, 143.74, 148.50, 162.83 (C═O); Anal. Calcd. for $C_9H_9N_5O_3S_2$ (299.01): C, 36.11; H, 3.03; N, 23.40; found C, 36.01; H, 3.10; N, 23.31.

5.1.4.6. 4-(3-(5-Phenyl-1,3,4-thiadiazol-2-yl)ureido)benzenesulfonamide ***12b***. Yellow crystal (yield 50%), m.p. 277–279 °C; IR (KBr, ν cm^{-1}): 3371, 3417, 3470 (NH_2, 2NH), 1708 (C═O) and 1150, 1318 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 7.46–7.59 (m, 5H; 2H of SO_2NH_2, H-3, H-4 and H-5 of $-C_6H_5$), 7.70 (d, 2H, H-3 and H-5 of $-C_6H_4-SO_2NH_2$, J = 8.0 Hz), 7.78 (d, 2H, H-2 and H-6 of $-C_6H_4-SO_2NH_2$, J = 8.0 Hz), 7.91 (d, 2H, H-2 and H-6 of $-C_6H_5$, J = 8.0 Hz), 9.20 (s, 1H, NH urea, D_2O exchangeable), 9.55 (s, 1H, NH urea, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz) δ *ppm*: 118.17, 118.71, 126.86, 126.92, 127.12, 127.33, 129.72, 129.85, 130.65, 131.06, 138.51, 142.22, 152.87, 161.16 (C═O); MS *m/z* [%]:375.27 [M$^+$, 74.13], 75.34 [100]; Anal. Calcd. for $C_{15}H_{13}N_5O_3S_2$ (375.05): C, 47.99; H, 3.49; N, 18.66; found C, 47.90; H, 3.44; N, 18.60.

5.1.4.7. 4-(3-(5-(4-Fluorophenyl)-1,3,4-thiadiazol-2-yl)ureido) benzenesulfonamide ***12c***. Buff crystal (yield 48%), m.p. 255–258 °C; IR (KBr, ν cm^{-1}): 3380, 3407, 3414 (NH_2, 2NH), 1710 (C=O) and 1147, 1325 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 7.28–7.40 (m, 4H; 2H of SO_2NH_2, H-3 and H-5 of 4-F-C_6H_4), 7.69 (d, 2H, H-3 and H-5 of $-C_6H_4-SO_2NH_2$, J = 8.0 Hz), 7.77 (d, 2H, H-2 and H-6 of $-C_6H_4-O_2NH_2$, J = 8.0 Hz), 7.79 (br s, 2H, H-2 and H-6 of 4-F-C_6H_4), 9.21 (s, 1H, NH urea, D_2O exchangeable), 9.56 (s, 1H, NH urea, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz) δ *ppm*: 116.80, 117.02, 118.21, 118.71, 126.90, 127.33, 129.41, 138.47, 142.18, 145.52, 154.10, 162.52 (C=O); MS *m/z* [%]: 393.14 [M^+, 43.52], 122.48 [100]; Anal. Calcd. for $C_{15}H_{12}FN_5O_3S_2$ (393.04): C, 45.80; H, 3.07; N, 17.80; found C, 45.70; H, 3.10; N, 17.83.

5.1.4.8. 4-(3-(5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-yl)ureido) benzenesulfonamide ***12d***. Buff crystal (yield 45%), m.p. 265–267 °C; IR (KBr, ν cm^{-1}): 3370, 3385, 3390 (NH_2, 2NH), 1714 (C=O) and 1145, 1327 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 7.48–7.60 (m, 4H; 2H of SO_2NH_2, H-3 and H-5 of 4-Cl-C_6H_4), 7.69 (d, 2H, H-3 and H-5 of $-C_6H_4-SO_2NH_2$, J = 8.0 Hz), 7.77 (d, 2H, H-2 and H-6 of $-C_6H_4-SO_2NH_2$, J = 8.0 Hz), 7.93 (d, 2H, H-2 and H-6 of 4-Cl-C_6H_4, J = 8.0 Hz), 9.22 (s, 1H, NH urea, D_2O exchangeable), 9.57 (s, 1H, NH urea, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz) δ *ppm*: 118.74, 12.33, 128.48, 128.81, 129.53, 129.70, 129.89, 135.56, 138.50, 142.13, 147.69, 152.80, 162.34 (C=O); Anal. Calcd. for $C_{15}H_{12}ClN_5O_3S_2$ (409.01): C, 43.96; H, 2.95; N, 17.09; found C, 43.87; H, 2.99; N, 17.13.

