





Design and synthesis of benzothiazole-based SLC-0111 analogues as new inhibitors for the cancer-associated carbonic anhydrase isoforms IX and XII

Tarfah Al-Warhi^a, Mostafa M. Elbadawi^b , Alessandro Bonardi^c, Alessio Nocentini^c , Ahmed A. Al-Karmalawy^d , Nada Aljaeed^a, Ohoud J. Alotaibi^a, Hatem A. Abdel-Aziz^e, Claudiu T. Supuran^c  and Wagdy M. Eldehna^b

^aDepartment of Chemistry, College of Science, Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia; ^bDepartment of Pharmaceutical Chemistry, Faculty of Pharmacy, Kafrelsheikh University, Kafr el-sheikh, Egypt; ^cDepartment of NE.UROFARBA, Section of Pharmaceutical and Nutraceutical Sciences, University of Florence, Polo Scientifico, Firenze, Italy; ^dDepartment of Pharmaceutical Medicinal Chemistry, Faculty of Pharmacy, Horus University-Egypt, Egypt; ^eDepartment of Applied Organic Chemistry, National Research Center, Dokki, Egypt

ABSTRACT

In this work, different series of benzothiazole-based sulphonamides **8a-c**, **10**, **12**, **16a-b** and carboxylic acids **14a-c** were developed as novel SLC-0111 analogues with the goal of generating potent carbonic anhydrase (CA) inhibitors. The adopted strategy involved replacing the 4-fluorophenyl tail in SLC-0111 with a benzothiazole motif that attached to the ureido linker to produce compounds **8c** and its regioisomers **8a-b**. In addition, the ureido spacer was elongated by methylene or ethylene groups to afford the counterparts **10** and **12**. In turn, the primary sulfamoyl zinc binding group (ZBG) was either substituted or replaced by carboxylic acid functionality in order to provide the secondary sulphonamide-based SLC-0111 analogues **16a-b**, and the carboxylic acid derivatives **14a-c**, respectively. All compounds (**8a-c**, **10**, **12**, **14a-c** and **16a-b**) were tested for their ability to inhibit CA isoforms CA I, II, IX and XII. Additionally, the *in vitro* anticancer properties of the developed CAs were evaluated.

ARTICLE HISTORY

Received 15 August 2022
Revised 5 September 2022
Accepted 9 September 2022

KEYWORDS

Metalloenzymes; sulphonamides; anticancer agents; hypoxic tumours; ureido derivatives





Introduction

Carbonic anhydrases (CAs, EC 4.2.1.1) are family of ubiquitous zinc-metalloenzymes present in the whole organisms¹. These enzymes catalyse the essential conversion of carbon dioxide and water to bicarbonate and proton in a crucial process accountable for diverse cellular activities such as electrolyte secretion, bone resorption, maintenance of acid-base balance, gluconeogenesis, CO₂ and pH homeostasis, calcification and tumorigenicity^{2–4}. The human CAs (*h*CAs) are relevant to α -CAs isozymes and sub-categorized into fifteen isoforms displaying distinct cellular distribution, levels of expression, kinetics and molecular features^{5,6}. Of special interest, the catalytic activity of CAs I-IV, VA, VB, VI, VII, IX, XII-XIV isoforms is due to the presence of three histidine residues in the active site in coordination with zinc⁷. Furthermore, *h*CAs are classified upon cellular distribution into cytosolic (*h*CAs I, II, III, VII and XIII), trans membrane (*h*CAs IV, IX, XII, and XIV), mitochondrial (*h*CAs VA and VB), and *h*CA VI is secreted in milk and saliva^{8,9}. The abnormal levels of these enzymes have been associated with many diseases; thus inhibitors of the CAs have potential applications in the treatment of glaucoma, edoema, obesity and mental problems^{1,7,9,10}.

Interestingly, the trans-membranal *h*CA IX and XII have shown diverse peculiarities over the other isoforms. *h*CA IX/XII isozymes have shown elevated expression in tumour cells and

are associated to hypoxic solid tumours inducing tumour growth and metastasis^{11,12}. Accordingly, the extremely desired selective inhibition of cancer-associated *h*CA IX/XII has been described as cutting-edge approach for the discovery of new small molecules for cancer treatment^{13–19}. As a consequence, several approaches have been employed to develop selective inhibitors for *h*CA IX/XII. Strikingly, tail approach stood out as the most successful and effective tool to improve the potency and selectivity of sulphonamide-based carbonic anhydrase inhibitors (CAIs)²⁰. In this context, tail approach has been devoted to generate selective sulphonamide CAIs which involves grafting of different molecular motifs (tails) to the aromatic/heterocyclic ring bearing a zinc binding group (ZBG) through a flexible spacer^{21,22}.

The ureido benzenesulfonamide SLC-0111 (Figure 1) has been developed utilising the tail approach as the first-in-class *h*CA IX inhibitor which, for the management of metastatic hypoxic solid tumours, is currently being investigated within phase I/II clinical trials^{3,23,24}. To date, numerous SLC-0111 analogues have been described with the prime aim to enhance potency and selectivity towards *h*CA IX exploiting bioisosteric replacement approach *via* replacement of the SLC-0111aryl tail by various sets of chemical scaffolds (Figure 1) like benzofuran **I**²³, thiazolo[3,2-*a*]benzimidazole **II**²⁵, triazine **III**²⁶, thiazole **IV**, and

CONTACT Claudiu T. Supuran  claudiu.supuran@unifi.it  Department of NE.UROFARBA, Section of Pharmaceutical and Nutraceutical Sciences, University of Florence, Polo Scientifico, Firenze 50121, Italy; Wagdy M. Eldehna  wagdy2000@gmail.com  Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Kafrelsheikh University, Kafr el-sheikh, Egypt

 Supplemental data for this article is available online at <https://doi.org/10.1080/14756366.2022.2124409>.

© 2022 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

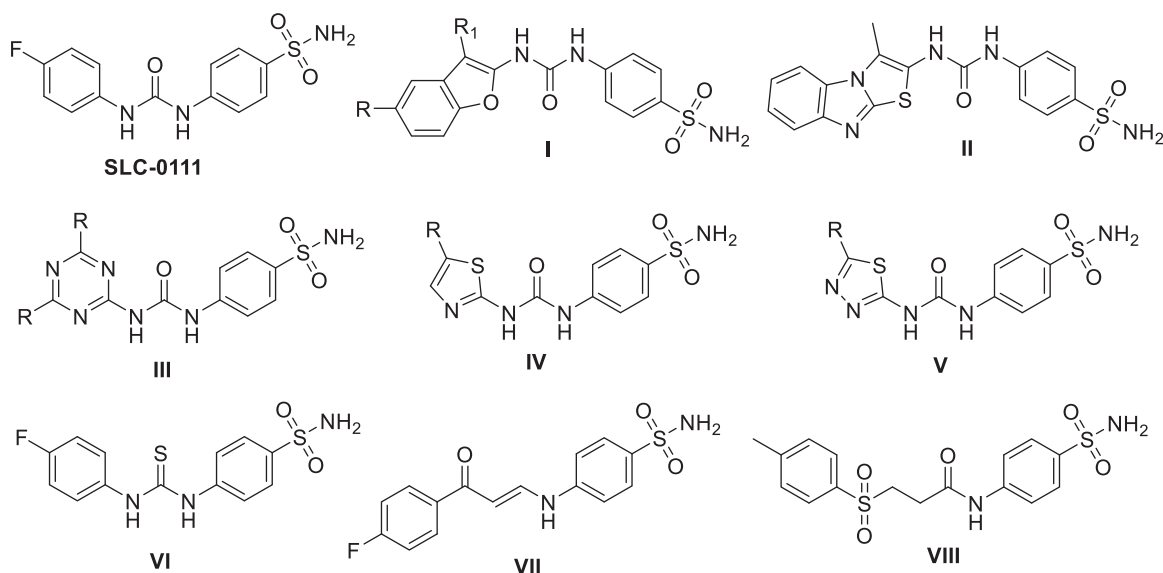


Figure 1. SLC-0111 chemical structure, and some of the previously reported SLC-0111 analogues.

