Accepted Manuscript

Synthesis of 4-(thiazol-2-ylamino)-benzenesulfonamides with carbonic anhydrase I, II and IX inhibitory activity and cytotoxic effects against breast cancer cell lines

Nagwa M. Abdel Gawad, Noha H. Amin, Mohammed T. Elsaadi, Fatma M.M. Mohamed, Andrea Angeli, Viviana De Luca, Clemente Capasso, Claudiu T. Supuran

PII: S0968-0896(16)30339-X
DOI: http://dx.doi.org/10.1016/j.bmc.2016.05.016
Reference: BMC 13000

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 9 April 2016
Revised Date: 4 May 2016
Accepted Date: 11 May 2016

Please cite this article as: Abdel Gawad, N.M., Amin, N.H., Elsaadi, M.T., Mohamed, F.M.M., Angeli, A., Luca, V.D., Capasso, C., Supuran, C.T., Synthesis of 4-(thiazol-2-ylamino)-benzenesulfonamides with carbonic anhydrase I, II and IX inhibitory activity and cytotoxic effects against breast cancer cell lines, *Bioorganic & Medicinal Chemistry* (2016), doi: http://dx.doi.org/10.1016/j.bmc.2016.05.016

Synthesis of 4-(thiazol-2-ylamino)-benzenesulfonamides with carbonic anhydrase I, II and IX inhibitory activity and cytotoxic effects against breast cancer cell lines

Nagwa M. Abdel Gawad,[a] Noha H. Amin,*[b] Mohammed T. Elsaadi,[b] Fatma M.M. Mohamed,[b] Andrea Angeli,[c] Viviana De Luca,[d] Clemente Capasso,[d] and Claudiu T. Supuran*[c]

[a]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Cairo University, Cairo, 11562, Egypt.*
[b]**Department of Medicinal Chemistry, Faculty of Pharmacy, Beni Suef University, Beni Suef, 62514, Egypt.*
[c]*Università degli Studi di Firenze, Dipartimento Neurofarba, Sezione di Scienze Farmaceutiche e Nutraceutiche, Via U. Schiff 6, 50019 Sesto Fiorentino, Florence, Italy;* E-mail: claudiu.supuran@unifi.it; Fax: +39055 457 3385; Tel: +39055 457 3005
[d]*Istituto di Bioscienze e Biorisorse, CNR, Via Pietro Castellino 81, Napoli, Italy*.

Abstract. A series of 4-(thiazol-2-ylamino)-benzenesulfonamides was synthesized and screened for their carbonic anhydrase (CA, EC 4.2.1.1) inhibitory and cytotoxic activity on human breast cancer cell line MCF-7. Human (h) CA isoforms I, II and IX were included in the study. The new sulfonamides showed excellent inhibition of all three isoforms, with K_Is in the range of 0.84-702 nM against hCA I, of 0.41 – 288 nM against hCA II and of 5.6 – 29.2 against the tumor-associated hCA IX, a validated anti-tumor target, with a sulfonamide (SLC-0111) in Phase I clinical trials for the treatment of hypoxic, metastatic solid tumors overexpressing CA IX. The new compounds showed micromolar inhibitory of growth efficacy against breast cancer MCF-7 cell lines.

Keywords*:* Carbonic anhydrase; inhibitor; sulfonamide, antitumor, cytotoxic agent.

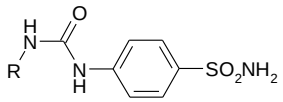
* Corresponding authors: E-mail:hani.noha@gmail.com (NHA); Phone: +39-055-4573005; fax: +39-055-4573385; E-mail: claudiu.supuran@unifi.it (CTS).