5.1.4.9. N′-((4-(3-(4-(4-Fluorophenyl)thiazol-2-yl)ureido)phenyl) sulfonyl)-N,N-dimethylformimidamide ***16a***. White crystal (yield 79%), m.p. 232–234 °C; IR (KBr, ν cm^{-1}): 3383, 3401 (2NH), 1711 (C=O) and 1140, 1355 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.91 (s, 3H, N−(CH_3)$_2$), 3.15 (s, 3H, N−(CH_3)$_2$), 7.22 (t, 2H, H-3, H-5 of 4-F-C_6H_4, J = 8.0 Hz), 7.51 (s, 1H, =CH), 7.61 (d, 2H, H-3, H-5 of $-C_6H_4-SO_2N$ J = 8.0 Hz), 7.69 (d, 2H, H-2, H-6 of $-C_6H_4-SO_2N$, J = 8.0 Hz), 7.91 (t, 2H, H-2, H-6 of 4-F-C_6H_4, J = 8.0 Hz), 8.19 (s, 1H, H-5 of thiazole ring), 9.85 (s, 1H, NH urea, D_2O exchangeable), 11.09 (s, 1H, NH urea, D_2O exchangeable); Anal. Calcd. for $C_{19}H_{18}FN_5O_3S_2$ (447.08): C, 51.00; H, 4.05; N, 15.65; found C, 51.10; H, 4.00; N, 15.60.

5.1.4.10. N′-((4-(3-(4-(4-Chlorophenyl)thiazol-2-yl)ureido)phenyl) sulfonyl)-N,N-dimethylformimidamide ***16b***. White crystal (yield 79%), m.p. 250–253 °C; IR (KBr, ν cm^{-1}): 3363, 3444 (2NH), 1701 (C=O) and 1141, 1338 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.91 (s, 3H, N−(CH_3)$_2$), 3.15 (s, 3H, N−(CH_3)$_2$), 7.46 (d, 2H, H-3, H-5 of 4-Cl-C_6H_4, J = 8.0 Hz), 7.60–7.62 (m, 3H; H-3, H-5 of $-C_6H_4-SO_2N$, 1H of =CH), 7.71 (d, 2H, H-2, H-6 of $-C_6H_4-SO_2N$, J = 8.0 Hz), 7.91 (d, 2H, H-2, H-6 of 4-Cl-C_6H_4, J = 8.0 Hz), 8.20 (s, 1H, H-5 of thiazole ring), 9.83 (s, 1H, NH urea, D_2O exchangeable), 11.09 (s, 1H, NH urea, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz) δ *ppm*: 35.47, 41.31 (*N*-(CH_3)$_2$), 108.51, 118.40, 127.71, 127.83, 129.15, 132.55, 133.62, 136.94, 142.30, 147.95, 152.34, 159.80, 160.01 (C=O of uera); HRMS (ESI) for $C_{19}H_{19}O_3N_5ClS_2$, calcd 464.06124, found 464.06145 $[M+H]^+$; Anal. Calcd. for $C_{19}H_{18}ClN_5O_3S_2$ (463.05): C, 49.19; H, 3.91; N, 15.10; found C, 49.10; H, 3.96; N, 15.01.