thiadiazole **V**²⁷. In addition, the replacement of the ureido linker in SLC-0111 with another spacers (Figure 1) for instance, thiourido **VI**¹⁴, enaminone **VII**²⁸ and sulfonylpropanamide **VIII**³ was adopted for developing novel SLC-0111 analogues. Remarkably, these strategies resulted in an enhancement of tumour-associated hCA IX inhibitory action.

The aforesaid results motivated our research interest to adopt the tail approach for the development of new SLC-0111 analogues featured with potent and selective hCA IX/XII inhibitory influence. In the herein study, the benzothiazole motif has been appended to the ureido linker rather than the phenyl tail in SLC-0111 in order to provide the target inhibitor **8c** (Figure 2). Thereafter, the regioisomers **8a-b** were designed through shifting of sulfamoyl functionality in **8c** to *ortho* and *para* positions, respectively (Figure 2). In addition to this, the ureido spacer in the SLC-0111 analogue **8c** was lengthened by either methylene or ethylene groups, which resulted in the counterparts **10** and **12**, respectively. Moreover, the functionality of the primary ZBG was either replaced by carboxylic acid or it was substituted in order to produce the carboxylic acid derivatives **14a-c** and the secondary sulphonamide-based SLC-0111 analogues **16a-b**, respectively.

The target benzothiazole-based SLC-0111 analogues **8a-c**, **10**, **12**, **14a-c** and **16a-b** were developed and screened for their inhibitory impact towards CA I, II, IX and XII isoforms. Furthermore, the designed CAIs were assessed for their potential *in vitro* anticancer effects.

Experimental

Chemistry

General

Uncorrected melting points were measured using a Stuart melting point device. In addition, the Shimadzu FT-IR 8400S spectrophotometer was used to record the IR spectra, whereas the Bruker spectrophotometer (400 MHz) was used to record the NMR spectra. ¹³C NMR spectra were run at 100 MHz in deuterated dimethylsulphoxide (DMSO-*d*₆). All coupling constant (*J*) values are reported in hertz. Both ethyl 1,3-benzothiazole-2-carboxylate **3** and benzo[*d*]thiazole-2-carbohydrazide **4** were prepared as previously reported²⁹.

Ethyl benzo[*d*]thiazole-2-carboxylate **3**

White crystals, m.p. = 69–72 °C (reported m.p. = 68–69 °C)²⁹, yield = 77%.

Benzo[*d*]thiazole-2-carbohydrazide **4**

White crystals, m.p. = 174–175 °C (reported m.p. = 173–174 °C)²⁹, yield = 84%.

General procedures for the preparation of target benzothiazole-derived sulphonamides **8a-c**, **10**, **12**, **16a-b** and the carboxylic acids **14a-c**

A mixture of benzo[*d*]thiazole-2-carbohydrazide **4** (0.58 g, 3 mmol) and sodium nitrite (0.41 g, 6 mmol) in glacial acetic acid was stirred in an ice bath for 2 h. Azide **4** was produced by air-drying the generated solid, washing it with water (3 × 4 mL), and collecting it using filtration. Azide **4** was then heated for 30 min at reflux in dry xylene. To the prepared xylene solution, the appropriate amine derivative (aminobenzenesulfonamides **7a-c**, 4-(aminomethyl)benzenesulfonamide **9**, 4-(2-aminoethyl)benzenesulfonamide **11**, aminobenzoic acids **13a-c**, and secondary sulphonamides **15a-b**) was added. After being refluxed for four hours, the reaction mixture was allowed to settle down to room temperature. The desired benzothiazole sulphonamides and carboxylic acids were obtained by filtering, washing the formed precipitate with methylene chloride (3 × 3 mL), drying it, and recrystallizing it from isopropyl alcohol.

Both spectral (NMR and IR) and elemental analysis for the newly prepared sulphonamides (**8a-c**, **10**, **12**, and **16a-b**) and carboxylic acids (**14a-c**) were described in the Supplementary Materials.

Biological evaluation

The procedures that were used for the conducted biological tests were carried out in the same manner that was stated earlier; stopped-flow CA^{30–34}, NCI-single dose^{35–37} and MTT cytotoxicity^{38–41} assays, and they were detailed in the Supplementary Materials.