1. Introduction

Cancer is a life threatening group of diseases in which deregulated proliferation of cells invades and disrupts the surrounding tissues, leading to dysfunctional metabolic, angiogenetic and immunologic functions.[1] According to World Health Organization (WHO), cancer figures among the leading cause of death worldwide, accounting for 8.2 million deaths in both developed and developing countries in 2012. Although a large number of anticancer drugs were launched ultimately, mainly kinase inhibitors,[2] their limited success, the drug resistance problems and severe side effects indicate that there is an imperative need of novel strategies for cancer management.[2] Thus, increasing efforts are being done towards developing novel such agents, with higher safety margins, possibly targeting novel pathways which exploit the particular biology/biochemistry of tumor cells.[3] Among them, hypoxia, a hallmark of many cancer types, leads to the overexpression of a host of proteins that drive adaptation of the cells to the hypoxic and acidotic conditions.[4] A large number of proteins (specifically overexpressed in tumors and with a limited diffusion in normal cells) participate in these processes, among which two isoforms of the metalloenzyme carbonic anhydrases (CA, EC 4.2.1.1), i.e., CA IX and XII.[5]

The CAs are a group of metalloenzymes involved in many physiologic processes, among which pH buffering of extra- and intracellular spaces, by catalyzing the reversible hydration of carbon dioxide to bicarbonate and a proton.[5-10] The family of human CAs (hCAs) comprises 16 different α-isoforms, of which several are cytosolic (CA I-III, CA VII and CA XIII), five are membrane-bound isoforms (CA IV, IX, XII, XIV and XV), two are mitochondrial (CA VA and VB), and one is secreted into saliva (CA VI). These enzymes are involved in various biochemical/metabolic processes, such as gluconeogenesis, lipogenesis, and ureagenesis.[5-10] Abnormal levels or activities of many CA isoforms have been associated with different diseases such as cancer (overexpression of CA IX/XII due to the hypoxia cascade activation),[4,5] epilepsy (abnormal levels/activities of brain CA isoforms),[6] obesity (dysregulation of the mitochondrial isoforms CA VA/B, involved among others in lipogenesis),[7] glaucoma (upregulation and high activity levels of CA II, IV and XII).[8,9] Furthermore, the renal excretion of anions is also mediated by several CA isoforms, and their inhibition with sulfonamides (the main class of CA inhibitors, CAIs)[4b,11,12] leads to a strong diuretic effect.[10] Although many sulfonamides are in clinical use as diuretics, antiglaucoma, antiepilepsy/antiobesity agents for decades,[4-12] only recently a

ureidosulfonamide acting as potent CA IX/XII inhibitor, SLC-0111 (**A**, Fig. 1), entered in Phase I clinical trials for the treatment of advanced solid, metastatic tumors.[12] **(**Fig.1**)**. SLC-0111 **A** and some of its congeners **B-E** (Fig. 1) are one of the many examples of isoform-selective sulfonamide CAIs discovered in the last period by using the tail approach.[11-15]



A: R = 4-F-C_6H_4 (SLC-0111); K_I = 960 nM
B: R = C_6F_5; K_I = 50 nM
C: R= 2(i-Pr-)-C_6H_4; K_I = 3.3nM
D: R = 3-O_2N-C_6H_4; K_I = 15 nM
E: R = cyclopentyl; K_I = 226 nM

Figure 1. Substituted ureido benzenesulfonamides **A-E**, some of which possessing substantial antitumor activity *in vitro* and/or *in vivo*, and their hCA II inhibitory activity.[13]

The structural requirements for the sulfonamide derivatives to show high affinity for CAs, are fully understood.[11-15] The sulfonamide moiety incorporates the essential nitrogen atom that is a zinc binding group (ZBG) (as sulfonamidate anion) and binds to the metal ion within the active site of the enzyme in a stable tetrahedral geometry of the zinc.[11-15] Furthermore, residues Thr 199 and Glu 106 in its neighborhood participate in hydrogen bonds to both with sulfonamide NH and oxygen atoms. In addition to that, the organic scaffold that is usually an aliphatic, aromatic, heterocyclic or sugar moiety interacts both with the hydrophilic and hydrophobic halves of the CA active site.[11-15]