5.1.4.11. N′-((4-(3-(4-(4-Methoxyphenyl)thiazol-2-yl)ureido)phenyl) sulfonyl)-N,N-dimethylformimidamide ***16c***. Yellow crystal (yield 79%), m.p. 223–225 °C; IR (KBr, ν cm^{-1}): 3360, 3390 (2NH), 1708 (C=O) and 1153, 1342 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.91 (s, 3H, N−(CH_3)$_2$), 3.15 (s, 3H, N−(CH_3)$_2$), 3.80 (s, 3H, OCH_3), 6.98 (d, 2H, H-3, H-5 of 4-CH_3O-C_6H_4, J = 8.0 Hz), 7.41 (s, 1H, =CH), 7.62 (d, 2H, H-2, H-6 of 4-CH_3O-C_6H_4, J = 8.0 Hz), 7.72 (d, 2H, H-3, H-5 of $-C_6H_4-SO_2N$, J = 8.0 Hz), 7.81 (d, 2H, H-2, H-6 of $-C_6H_4-SO_2N$, J = 8.0 Hz), 8.21 (s, 1H, H-5 of thiazole ring), 9.27 (s, 1H, NH urea, D_2O exchangeable), 10.78 (s, 1H, NH urea, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz) δ *ppm*: 35.48, 41.32 (*N*-(CH_3)$_2$), 55.61 (OCH_3), 105.79, 114.54, 118.60, 125.77, 127.43, 127.69, 128.66, 129.36, 137.15, 142.13, 151.88, 159.43, 160.02 (C=O of uera); Anal. Calcd. for $C_{20}H_{21}N_5O_4S_2$ (459.10): C, 52.27; H, 4.61; N, 15.24; found C, 52.11; H, 4.67; N, 15.27.

5.1.4.12. N′-((4-(3-(1,3,4-Thiadiazol-2-yl)ureido)phenyl)sulfonyl)-N,N-dimethylformimidamide ***17a***. White crystal (yield 79%), m.p. 230–231 °C; IR (KBr, ν cm^{-1}): 3373, 3435 (2NH), 1709 (C=O) and 1141, 1338 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.91 (s, 3H, N−(CH_3)$_2$), 3.15 (s, 3H, N−(CH_3)$_2$), 7.66 (d, 2H, H-3, H-5 of $-C_6H_4-SO_2N$, J = 8.0 Hz), 7.95 (d, 2H, H-2, H-6 of $-C_6H_4-SO_2N$, J = 8.0 Hz), 8.19 (s, 1H, =CH), 8.94 (s, 1H, H-5 of thiazole ring), 9.20 (s, 1H, NH urea, D_2O exchangeable), 11.96 (s, 1H, NH urea, D_2O exchangeable); ^{13}C NMR (DMSO-d_6,100 MHz) δ *ppm*: 35.44, 41.29 (N−(CH_3)$_2$), 117.82, 127.45, 135.37, 144.51, 146.58, 156.99, 160.00, 166.94 (C=O); Anal. Calcd. for $C_{12}H_{14}N_6O_3S_2$ (354.06): C, 40.67; H, 3.98; N, 23.71; found C, 40.72; H, 3.91; N, 23.65.

5.1.4.13. N,N-Dimethyl-N′-((4-(3-(5-phenyl-1,3,4-thiadiazol-2-yl)ureido) phenyl)sulfonyl)formimidamide ***17b***. White crystal (yield 79%), m.p. 240–241 °C; IR (KBr, ν cm^{-1}): 3383, 3401 (2NH), 1711 (C=O) and 1147, 1345 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.94 (s, 3H, N−(CH_3)$_2$), 3.18 (s, 3H, N−(CH_3)$_2$), 7.42–7.56 (m, 3H, H-3, H-4, H-5 of $-C_6H_5$), 7.71–7.77 (m, 2H, H-3, H-5 of $-C_6H_4-SO_2N$), 7.87–8.02 (m, 4H; H-2, H-6 of $-C_6H_4-SO_2N$, H-2, H-6 of $-C_6H_5$), 8.23 (s, 1H, =CH), 8.50 (s, 1H, NH urea, D_2O exchangeable), 11.62 (s, 1H, NH urea, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz) δ *ppm*: 35.45, 41.30 (N−(CH_3)$_2$), 117.92, 126.87, 127.48, 127.99, 129.66, 130.05, 130.38, 131.92, 135.49, 144.38, 156.07, 159.46, 159.92, 165.15 (C=O); Anal. Calcd. for $C_{18}H_{18}N_6O_3S_2$ (430.09): C, 50.22; H, 4.21; N, 19.52; found C, 50.15; H, 4.23; N, 19.54.