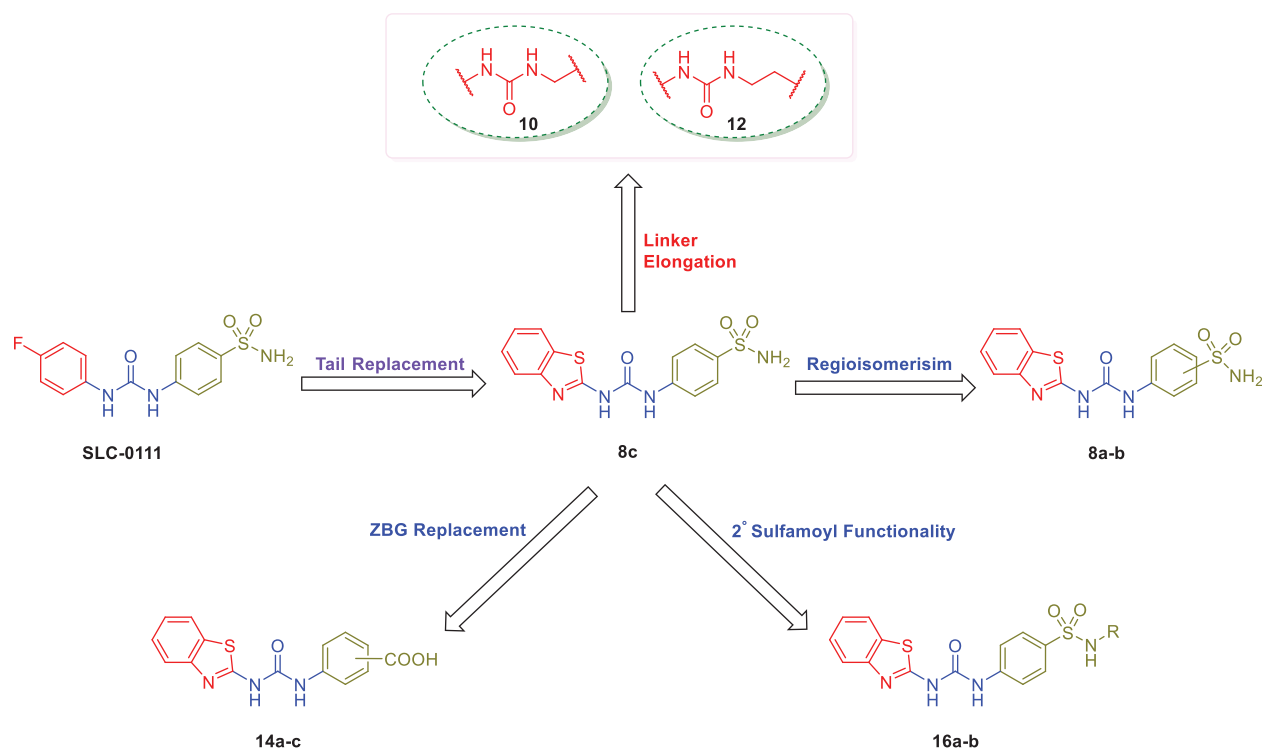


Figure 2. Design of the target benzothiazole-based SLC-0111 analogues as CAIs.

Results and discussion

Chemistry

Schemes 1, 2, and 3 illustrate the synthetic routes that were used in order to prepare the target benzothiazole-derived sulphonamides **8a-c**, **10**, **12**, and **16a-b**, as well as the carboxylic acids **14a-c**.

Synthesis was started by cyclisation of 2-aminothiophenol **1** with diethyl oxalate **2** to afford ethyl benzo[*d*]thiazole-2-carboxylate **3**. Hydrolysis of ester **2**, via refluxing with hydrazine hydrate in ethanol, afforded benzo[*d*]thiazole-2-carbohydrazide **4** in 84% yield. Thereafter, stirring of intermediate **4** with sodium nitrite in glacial acetic acid at 0°C produced the corresponding azide derivative **5**. In the last step, preparation of target benzothiazole-derived sulphonamides **8a-c** was performed via the addition of the appropriate aminobenzenesulfonamide derivative **7a-c** to a pre-heated azide **4** solution in xylene (Scheme 1).

Furthermore, 2-isocyanatobenzothiazole intermediate **6** was reacted with 4-(aminomethyl)benzenesulfonamide **9** and 4-(2-aminoethyl)benzenesulfonamide **11** in refluxing xylene to yield target benzothiazole-derived sulphonamides **10** and **12**, respectively (Scheme 2).

In the last Scheme, benzothiazole-based carboxylic acids **14a-c** and sulphonamides **16a-b** were obtained through a nucleophilic addition reaction of 2-isocyanatobenzothiazole intermediate **6** with aminobenzoic acids **13a-c** and secondary sulphonamides **15a-b**, respectively (Scheme 3).

Biological evaluation

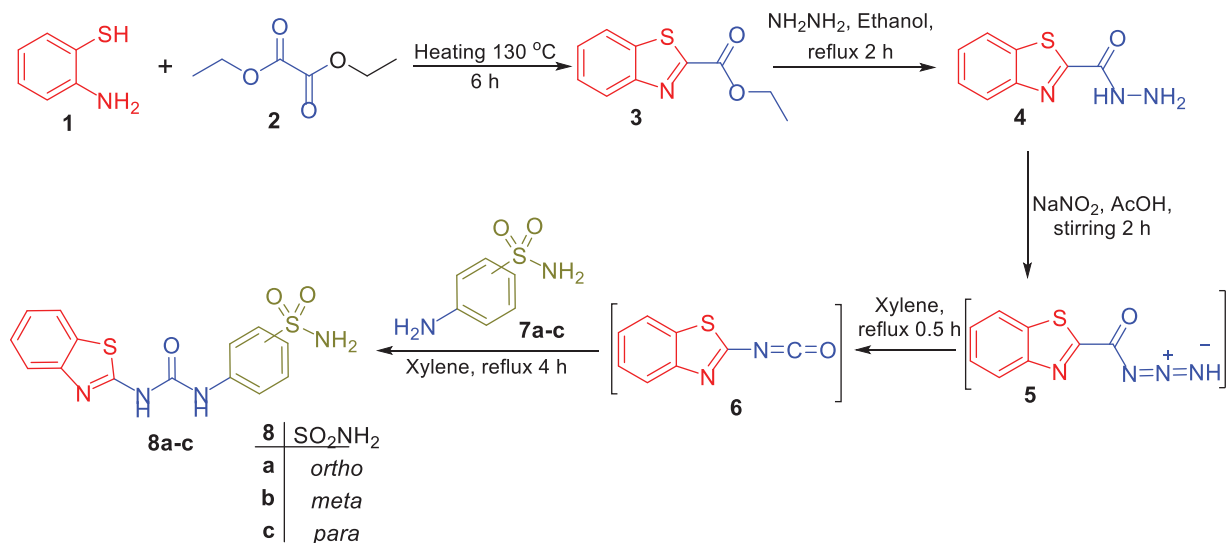
Carbonic anhydrases inhibition

The herein synthesised benzothiazole-derived sulphonamides **8a-c**, **10**, **12**, **16a-b** and the carboxylic acids **14a-c** were assessed for their inhibitory action against the widespread cytosolic *hCA* I and II, and cancer-related IX and XII isoforms employing a stopped

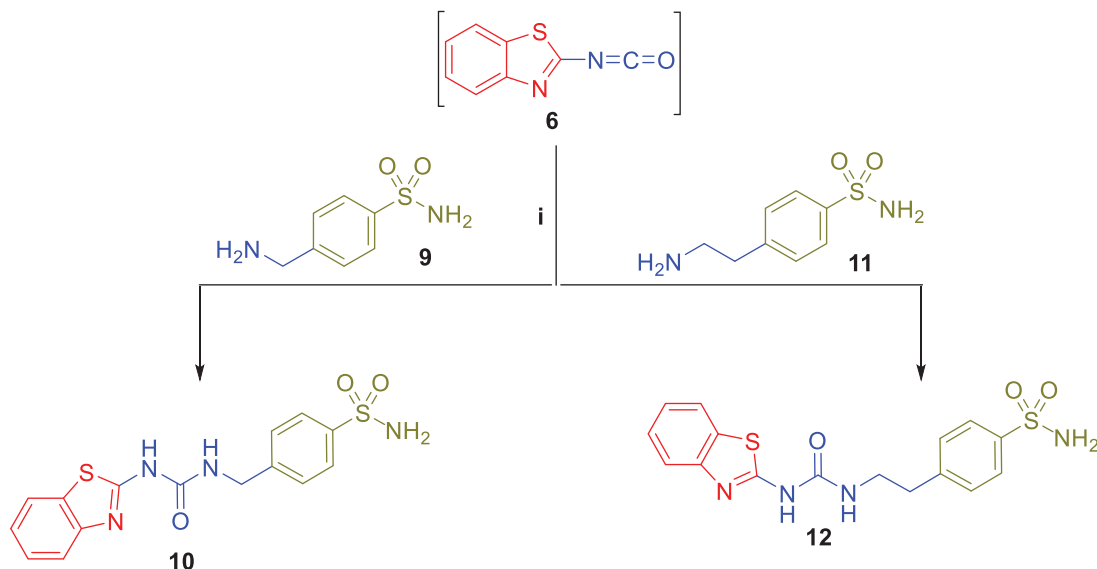
flow CO₂ hydrase assay and the CAI acetazolamide (**AAZ**) was adopted as a control³⁰. The provided inhibition constants (K_i) manifested in Table 1 can be exploited to delineate the structure activity relationships (SARs).