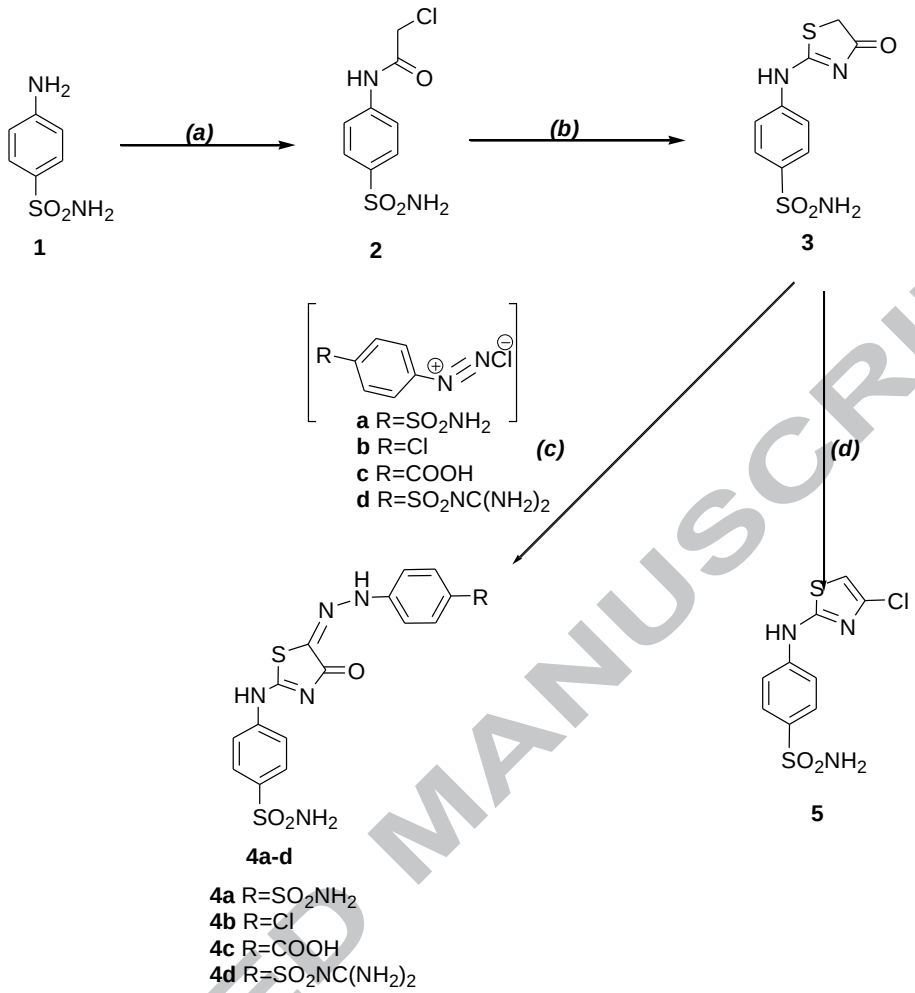
Continuing our interest in sulfonamide CAIs, in the present study we report the design of novel 4-/5-substituted thiazolylbenzenesulfonamides, in an attempt to obtain selective inhibitors for the tumor-associated isoform CA IX, which were further explored for their cytotoxic effects on human breast cancer (MCF-7) cell lines, which overexpress CA IX.[16]