5.1.4.14. N′-((4-(3-(5-(4-Fluorophenyl)-1,3,4-thiadiazol-2-yl)ureido) phenyl)sulfonyl)-N,N-dimethylformimidamide ***17c***. Buff crystal (yield 79%), m.p. 249–250 °C; IR (KBr, ν cm^{-1}): 3375, 3395 (2NH), 1720 (C=O) and 1150, 1348 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.97 (s, 3H, *N*−(CH_3)$_2$), 3.20 (s, 3H, N−(CH_3)$_2$), 7.37–7.69 (m, 4H, 2H, H-3, H-5 of 4-F-C_6H_4, 2H, H-3, H-5 of $-C_6H_4-SO_2N$), 7.73–7.86 (m, 2H, H-2, H-6 of $-C_6H_4-SO_2N$), 8.0–8.10 (m, 2H, H-2, H-6 of 4-F-C_6H_4), 8.27 (s, 1H, =CH), 8.65 (s, 1H, NH urea, D_2O exchangeable), 11.48 (s, 1H, NH urea, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz) δ *ppm*: 35.51, 42.09 (*N*−(CH_3)$_2$), 116.91, 118.08, 123.68, 125.69, 127.67, 129.16, 131.63, 133.16, 136.15, 144.17, 152.49, 158.64, 159.95, 162.15 (C=O); Anal. Calcd. for $C_{18}H_{17}FN_6O_3S_2$ (448.08): C, 48.21; H, 3.82; N, 18.74; found C, 48.10; H, 3.84; N, 18.71.

5.1.4.15. N′-((4-(3-(5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-yl)ureido) phenyl)sulfonyl)-N,N-dimethylformimidamide ***17d***. White crystal (yield 79%), m.p. 225–228 °C; IR (KBr, ν cm^{-1}): 3388, 3441 (2NH), 1717 (C=O) and 1149, 1342 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.91 (s, 3H, N−(CH_3)$_2$), 3.14 (s, 3H, N−(CH_3)$_2$), 7.55 (d, 2H, H-3, H-5 of 4-Cl-C_6H_4, J = 8.0 Hz), 7.64 (d, 2H, H-3, H-5 of $-C_6H_4-SO_2N$, J = 8.0 Hz), 7.81 (d, 2H, H-2, H-6 of $-C_6H_4-SO_2N$, J = 8.0 Hz), 7.90 (d, 2H, H-2, H-6 of 4-Cl-C_6H_4, J = 8.0 Hz), 8.18 (s, 1H, =CH), 8.45 (s, 1H, NH urea, D_2O exchangeable), 11.36 (s, 1H, NH urea, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz) δ *ppm*: 35.45, 41.29 (N−(CH_3)$_2$), 117.80, 124.03, 127.42, 128.30, 129.64, 130.96, 131.86, 134.19, 135.20, 144.67, 156.00, 157.83, 159.89, 161.21 (C=O); Anal. Calcd. for $C_{18}H_{17}ClN_6O_3S_2$ (464.05): C, 46.50; H, 3.69; N, 18.08; found C, 46.40; H, 3.64; N, 18.01.

5.2. Biological evaluation

5.2.1. CA inhibitory assay

An Applied Photophysics stopped-flow instrument has been used for

assaying the CA catalysed CO_2 hydration activity [19]. 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 [24–28], and represent the mean from at least three different determinations. The four tested CA isofoms were recombinant ones obtained in-house as reported earlier [24–28].