The herein tested benzothiazole-based sulphonamides **8a-c**, **10**, **12**, **16a-b** and the carboxylic acids **14a-c** displayed diverse off-target *hCA* I inhibition profile spanning from nanomolar to high micromolar inhibitory constants (K_i s = 61.5 nM to > 100 μM), Table 1. In the regard of benzothiazole-bearing sulphonamides with ureido linker **8a-c**, they exerted low to moderate inhibitory activities towards the dominant *hCA* I with K_i s ranged from 361.7 nM to 12.59 μM. Noteworthy, the switching of sulphonamide anchoring moiety from *ortho*- and *meta* positions (**8a-b**; K_i s = 12.59 and 4.04 μM, respectively) to *para* position potentially elevated the inhibition constant to the nanomolar level (**8c**; K_i = 361.7 nM). Notably, the elongation of the ureido spacer in the *p*-benzenesulfonamide counterpart **8c** (K_i = 361.7 nM) by one carbon sharply reduced *hCA* I inhibitory potency (**10**; K_i = 945.9 nM), whereas its elongation by two carbons led to sensible enhancement in *hCA* I inhibitory impact providing the most potent *hCA* I inhibitor within the current study (**12**; K_i = 61.5 nM). In contrast, the bioisosteric replacement of the sulphonamide zinc binding functionality in **8a-c** by carboxylic acid moiety **14a-c** dramatically diminished *hCA* I inhibition constants to high micromolar values (**14a-c**; K_i s = 68.09, 82.75 and 11.82 μM, respectively). Furthermore, it was noted that the inclusion of secondary sulfamoyl functionality totally abolished *hCA* I inhibitory effect (**16a-b**; K_i s > 100 μM) compared to the primary sulfamoyl-appended sulphonamides **8a-c**.

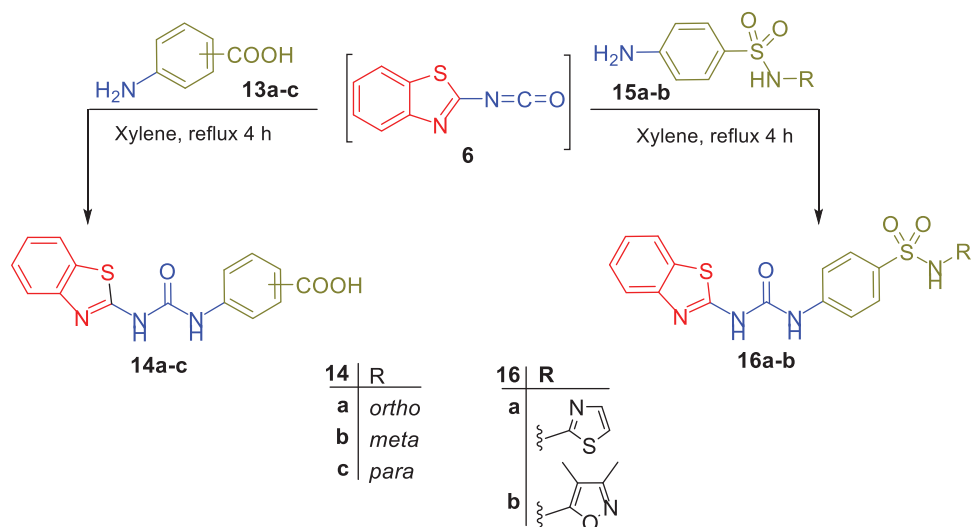
Interestingly, the *in vitro* kinetic data towards the physiologically relevant *hCA* II isoform presented inhibition pattern similar to *hCA* I, Table 1. In a similar fashion, the benzothiazole-derived analogues **8a-c** exhibited moderate to high *hCA* II inhibitory potential with K_i s spanning between 54.1 and 785.2 nM. While the incorporation of *ortho* or *meta* sulphonamide demonstrated moderate *hCA* II inhibition (**8a-b**; K_i = 785.2 and 652.7 nM, respectively), the



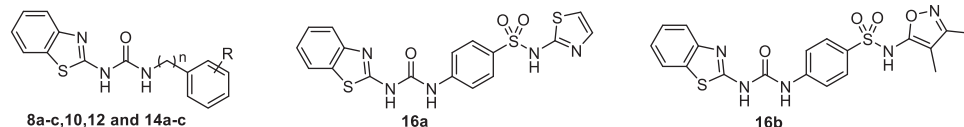
Scheme 1. Synthesis of target benzothiazole-derived sulphonamides 8a-c.



Scheme 2. Synthesis of target benzothiazole-derived sulphonamides 10 and 12.



Scheme 3. Synthesis of target benzothiazole-derived carboxylic acids 14a-c and sulphonamides 16a-b.

Table 1. Inhibition constants for benzothiazole-based derivatives (**8a-c**, **10**, **12**, **14a-c** and **16a-b**) and the standard sulphonamide inhibitor acetazolamide (**AAZ**) towards *hCA* I, II, IX and XII, determined with a stopped-flow CO₂ hydrase assay.


Cmpd	<i>n</i>	R	<i>K_i</i> (nM) ^a			
			<i>hCA</i> I	<i>hCA</i> II	<i>hCA</i> IX	<i>hCA</i> XII
8a	0	<i>o</i> -SO ₂ NH ₂	12590	785.2	65.3	41.2
8b	0	<i>m</i> -SO ₂ NH ₂	4040	652.7	48.9	57.5
8c	0	<i>p</i> -SO ₂ NH ₂	361.7	54.1	31.5	29.3
10	1	<i>p</i> -SO ₂ NH ₂	945.9	204.3	58.8	51.2
12	2	<i>p</i> -SO ₂ NH ₂	61.5	28.5	16.4	34.7
14a	0	<i>o</i> -COOH	68090	75940	44620	39860
14b	0	<i>m</i> -COOH	82750	94130	16280	9140
14c	0	<i>p</i> -COOH	11820	9470	2410	8540
16a	-	-	>100000	>100000	56090	32500
16b	-	-	>100000	>100000	>100000	>100000
AAZ			250.0	12.5	25.0	5.7
SLC-0111			5080.0	960.0	45.0	4.5

^aMean from three different assays (errors were in the range of \pm 5–10% of the reported values).

shifting of this sulfamoyl to *para* position interestingly improved *hCA* II inhibition constant to two-digits value (**8c**; *K_i* = 54.1 nM). To explore the impact of linker length, the obtained results revealed that elongation of the ureido linker in **8c** (*K_i* = 54.1 nM) by one carbon significantly decreased the inhibitory action against *hCA* II by 4-fold (**10**; *K_i* = 204.3 nM), whereas the elongation of this linker by two carbons was more advantageous furnishing the most powerful *hCA* II inhibitor within this series (*K_i* = 28.5 nM) in a similar way to the *hCA* I inhibition profile. Similarly, the appending of carboxyl group as a zinc anchoring moiety **14a-c** in place of the sulfamoyl functionality **8a-c** (*K_i*s range 54.1–785.2 nM) drastically declined *hCA* II inhibition to micromolar level (**14a-c**; *K_i*s equal 75.94, 94.13 and 9.47 μ M, respectively). In addition, the applying of secondary sulfamoyl group **16a-b** completely abolished *hCA* II inhibitory power similar to *hCA* I inhibition data.