2. Results and discussion

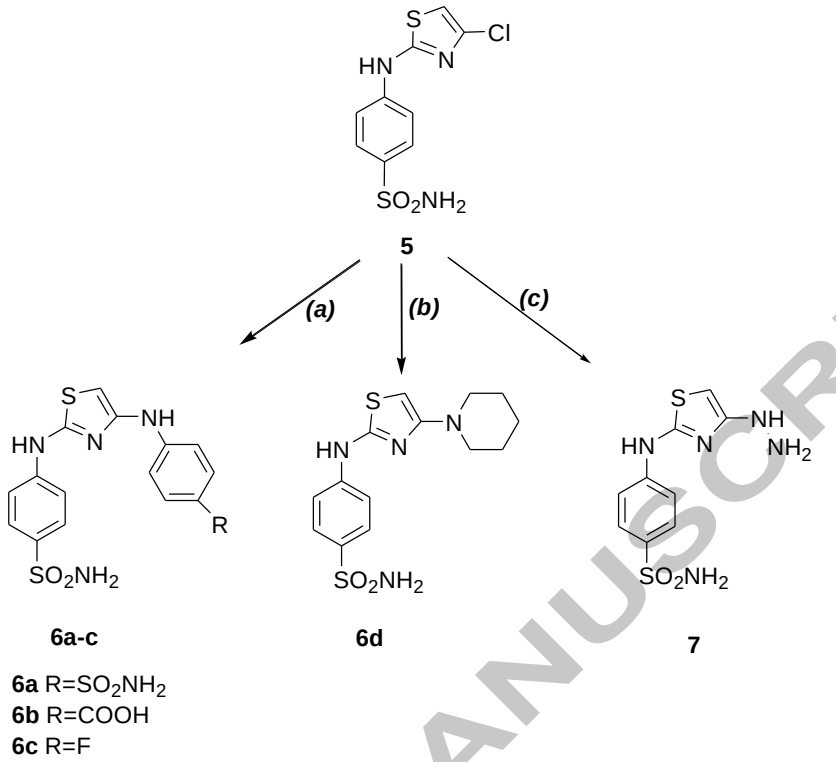
2.1. Chemistry

The drug design strategy of our work used the SLC-0111 series of compounds (Fig. 1) as leads, in which the ureido moiety present in **A-B** was replaced by a five-membered heterocylic scaffold, the thiazole/ thiazolidinone, which incorporates the isosteric thioureido moiety present in the lead compounds within the heterocyle. In fact a recent X-ray/kinetic study of thioureido analog of SLC-0111 showed that this isosteric replacement may lead to highly effective CAIs for the tumor-associated isoforms.[17] Thus, we designed various strategies for synthesis of -benzenesulfonamide derivatives incorporating thiazole/ thiazolidine scaffolds, which are shown in Schemes 1-4. Three key intermediates, were synthesized, the first of which was a thiazolidinone, i.e., 4- ((4-oxo-4,5-dihydrothiazol-**2**-yl)amino)benzenesulfonamide **3.** It has been prepared by treating previously prepared chloroacetamide derivative **2**[18] with ammonium thiocyanate in ethanol via an intramolecular cyclization rearrangement reaction.[18] Furthermore, compound **3** underwent a nucleophilic substitution reaction with phosphorus oxychloride in presence of pyridine to yield 4-(4-Chloro-thiazol-2-ylamino)-benzenesulfonamide **5.**[19] Coupling reactions were conducted between previously prepared diazonium salts of selected primary aromatic amines (e.g., sulfanilamide, *para*-chloroaniline, *para*-aminobenzoic acid or sulfaguanidine) and C5 active thiazole methylene group of intermediate **3** in order to synthesize 5-phenylhydrazonothiazol-2-yl-aminobenzenesulfonamides **4a-d. (**Scheme 1)**.**

Compound **5**, which possesses a reactive chlorine atom in position 4 of the heterocyclic ring, was refluxed with commercially available hydrazine hydrate in ethanol to yield 4-((4-hydrazinylthiazol-2-yl)amino)benzenesulfonamide **7.** By refluxing compound **5** with selected amines (sulfanilamide, *para*-benzoic acid or *para*- fluoro aniline) or piperidine in the presence of triethylamine, the 4-phenylthiazol-2-yl-aminobenzenesulfonamides **6a-6d,** were obtained **(**Scheme 2**).**