5.3. Molecular docking simulation

The crystal structure of hCA IX (PDB 3IAI [29]) was downloaded from protein databank for docking calculations using the Protein Preparation wizard in Maestro - Schrödinger suite 2017–1, using the default values in the preprocess. The water molecules were deleted and the default input parameters and Prime were used for adding missing side chains or loops, also, optimization of the hydrogen bonds was done with refinement process by running a restrained minimization (OPLS3 force field) which was controlled by cutoff RMSD value of heavy atoms reached 0.30 Å. The 3D ligand structures were generated with Maestro (Schrödinger suite). LigPrep was used to prophesy ionization form of the ligands using a pH of 7.0 ± 0.5 with Epik. In docking simulations using Glide, the protein was treated as rigid, compounds were flexibly docked, and scoring was assigned according to the extra precision (XP) mode. Before docking process, the grid box was prepared according to the default size. The constrained were assigned using the metallic bond for Zn metal [30–32].

References

[1] (a) A. Innocenti, I. Gülçin, S.B.Ö. Sarıkaya, C.T. Supuran, Carbonic anhydrase inhibitors. Inhibition of mammalian isoforms I-XIV with a series of natural product polyphenols and phenolic acids, Bioorg. Med. Chem. 18 (2010) 2159–2164; (b) A. Innocenti, I. Gülçin, A. Scozzafava, C.T. Supuran, Carbonic anhydrase inhibitors. Antioxidant polyphenols effectively inhibit mammalian isoforms I–XV, Bioorg. Med. Chem. Lett. 20 (2010) 5050–5053.

[2] (a) F. Turkan, A. Cetin, P. Taslimi, M. Karaman, İ. Gulçin, Synthesis, biological evaluation and molecular docking of novel pyrazole derivatives as potent carbonic anhydrase and acetylcholinesterase inhibitors, Bioorg. Chem. 86 (2019) 420–427; (b) K. Kucukoglu, H. InciGul, P. Taslimi, İ. Gulçin, C.T. Supuran, Investigation of inhibitory properties of some hydrazone compounds on hCA I, hCA II and AChE enzymes, Bioorg. Chem. 86 (2019) 316–321.

[3] C.T. Supuran, Carbonic anhydrases: novel therapeutic applications for inhibitors and activators, Nat. Rev. Drug Discovery 7 (2008) 168–181.

[4] V. Alterio, A. Di Fiore, K. D'Ambrosio, C.T. Supuran, G. De Simone, Multiple binding modes of inhibitors to carbonic anhydrases: how to design specific drugs targeting 15 different isoforms? Chem. Rev. 112 (2012) 4421–4468.

[5] D. Neri, C.T. Supuran, Interfering with pH regulation in tumours as a therapeutic strategy, Nat. Rev. Drug Discov. 10 (2011) 767–777.

[6] G. De Simone, C.T. Supuran, Carbonic anhydrase IX: biochemical and crystallographic characterization of a novel antitumor target, Biochim. Biophys. Acta 2010 (1804) 404–409.

[7] Y. Lou, P.C. McDonald, A. Oloumi, S. Chia, C. Ostlund, A. Ahmadi, A. Kyle, U. Auf dem Keller, S. Leung, D. Huntsman, B. Clarke, B.W. Sutherland, D. Waterhouse, M. Bally, C. Roskelley, C.M. Overall, A. Minchinton, F. Pacchiano, F. Carta, A. Scozzafava, N. Touisni, J.Y. Winum, C.T. Supuran, S. Dedhar, Targeting tumor hypoxia: suppression of breast tumor growth and metastasis by novel carbonic anhydrase IX inhibitors, Cancer Res. 71 (2011) 3364–3376.

[8] F. Pacchiano, M. Aggarwal, B.S. Avvaru, A.H. Robbins, A. Scozzafava, R. McKenna, C.T. Supuran, Selective hydrophobic pocket binding observed within the carbonic anhydrase II active site accommodate different 4-substituted-ureido-benzenesulfonamides and correlate to inhibitor potency, Chem. Commun. (Camb) 46 (2010) 8371–8373.

[9] F. Pacchiano, F. Carta, P.C. McDonald, Y. Lou, D. Vullo, A. Scozzafava, S. Dedhar, C.T. Supuran, Ureido-substituted benzenesulfonamides potently inhibit carbonic anhydrase IX and show antimetastatic activity in a model of breast cancer metastasis, J. Med. Chem. 54 (2011) 1896–1902.