Concerning the inhibitory influence of the here evaluated benzothiazole-based SLC-0111 analogues towards cancer-associated *hCA* IX isozyme (Table 1), the primary sulfamoyl-bearing derivatives **8a-c**, **10** and **12** exerted the most efficient potencies within this study against such enzyme displaying two-digits nanomolar *K_i*s spanning a range between 16.4 and 65.3 nM. It is worth to mention that switching of the sulphonamide from *ortho*- and *meta* positions **8a-b** (*K_i*s equal 65.3 and 48.9 nM, respectively) to *para* position **8c** enhanced the *hCA* IX inhibitory power (*K_i* equals 31.5 nM), while the elongation of ureido linker in **8c** by one carbon decreased the inhibition potency by the half (**10**; *K_i* = 58.8 nM). On the other hand, extending such a linker by two carbons resulted in the production of the most efficient *hCA* IX inhibitor within the scope of the present study (**12**; *K_i* = 16.4 nM). Additionally, the replacement of the sulfamoyl zinc binding group in **8a-c** (*K_i* range 31.5 to 65.3 nM) by carboxylic acid functionality sharply lowered the *hCA* IX inhibition constants (**14a-c**; *K_i* = 44.62, 16.28 and 2.41 μ M, respectively). Like the *hCA* I and II inhibition outcomes, the incorporation of 3,5-dimethyl-1,2-oxazole-bearing secondary sulphonamide entirely revoked the *hCA* IX inhibitory efficiency (**16b**; *K_i* > 100 μ M), whereas the inclusion of thiazole-appended secondary sulphonamide **16a** resulted in very weak *hCA* IX inhibition (*K_i* of 56.09 μ M).

In the context of inhibitory activities towards the second tumour-related *hCA* XII isoform, the herein assessed

benzothiazole-derived SLC-0111 analogues demonstrated diverse potencies in a similar behaviour as *hCA* I, II and IX isoforms as depicted in Table 1. The primary sulfamoyl-bearing analogues **8a-c**, **10** and **12** showed the most favourable inhibition profile against *hCA* XII isoform demonstrating inhibition constants *K_i*s ranged from 29.3 to 57.5 nM. For the ureido linker-grafted sulphonamides **8a-c**, the *para* regioisomer **8c** was the most potent *hCA* XII inhibitor with *K_i* = 29.3 nM, similarly the *ortho* and *meta* regioisomers exhibited potential *hCA* XII inhibition (**8a-b**; *K_i*s equal 41.2 and 57.5 nM, respectively). Moreover, the elongation of the ureido linker in **8c** (*K_i* = 29.3 nM) by one or two carbons slightly reduced the inhibition constants (**10** and **12**; *K_i*s of 51.2 and 34.7 nM, respectively). As obtained from *hCA* I, II and IX inhibitory investigations, the bioisosteric replacement of the sulphonamide functionality in **8a-c** (*K_i*s range 29.3–57.5 nM) with carboxylic acid group dramatically decreased the *hCA* XII inhibitory effect (**14a**; *K_i* = 39.86 μ M, **14b**; *K_i* = 9.14 μ M and **14c**; *K_i* = 8.54 μ M). Furthermore, it was noted that the inclusion of secondary sulfamoyl functionality **16a-b** markedly declined *hCA* XII inhibition data, while the grafting of thiazole-bearing sulfamoyl **16a** resulted in *K_i* = 32.5 μ M, the introduction of 3,5-dimethyl-1,2-oxazole-bearing secondary sulphonamide **16b** completely abolished the *hCA* XII inhibition (*K_i* > 100 μ M).

Collectively, the elicited SAR hinted out that the replacement of 4-fluorophenyl tail in SLC-0111 with benzothiazole motif while maintaining the *para* primary sulfamoyl functionality in conjunction with the elongation of its ureido linker by two carbons furnished the most potent inhibitors towards the tumour-related *hCA* IX with inhibition constants better than the lead SLC-0111 (**8c**, *K_i* = 31.5 nM; **12**, *K_i* = 16.4 nM vs SLC-0111, *K_i* = 45 nM). Undesirably such improvement in *hCA* IX inhibition for the promising candidates (**8c** and **12**) was concomitant with the enhancement in inhibition of the ubiquitous *hCA* I (IX/I *S.I.* = 11.5, 3.75, respectively) and II isoforms (IX/II *S.I.* = 1.7 for both) compared to SLC-0111 (IX/I *S.I.* = 112.9; IX/II *S.I.* = 21.3). Consequently, the resulting potent benzothiazole-tethered SLC-0111 analogues **8c** and **12** can be employed as leads for further optimisation to develop promising candidates with superior potency and selectivity towards the cancer-associated isozymes over the physiologically dominant *hCA* I and II isoforms.

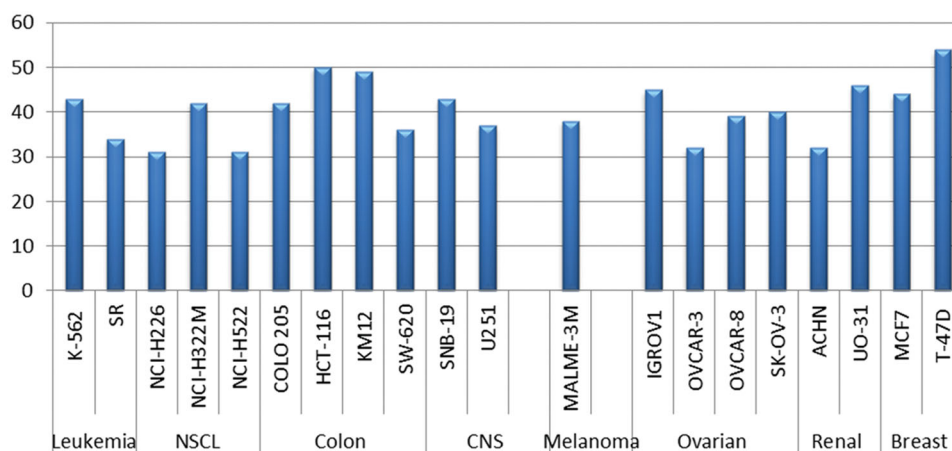


Figure 3. The cancer cell lines that are most vulnerable to the effects of sulphonamide **8b**.

Antitumor activity towards NCI-60 cancer cell lines

All herein developed benzothiazole-derived sulphonamides **8a-c**, **10**, **12**, **16a-b** and the carboxylic acids **14a-c** were explored for their potential antitumor activities at the National Cancer Institute (NCI-USA) within the Developmental Therapeutic Program, utilising the US-NCI protocol and the sulforhodamine B (SRB) colorimetric assay for cell growth and viability evaluation^{32,37,42,43}.