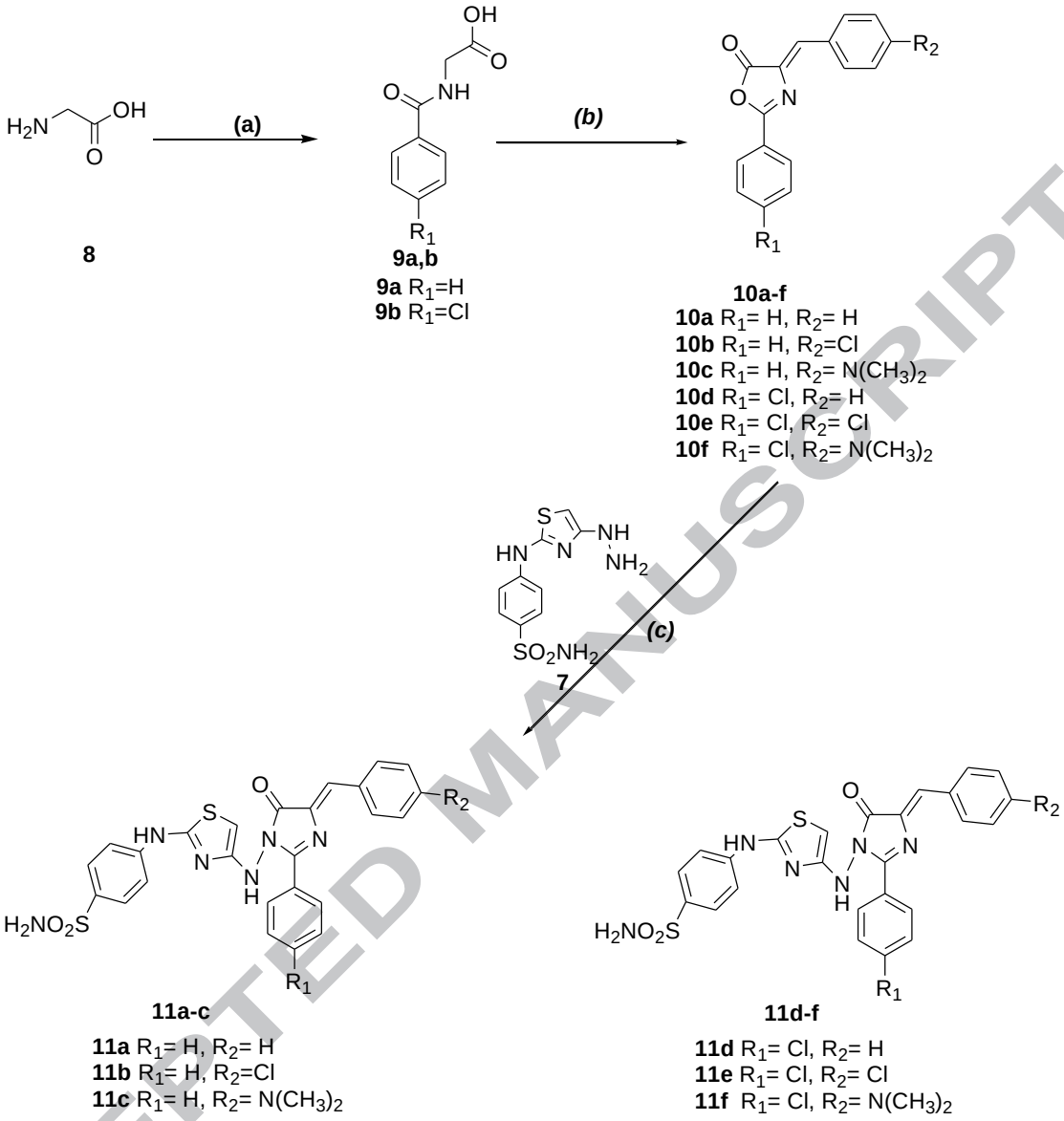


Scheme 1. Synthesis of target compounds **2-5**: **(*a*)** chloroacetyl chloride, dry DMF, rt, 2 h; ***(b)*** ammonium thiocyanate, absolute ethanol, reflux, 1 h; ***(c)*** 2N HCl, sodium acetate, rt, 18 h; ***(d)*** phosphorus oxychloride, pyridine, reflux, 3 h.



Scheme 2. Synthesis of target compounds **6** and **7**: ***(a)*** sulfanilamide or *p*-amino benzoic acid or *p*-fluoro aniline, TEA, DMF, reflux, 24 h; ***(b)*** piperidine, TEA, dry DMF, reflux, 24 h; ***(c)*** hydrazine hydrate 99.9%, absolute ethanol, reflux, 24 h.