[10] Y. Lou, P.C. McDonald, A. Oloumi, S.K. Chia, C. Ostlund, A. Ahmadi, A. Kyle, U.A. Keller, S. Leung, D.G. Huntsman, B. Clarke, B.W. Sutherland, D. Waterhouse, M.B. Bally, C.D. Roskelley, C.M. Overall, A. Minchinton, F. Pacchiano, F. Carta, A. Scozzafava, N. Touisni, J.Y. Winum, C.T. Supuran, S. Dedhar, Targeting tumor hypoxia: suppression of breast tumor growth and metastasis by novel carbonic anhydrase IX inhibitors, Cancer Res. 71 (2011) 3364–3376.

[11] S.M. Monti, C.T. Supuran, G. De Simone, Anticancer carbonic anhydrase inhibitors: a patent review (2008 e 2013), Expert Opin. Ther. Pat. 23 (2013) (2008) 737–749.

[12] P.C. McDonald, J.Y. Winum, C.T. Supuran, S. Dedhar, Recent developments in targeting carbonic anhydrase IX for cancer therapeutics, Oncotarget 3 (2012) 84–97.

[13] C.T. Supuran, Carbonic anhydrases: from biomedical applications of the inhibitors and activators to biotechnological use for CO2 capture, J. Enzyme Inhib. Med. Chem. 28 (2013) 229–230.

[14] W.M. Eldehna, M.F. Abo-Ashour, E. Berrino, D. Vullo, H.A. Ghabbour, S.T. Al-Rashood, G.S. Hassan, H.M. Alkahtani, A.A. Almehizia, A. Alharbi, SLC-0111 enaminone analogues, 3/4-(3-aryl-3-oxopropenyl) aminobenzenesulfonamides, as novel selective subnanomolar inhibitors of the tumor-associated carbonic anhydrase isoform IX, Bioorg. Chem. 83 (2019) 549–558.

[15] W.M. Eldehna, M.F. Abo-Ashour, A. Nocentini, R.S. El-Haggar, S. Bua, A. Bonardi, S.T. Al-Rashood, G.S. Hassan, P. Gratteri, H.A. Abdel-Aziz, Enhancement of the tail hydrophobic interactions within the carbonic anhydrase IX active site via structural extension: Design and synthesis of novel *N*-substituted isatins-SLC-0111 hybrids as carbonic anhydrase inhibitors and antitumor agents, Eur. J. Med. Chem. 162 (2019) 147–160.

[16] A. Angeli, D. Tanini, T.S. Peat, L. Di Cesare Mannelli, G. Bartolucci, A. Capperucci, C. Ghelardini, C.T. Supuran, F. Carta, Discovery of new selenoureido analogues of 4-(4-fluorophenylureido) benzenesulfonamide as carbonic anhydrase inhibitors, ACS medicinal chemistry letters 8 (2017) 963–968.

[17] N. Lolak, S. Akocak, S. Bua, M. Koca, C.T. Supuran, Design and synthesis of novel 1, 3-diaryltriazene-substituted sulfonamides as potent and selective carbonic anhydrase II inhibitors, Bioorg. Chem. 77 (2018) 542–547.

[18] S. Akocak, N. Lolak, S. Bua, I. Turel, C.T. Supuran, Synthesis and biological evaluation of novel N, N′-diaryl cyanoguanidines acting as potent and selective carbonic anhydrase II inhibitors, Bioorg. Chem. 77 (2018) 245–251.

[19] R.G. Khalifah, The carbon dioxide hydration activity of carbonic anhydrase I. Stopflow kinetic studies on the native human isoenzymes B and C, J. Biol. Chem. 246 (1971) 2561–2573.

[20] M. Boztas, Y. Cetinkaya, M. Topal, İ. Gülçin, A. Menzek, E. Sahin, M. Tanc, C.T. Supuran, Synthesis and carbonic anhydrase isoenzymes I, II, IX, and XII inhibitory effects of dimethoxybromophenol derivatives incorporating cyclopropane moieties, J. Med. Chem 58 (2014) 640–650.