The obtained results revealed that the examined molecules have weak or non-significant antitumor activities towards most NCI cancer cell lines, except **8b**. Sulphonamide **8b** displayed selective anti-proliferative activity (GI > 30%) towards twenty cancer cell lines belonging to all tumour subpanels, except the prostate cancer subpanel, with GI% range of 31–54% (Figure 3). The best growth inhibitory activity of **8b** (GI = 54%) was observed for the breast T-47D cancer cell line. Figure 3 provides a summary of the cancer cell lines that are most vulnerable to the effects of benzothiazole-derived sulphonamide **8b**.

Sulphonamide **8c** exerted cell growth inhibition (GI) equals about 20% towards non-small cell lung cancer (EKVX) and breast cancer (MCF7 and T-47D) cell lines, as well as GI = 24% towards renal cancer (UO-31) cell line. Sulphonamide **10** displayed GI equals about 20% against non-small cell lung cancer (NCI-H226 and EKVX), and breast cancer (MCF7 and T-47D) cell lines, in addition, it exerted about 25% GI towards ovarian cancer (IGROV1) and CNS cancer (SNB-75) cell lines. Superiorly, the renal cancer (UO-31) cell line was the most sensitive one to the effect of sulphonamide **10** with a GI value of 33%. Moreover, non-small cell lung cancer (EKVX), ovarian cancer (IGROV1) and CNS cancer (SNB-75), and renal cancer (UO-31) and breast cancer (MCF7 and T-47D) cell lines were the most susceptible cells to the impact of sulphonamides **16a** and **16b** with GI % = 27, 28, 29, 37, 24, 22 for **16a**, and GI % = 19, 24, 37, 29, 24, 16 for **16b**.

Regarding the anticancer activities of series **14**, carboxylic acid derivative **14a** was found to possess a moderate growth inhibitory effect against non-small cell lung cancer (EKVX), ovarian cancer (IGROV1), CNS cancer (SNB-75), and renal cancer (CAKI-1 and UO-31) cell lines with inhibition % 22, 27, 20, 20, and 32, respectively. Also, carboxylic acid derivative **14b** displayed GI more than 20% against ovarian cancer (IGROV1), CNS cancer (SNB-75), and renal cancer (UO-31) cell lines, whereas compound **14c** exerted GI more than 20% against ovarian cancer (IGROV1), non-small cell lung cancer (EKVX), renal cancer (UO-31) and breast cancer (MCF7 and T-47D) cell lines.

Anti-proliferative activity towards breast cancer cell lines

The inhibition constants presented in Table 1 highlighted that sulphonamide **8b** elicited an excellent selectivity towards *hCA* IX and XII over the off-target *hCA* I, with selective indexes (SIs) equal 82.6 and 70.2, respectively. In addition, only sulphonamide **8b** demonstrated excellent selectivity towards *hCA* IX and XII in comparison to *hCA* II, with SI values of 13.3 and 11.3, respectively. As a consequence of this, sulphonamide **8b** maintained both its activity and its selectivity with regard to the *hCA* IX and XII isoforms that are associated with cancer. In addition, according to the findings of the US-NCI assay that was discussed before, sulphonamide **8b** was determined to be the most effective anticancer molecules herein reported. As a consequence, sulphonamide **8b** was evaluated for its anticancer effect towards breast cancer (T-47D and MCF-7) cell lines within the hypoxic conditions, using the SRB assay. The assay results that presented in Table 2 showed that sulphonamide **8b** had good IC₅₀ values (6.73 ± 0.28 and 9.16 ± 0.70) against T-47D and MCF-7 cells, respectively.

Conclusions

This study developed different series of benzothiazole-based sulphonamides **8a-c**, **10**, **12**, **16a-b** and carboxylic acids as novel SLC-0111 analogues, and assessed their CA inhibitory effects towards CA I, II, IX and XII isoforms. Different drug design approaches were utilised. The benzothiazole motif was appended to the ureido linker instead of the SLC-0111 fluorophenyl tail to produce compounds **8c** and its regioisomers **8a-b**. In addition, the ureido spacer was elongated by methylene or ethylene groups to afford the counterparts **10** and **12**. In turn, the functionality of the primary ZBG was either replaced by carboxylic acid or it was substituted in order to produce the carboxylic acid derivatives **14a-c** and the secondary sulphonamide-based SLC-0111 analogues **16a-b**, respectively. The elicited SAR, from this study, hinted out that the introduction of *para* primary sulfamoyl functionality along with elongation of the ureido linker by two carbons were more beneficial for the cancer-related *hCA* IX and XII inhibition. The primary sulfamoyl-bearing analogues **8a-c**, **10** and **12** disclosed the most favourable inhibition profile against tumour-related *hCA* IX and XII isoforms demonstrating inhibition constants ranged from 16.4 to 65.3 nM and from 29.3 to 57.5 nM, respectively. Moreover, all herein developed benzothiazole-derived sulphonamides **8a-c**,

Table 2. Anticancer activity of target benzothiazole-based sulphonamide **8b** towards breast T-47D and MCF-7 cancer cell lines under hypoxic conditions.

Com.	IC ₅₀ (μM)	
	T-47D	MCF-7
8b	6.73 ± 0.28	9.16 ± 0.70
Doxorubicin	7.31 ± 0.49	6.52 ± 0.38

10, 12, 16a-b and the carboxylic acids **14a-c** were explored for their potential antitumor activities at the NCI-National Cancer Institute. The examined molecules have weak or non-significant antitumor activities towards most NCI cancer cell lines, except sulphonamide **8b** which displayed selective anti-proliferative activity (GI > 30%) towards twenty cancer cell lines belonging to all tumour subpanels, except the prostate cancer subpanel. It is interesting to mention that **8b** elicited an excellent selectivity towards hCA IX and XII over the off-target hCA I, with selective indexes equal to 82.6 and 70.2, respectively. Additionally, it demonstrated good selectivity towards hCA IX and XII over hCA II with selective indexes of 13.3 and 11.3, respectively. Furthermore, the cytotoxicity SRB assay showed that sulphonamide **8b** had good IC₅₀ values (6.73 ± 0.28 and 9.16 ± 0.70) against breast cancer T-47D and MCF-7 cells, respectively.

Disclosure statement

CT Supuran is Editor-in-Chief of the Journal of Enzyme Inhibition and Medicinal Chemistry. He was not involved in the assessment, peer review, or decision-making process of this paper. The authors have no relevant affiliations of financial involvement with any organisation or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Funding

This work is funded by the Deanship of Scientific Research at Princess Nourah bint Abdulrahman University, through the Research Groups Program Grant no [RGP-1440 -0025](2).