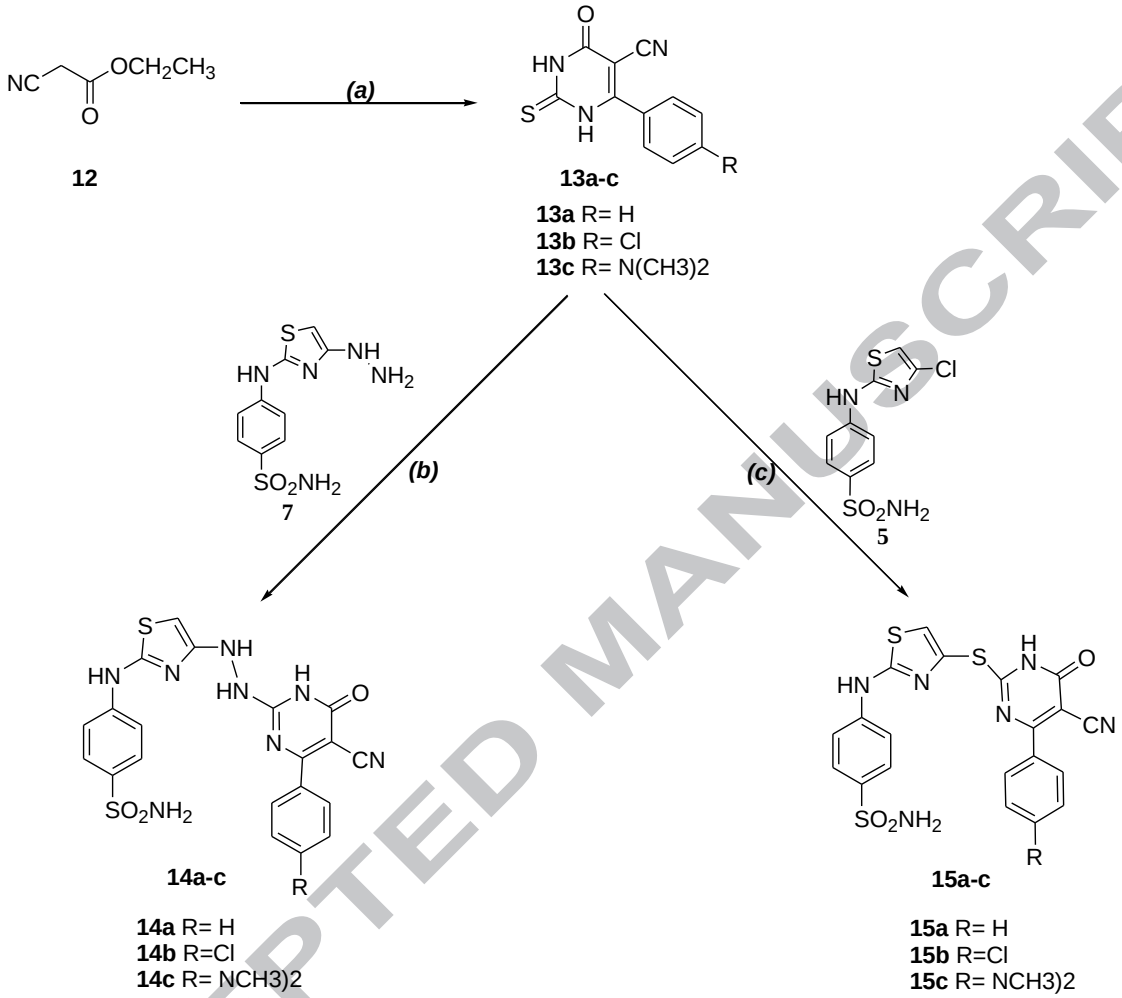
A multistep reaction was then applied for obtaining sulfonamides **11** (Scheme 1). The preparation of 2-benzamidoacetic acid **9a** or 2-(4-chlorobenzamido)acetic acid **9b** from glycine **8** was done by benzoylation with benzoyl chloride or *para*-chlorobenzoyl chloride, followed by Erlenmeyer reaction with appropriate aldehydes (benzaldehyde, *para*- chlorobenzaldehyde or *para*- dimethylaminobenzaldehyde) in acetic anhydride to yield oxazolone derivatives **10a-f**.[20] Compounds **10a-f** were reacted with the hydrazinyl derivative **7** and dry dimethylformamide in glacial acetic acid to give imidazolones **11a-f** (Scheme 3).



Scheme 3. Synthesis of target compounds **9-11**: ***(a)*** benzoyl chloride or *p*-chloro benzoyl chloride, 10% NaOH, rt, 2 h; ***(b)*** benzaldehyde or *p*-chlorobenzaldehyde or *p*-dimethylaminobenzaldehyde, fused sodium acetate , acetic anhydride, boiling water bath, 2 h; ***(c)*** glacial acetic acid, dry DMF, boiling water bath, 10 h.