[21] J. Ramprasad, N. Nayak, U. Dalimba, P. Yogeeswari, D. Sriram, S. Peethambar, R. Achur, H.S. Kumar, Synthesis and biological evaluation of new imidazo [2, 1-b] [1, 3, 4] thiadiazole-benzimidazole derivatives, Eur. J. Med. Chem. 95 (2015) 49–63.

[22] A. Geronikaki, G. Theophilidis, Synthesis of 2-(aminoacetylamino) thiazole derivatives and comparison of their local anaesthetic activity by the method of action potential, Eur. J. Med. Chem. 27 (1992) 709–716.

[23] J. Banothu, K. Vaarla, R. Bavantula, P.A. Crooks, Sodium fluoride as an efficient catalyst for the synthesis of 2, 4-disubstituted-1, 3-thiazoles and selenazoles at ambient temperature, Chin. Chem. Lett. 25 (2014) 172–175.

[24] W.M. Eldehna, M. Fares, M. Ceruso, H.A. Ghabbour, S.M. Abou-Seri, H.A. Abdel-Aziz, D.A. El Ella, C.T. Supuran, Amido/ureidosubstituted benzenesulfonamides-isatin conjugates as low nanomolar/subnanomolar inhibitors of the tumor-associated carbonic anhydrase isoform XII, Eur. J. Med. Chem. 110 (2016) 259–266.

[25] W.M. Eldehna, M.F. Abo-Ashour, A. Nocentini, P. Gratteri, H.I. Eissa, M. Fares, O.E. Ismael, H.A. Ghabbour, M.M. Elaasser, H.A. Abdel-Aziz, C.T. Supuran, Novel 4/3-((4-oxo-5-(2-oxoindolin-3-ylidene) thiazolidin-2-ylidene) amino) benzenesulfonamides: synthesis, carbonic anhydrase inhibitory activity, anticancer activity and molecular modelling studies, Eur. J. Med. Chem. 139 (2017) 250–262.

[26] W.M. Eldehna, G.H. Al-Ansary, S. Bua, A. Nocentini, P. Gratteri, A. Altoukhy, H. Ghabbour, H.Y. Ahmed, C.T. Supuran, Novel indolin-2-one-based sulfonamides as carbonic anhydrase inhibitors: synthesis, in vitro biological evaluation against carbonic anhydrases isoforms I, II, IV and VII and molecular docking studies, Eur. J. Med. Chem. 127 (2017) 521–530.

[27] M.F. Abo-Ashour, W.M. Eldehna, A. Nocentini, H.S. Ibrahim, S. Bua, S.M. Abou-Seri, C.T. Supuran, Novel hydrazido benzenesulfonamides-isatin conjugates: synthesis, carbonic anhydrase inhibitory activity and molecular modeling studies, Eur. J. Med. Chem. 157 (2018) 28–36.

[28] M. Fares, R.A. Eladwy, A. Nocentini, S.R.A. El Hadi, H.A. Ghabbour, A. Abdel-Megeed, W.M. Eldehna, H.A. Abdel-Aziz, C.T. Supuran, Synthesis of bulky-tailed

sulfonamides incorporating pyrido [2, 3-d][1, 2, 4] triazolo [4, 3-a] pyrimidin-1 (5H)-yl) moieties and evaluation of their carbonic anhydrases I, II, IV and IX inhibitory effects, Bioorg. Med. Chem. 25 (2017) 2210–2217.
[29] V. Alterio, M. Hilvo, A. Di Fiore, C.T. Supuran, P. Pan, S. Parkkila, A. Scaloni, J. Pastorek, S. Pastorekova, C. Pedone, Crystal structure of the catalytic domain of the tumor-associated human carbonic anhydrase IX, Proc. Natl. Acad. Sci. 106 (2009) 16233–16238.
[30] L. Schrödinger, PyMOL Molecular Graphics System, Version, Schrödinger, LLC, New York, NY: 2015, 2016.
[31] Schrödinger Release 2018-1, Maestro, Schrödinger, LLC, New York, NY, 2018.
[32] Schrödinger Release 2018-1, LigPrep, Schrödinger, LLC, New York, NY, 2018.