ORCID

Mostafa M. Elbadawi  <http://orcid.org/0000-0002-9073-4176>
Alessio Nocentini  <http://orcid.org/0000-0003-3342-702X>
Ahmed A. Al-Karmalawy  <http://orcid.org/0000-0002-8173-6073>
Claudiu T. Supuran  <http://orcid.org/0000-0003-4262-0323>

References

- Alterio V, Di Fiore A, D'Ambrosio K, Supuran CT, De Simone G. Multiple binding modes of inhibitors to carbonic anhydrases: How to design specific drugs targeting 15 different isoforms? *Chem Rev.* 2012;112(8):4421–4468.
- Kumar S, Rulhania S, Jaswal S, Monga V. Recent advances in the medicinal chemistry of carbonic anhydrase inhibitors. *Eur J Med Chem.* 2021;209:112923.
- Elbadawi MM, Eldehna WM, Nocentini A, Abo-Ashour MF, Elkaeed EB, Abdelgawad MA, Alharbi KS, Abdel-Aziz HA, Supuran CT, Gratteri P, et al. Identification of N-phenyl-2-(phenylsulfonyl) acetamides/propanamides as new SLC-0111 analogues: synthesis and evaluation of the carbonic anhydrase inhibitory activities. *Eur J Med Chem.* 2021;218:113360.
- Supuran CT. Structure and function of carbonic anhydrases. *Biochem J.* 2016;473(14):2023–2032.
- Buabeng ER, Henary M. Developments of small molecules as inhibitors for carbonic anhydrase isoforms. *Bioorg Med Chem.* 2021;39:116140.
- Nocentini A, Supuran CT. Carbonic anhydrase inhibitors as antitumor/antimetastatic agents: a patent review (2008–2018). *Expert Opin Ther Pat.* 2018;28(10):729–740.
- Angeli A, Carta F, Nocentini A, Winum J-Y, Zalubovskis R, Akdemir A, Onnis V, Eldehna WM, Capasso C, Simone GD, et al. Carbonic anhydrase inhibitors targeting metabolism and tumor microenvironment. *Metabolites.* 2020;10(10):412.
- Mishra CB, Tiwari M, Supuran CT. Progress in the development of human carbonic anhydrase inhibitors and their pharmacological applications: Where are we today? *Med Res Rev.* 2020;40(6):2485–2565.
- Eldehna WM, Taghour MS, Al-Warhi T, Nocentini A, Elbadawi MM, Mahdy HA, Abdelrahman MA, Alotaibi OJ, Aljaeed N, Elimam DM, et al. Discovery of 2,4-thiazolidinedione-tethered coumarins as novel selective inhibitors for carbonic anhydrase IX and XII isoforms. *J Enzyme Inhib Med Chem.* 2022;37(1):531–541.
- Elimam DM, Eldehna WM, Salem R, Bonardi A, Nocentini A, Al-Rashood ST, Elaasser MM, Gratteri P, Supuran CT, Allam HA. Natural inspired ligustrazine-based SLC-0111 analogues as novel carbonic anhydrase inhibitors. *Eur J Med Chem.* 2022;228:114008.
- Narella SG, Shaik MG, Mohammed A, Alvala M, Angeli A, Supuran CT. Synthesis and biological evaluation of coumarin-1,3,4-oxadiazole hybrids as selective carbonic anhydrase IX and XII inhibitors. *Bioorg Chem.* 2019;87:765–772.
- Zengin Kurt B, Sonmez F, Ozturk D, Akdemir A, Angeli A, Supuran CT. Synthesis of coumarin-sulfonamide derivatives and determination of their cytotoxicity, carbonic anhydrase inhibitory and molecular docking studies. *Eur J Med Chem.* 2019;183:111702.
- Supuran CT. How many carbonic anhydrase inhibition mechanisms exist? *J Enzyme Inhib Med Chem.* 2016;31(3):345–360.
- Angeli A, Tanini D, Peat TS, Di Cesare Mannelli L, Bartolucci G, Capperucci A, Ghelardini C, Supuran CT, Carta F. Discovery of new Selenoureido Analogues of 4-(4-Fluorophenylureido)benzenesulfonamide as Carbonic Anhydrase Inhibitors. *ACS Med Chem Lett.* 2017;8(9):963–968.
- Pastorek J, Pastorekova S. Hypoxia-induced carbonic anhydrase IX as a target for cancer therapy: from biology to clinical use. *Semin Cancer Biol.* 2015;31:52–64.
- Narella SG, Singh P, Arifuddin M, Supuran CT. Anticancer carbonic anhydrase inhibitors: a patent and literature update 2018–2022. *Expert Opin Ther Pat.* 2022;32(8):833–847.
- McDonald PC, Chafe SC, Supuran CT, Dedhar S. Cancer therapeutic targeting of hypoxia induced carbonic anhydrase IX: from bench to bedside. *Cancers.* 2022;14(14):3297.
- Peerzada MN, Hamel E, Bai R, Supuran CT, Azam A. Deciphering the key heterocyclic scaffolds in targeting microtubules, kinases and carbonic anhydrases for cancer drug development. *Pharmacol Ther.* 2021;225:107860.