The one pot ternary condensation reaction of aldehydes, ethyl cyanoacetate and thiourea was first reported by Kambe *et al*. to yield carbonitriles.[21] We applied this reaction by using equimolar amounts of benzaldehyde/ *para*- substituted benzaldehydes and thiourea to obtain the new carbonitriles **13a-c** (Scheme 4). Reaction of carbonitriles **13a-c** with intermediates **7** and **5,** led to 4-{4-[N'-(5-Cyano-6-oxo-4-phenyl-1,6-dihydro-pyrimidin-2-yl)-hydrazino]-thiazol-2-ylamino}-benzenesulfonamide derivatives **14a-c** and 4-[4-(5-Cyano-6-oxo-4-phenyl-1,6-

dihydro-pyrimidin-2-ylsulfanyl)-thiazol-2-ylamino]-benzenesulfonamide **15a-c,** respectively, by procedures already reported in the literature[22] **(**Scheme 4**).**



Scheme 4. Synthesis of target compounds **12-15**: ***(a)*** benzaldehyde or *p*-chlorobenzaldehyde or *p*-dimethylaminobenzaldehyde, thiourea, potassium carbonate, absolute ethanol, reflux, 5h; ***(b)*** TEA, dry DMF, reflux, 24 h; ***(c)*** TEA, dry DMF, reflux, 24h.

All compounds were extensively characterized by physico-chemical and spectroscopic procedures which confirmed their structures (see Experimental for details).

2.2. CA inhibition

The CA inhibition assay was conducted on human (h) CA isoforms hCA I, II and IX with a selection of the new sulfonamides reported here, i.e., compounds **4a-d, 6a-**

d, 11b, 14a, and acetazolamide (AAZ) as a standard inhibitor, by a stopped- flow CO_2 hydrase assay[23] and the results, as inhibition constants K_I (nM), are shown in Table 1.

Table 1. Inhibition data of human (h) CA isoforms hCA I, II and IX with compounds **4a-d, 6a-d, 11b, 14a** and acetazolamide (AAZ)/SLC-0111 as standard inhibitors, by a stopped-flow CO_2 hydrase assay. [23]

Compound	K_I (nM)*		
	hCA I	hCA II	hCA IX
AAZ	250	12.1	25.8
SLC-0111	5080	960	45.0
4a	6.4	5.5	5.8
4b	7.9	4.1	7.3
4c	7.6	4.4	6.4
4d	0.85	0.53	8.5
6a	6.5	0.41	7.3
6b	6.6	0.62	8.8
6c	3.2	0.58	5.6
6d	0.84	0.47	6.7
11b	702	288	29.2
14a	41	7.1	6.5

** Mean from 3 different assays, errors in the range of ± 10 % of the reported values (data not shown).*

The following structure activity relationship (SAR) is evident from the data of Table 1:

(i) The slow isoform hCA I was highly inhibited by the investigated sulfonamides, with K_Is ranging between 0.84 and 41 nM, except **11b** which was a poor inhibitor (K_I of 702 nM). **AAZ** and SLC-0111, poorly inhibit this isoform (K_I of 250 nM for AAZ and of > 5 μM for the second standard). As the physiological role of hCA I is poorly understood to date,[11,12] the sulfonamides reported here may be considered interesting

tools to better understand whether hCA I inhibition in some biological systems has physiologic consequences. For the congeneric subseries of compounds **4**, it is interesting to note that a second primary sulfonamide moiety (in addition to the sulfanilamide one), as in **4a**, does not improve the CA inhibitory properties, whereas the best inhibitor was **4d**, which has a sulfaguanidine such moiety (which is not a good ZBG for CAIs).[11,12] Compounds **6c** and **14a**, although possessing bulkier scaffolds compared to derivatives **4**, showed similar inhibitory activity, whereas **11b** is a poor inhibitor probably due to the fact that its scaffold became too bulky.