19. Mishra CB, Mongre RK, Prakash A, Jeon R, Supuran CT, Lee M-S. Anti-breast cancer action of carbonic anhydrase IX inhibitor 4-[4-(4-Benzo [1, 3] dioxol-5-ylmethyl-piperazin-1-yl)-benzylidene-hydrazinocarbonyl]-benzenesulfonamide (BSM-0004): *in vitro* and *in vivo* studies. *J Enzyme Inhib Med Chem.* **2021**;36(1):954–963.
20. Tanpure RP, Ren B, Peat TS, Bornaghi LF, Vullo D, Supuran CT, Poulsen S-A. Carbonic anhydrase inhibitors with dual-tail moieties to match the hydrophobic and hydrophilic halves of the carbonic anhydrase active site. *J Med Chem.* **2015**; 58(3):1494–1501.
21. Elbadawi MM, Eldehna WM, Nocentini A, Soma WR, Al-Rashood ST, Elkaeed EB, El Hassab MA, Abdel-Aziz HA, Supuran CT, Fares M. Development of 4-((3-oxo-3-phenyl-propyl) amino) benzenesulfonamide derivatives utilizing tail/dual-tail approaches as novel carbonic anhydrase inhibitors. *Eur J Med Chem.* **2022**;238:114412.
22. Fares M, Eldehna WM, Bua S, Lanzi C, Lucarini L, Masini E, Peat TS, Abdel-Aziz HA, Nocentini A, Keller PA, et al. Discovery of potent dual-tailed benzenesulfonamide inhibitors of human carbonic anhydrases implicated in glaucoma and *in vivo* profiling of their intraocular pressure-lowering action. *J Med Chem.* **2020**;63(6):3317–3326.
23. Shaldam M, Eldehna WM, Nocentini A, Elsayed ZM, Ibrahim TM, Salem R, El-Domany RA, Capasso C, Abdel-Aziz HA, Supuran CT. Development of novel benzofuran-based SLC-0111 analogs as selective cancer-associated carbonic anhydrase isoform IX inhibitors. *Eur J Med Chem.* **2021**;216: 113283.
24. Sarnella A, Ferrara Y, Auletta L, Albanese S, Cerchia L, Alterio V, De Simone G, Supuran CT, Zannetti A. Inhibition of carbonic anhydrases IX/XII by SLC-0111 boosts cisplatin effects in hampering head and neck squamous carcinoma cell growth and invasion. *J Exp Clin Cancer Res.* **2022**;41(1):1–16.
25. Alkhalidi AAM, Al-Sanea MM, Nocentini A, Eldehna WM, Elsayed ZM, Bonardi A, Abo-Ashour MF, El-Damasy AK, Abdel-Maksoud MS, Al-Warhi T, et al. 3-Methylthiazolo[3,2-a]benzimidazole-benzenesulfonamide conjugates as novel carbonic anhydrase inhibitors endowed with anticancer activity: design, synthesis, biological and molecular modeling studies. *Eur J Med Chem.* **2020**;207:112745.
26. Lolak N, Akocak S, Bua S, Sanku RKK, Supuran CT. Discovery of new ureido benzenesulfonamides incorporating 1,3,5-triazine moieties as carbonic anhydrase I, II, IX and XII inhibitors. *Bioorg Med Chem.* **2019**;27(8):1588–1594.
27. Abo-Ashour MF, Eldehna WM, Nocentini A, Ibrahim HS, Bua S, Abdel-Aziz HA, Abou-Seri SM, Supuran CT. Novel synthesized SLC-0111 thiazole and thiadiazole analogues: determination of their carbonic anhydrase inhibitory activity and molecular modeling studies. *Bioorg Chem.* **2019**;87:794–802.
28. Eldehna WM, Abo-Ashour MF, Berrino E, Vullo D, Ghabbour HA, Al-Rashood ST, Hassan GS, Alkahtani HM, Almehezia AA, Alharbi A, et al. SLC-0111 enaminone analogs, 3/4-(3-aryl-3-oxopropenyl) aminobenzenesulfonamides, as novel selective subnanomolar inhibitors of the tumor-associated carbonic anhydrase isoform IX. *Bioorg Chem.* **2019**;83:549–558.
29. (a) Liu K, Ding Y, Kang C. Synthesis and antiproliferative activity of new n-acylhydrazone derivatives containing Benzothiazole and indole based moiety. *Pharm Chem J.* **2020**;54(4):345–352.(b) Al-Rashood KA, Abdel-Aziz HA. Thiazolo [3, 2-a] benzimidazoles: synthetic strategies, chemical transformations and biological activities. *Molecules.* **2010**;15(6):3775–3815.
30. Khalifah RG. The carbon dioxide hydration activity of carbonic anhydrase: I. stop-flow kinetic studies on the native human isoenzymes B and C. *J Biol Chem.* **1971**;246(8): 2561–2573.
31. (a) Alafeefy AM, Abdel-Aziz HA, Vullo D, Al-Tamimi A-MS, Awaad AS, Mohamed MA, Capasso C, Supuran CT. Inhibition of human carbonic anhydrase isozymes I, II, IX and XII with a new series of sulfonamides incorporating aroylhydrazone-[1,2,4]triazolo[3, 4-b][1, 3,4] thiadiazinyl-or 2-(cyanophenyl-methylene)-1,3,4-thiadiazol-3(2H)-yl moieties. *J Enzyme Inhib Med Chem.* **2015**;30(1):52–56.(b) Ibrahim HS, Abdelrahman MA, Nocentini A, Bua S, Abdel-Aziz HA, Supuran CT, Abou-Seri SM, Eldehna WM. Insights into the effect of elaborating coumarin-based aryl enaminones with sulfonamide or carboxylic acid functionality on carbonic anhydrase inhibitory potency and selectivity. *Bioorg Chem.* **2022**;126:105888.
32. Tawfik HO, Shaldam MA, Nocentini A, Salem R, Almahli H, Al-Rashood ST, Supuran CT, Eldehna WM. Novel 3-(6-methylpyridin-2-yl) coumarin-based chalcones as selective inhibitors of cancer-related carbonic anhydrases IX and XII endowed with anti-proliferative activity. *J Enzyme Inhib Med Chem.* **2022**;37(1):1043–1052.
33. Elimam DM, Elgazar AA, Bonardi A, Abdelfadil M, Nocentini A, El-Domany RA, Abdel-Aziz HA, Badria FA, Supuran CT, Eldehna WM. Natural inspired piperine-based sulfonamides and carboxylic acids as carbonic anhydrase inhibitors: design, synthesis and biological evaluation. *Eur J Med Chem.* **2021**;225:113800.
34. Eldehna WM, Nocentini A, Elsayed ZM, Al-Warhi T, Aljaeed N, Alotaibi OJ, Al-Sanea MM, Abdel-Aziz HA, Supuran CT. Benzofuran-based carboxylic acids as carbonic anhydrase inhibitors and antiproliferative agents against breast cancer. *ACS Med Chem Lett.* **2020**;11(5):1022–1027.
35. Boyd MR, Paull KD. Some practical considerations and applications of the National Cancer Institute *in vitro* anticancer drug discovery screen. *Drug Dev Res.* **1995**;34(2):91–109.
36. Elbadawi MM, Eldehna WM, Wang W, Agama KK, Pommier Y, Abe M. Discovery of 4-alkoxy-2-aryl-6, 7-dimethoxyquinolines as a new class of topoisomerase I inhibitors endowed with potent *in vitro* anticancer activity. *Eur J Med Chem.* **2021**;215:113261.
37. Eldehna WM, Salem R, Elsayed ZM, Al-Warhi T, Knany HR, Ayyad RR, Traiki TB, Abdulla M-H, Ahmad R, Abdel-Aziz HA, et al. Development of novel benzofuran-isatin conjugates as potential antiproliferative agents with apoptosis inducing mechanism in Colon cancer. *J Enzyme Inhib Med Chem.* **2021**;36(1):1423–1434.
38. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods.* **1983**;65(1–2):55–63.
39. Taghour MS, Elkady H, Eldehna WM, El-Deeb NM, Kenawy AM, Elkaeed EB, Alsouk AA, Alesawy MS, Metwaly AM, Eissa IH. Design and synthesis of thiazolidine-2, 4-diones hybrids with 1, 2-dihydroquinolones and 2-oxindoles as potential VEGFR-2 inhibitors: *in-vitro* anticancer evaluation and *in-silico* studies. *J Enzyme Inhib Med Chem.* **2022**;37(1): 1903–1917.
40. Yousef RG, Ibrahim A, Khalifa MM, Eldehna WM, Gobaara IMM, Mehany AB, Elkaeed EB, Alsouk AA, Metwaly AM, Eissa IH. Discovery of new nicotinamides as apoptotic VEGFR-2 inhibitors: virtual screening, synthesis, anti-proliferative, immunomodulatory, ADMET, toxicity, and molecular

- dynamic simulation studies. *J Enzyme Inhib Med Chem.* [2022](#);37(1):1389–1403.
41. Eldehna WM, El Hassab MA, Elsayed ZM, Al-Warhi T, Elkady H, Abo-Ashour MF, Abourehab MA, Eissa IH, Abdel-Aziz HA. Design, synthesis, in vitro biological assessment and molecular modeling insights for novel 3-(naphthalen-1-yl)-4, 5-dihydropyrazoles as anticancer agents with potential EGFR inhibitory activity. *Sci Rep.* [2022](#);12(1):1–13.
42. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR. New colorimetric cytotoxicity assay for anticancer-drug screening. *J Natl Cancer Inst.* [1990](#);82(13):1107–1112.
43. Dweedar HE, Mahrous H, Ibrahim HS, Abdel-Aziz HA. Analogue-based design, synthesis and biological evaluation of 3-substituted-(methylenediazono) indolin-2-ones as anticancer agents. *Eur J Med Chem.* [2014](#);78:275–280.