(ii) A similar inhibition profile with these sulfonamides was also observed against the second cytosolic isoform, hCA II, with compound **11b** a poor inhibitor (K_I of 288 nM) and the remaining ones highly effective CAIs, with K_Is ranging between 0.41 and 5.5 nM. Thus all the substitution patterns present in these derivatives led to highly effective, low nanomolar or subnanomolar inhibitors. Probably the rigid thiazole scaffold present in the new derivatives reported here (compared to the ureido/thioureido-substituted such componds reported earlier,[13,17] which have a greater rotational flexibility) leads to the observed incerase in hCA II inhibitory properties (but also to a lack of selectivity for inhibiting hCA IX over hCA II, see Table 1).

(iii) The tumor-associated, transmembrane isoform hCA IX was effectively inhibited by all the investigated sulfonamides, with K_Is ranging between 5.6 and 29.2 nM (AAZ, a compound used clinically which showed antitumor effects in some models, has a K_I of 25 nM). Thus, as for hCA I and II inhibition, all substitution patterns present in these sulfonamides lead to low nanomolar hCA IX inhibitors, except **11b**, which has a potency similar to that of AAZ, with a K_I of 29.2 nM (Table 1).

(iv) The main drawback of these compounds is that they potently inhibit both cytosolic as well as transmembrane CA isoforms, with no isoform selectivity at all being present for any member of the series.

2.3. Cytotoxicity studies

The newly obtained compounds were screened for their cytotoxic activity against human breast cancer cell line MCF-7,[24] obtained from the American Type Culture Collection (ATCC, Rockville, MD), which has been known to be a good predictor of clinically useful drugs. Concentrations required to cause cytotoxic effects in 50% of

intact cells (IC_{50}), were estimated from graphic plots and shown in Table 2. The results of table 2 reveal that compounds **4a-d** had IC_{50} ranging between 2.79 and 11.9 μM, whereas **6a-d** had IC_{50} of 12 to 24.3 μM. On the other hand compounds **11a-f**, **14a-c** and **15a-c** had IC_{50} ranging between 36.2 to and ›150 μM. The reference drug 5-fluorouracil (5-FU) with an IC_{50} of 11.1 μM, was also included in the tests. One of the most active compounds, **4a** (IC_{50} of 2.79 μM), was four times as active as 5-FU. A closer look at compounds **4a-d** has revealed that superior cytotoxic activity due to substitution on the phenyl ring at C5 of thiazole was owed to the sulfamoyl moiety or its isosteric COOH group as in compounds **4a** and **4c**, respectively, whereas substitution with electron withdrawing chloride group **(4b)** decreased the activity, which is further decreased if substituted with a bulkier less acidic sulfaguanidino moiety **(4d)**. As for the C4 ring substitution of the thiazole ring, maximum activity was observed for the phenylamino derivatives **6a-c** and the piperdinyl one **6d**, followed by the heterocyclic 5-membered imidazolohydrazono derivatives **11a-f** and finally the heterocyclic 6-membered dihydropyrimidino derivatives **14a-c** and **15a-c.** A closer look at compounds **6a-d** has shown that the fluorophenyl derivative **6c** possessed superior cytotoxic activity within this class. Also, it was worthy to highlight the effect of increasing the bulkiness of the thiazole substituents, which was not in favor for the cytotoxic activity (poor CA IX inhibitory effects), as proved by significant cytotoxic activity decline generally noticed in the derivatives of compounds **11, 14** and **15** if compared to simpler C5 or C4 derivatives of compounds **4** and **6**. Investigating other steric concepts, ring contraction of the 4-oxo-pyrimidine ring **14a-c** to imidazol-5(4*H*)-one gave more promising analogues. Thus, the best CA IX inhibitors were also the most cytotoxic derivatives, according to data of tables 1 and 2.

Table 2**:** IC_{50} values of 5-fluorouracil (5-FU) and all the newly synthesized compounds against human breast cancer cell line (MCF7).[24]

Compound	**IC_{50} (μM)**
5-FU	11.1
4a	2.79
4b	10.7
4c	5.45
4d	11.9
6a	21.5
6b	22.1

6c	12
6d	24.3
11a	45.87
11b	›150
11c	38.9
11d	›150
11e	136.2
11f	44.5
14a	›150
14b	›150
14c	89.7
15a	›150
15b	86.6
15c	85

biological Pharmacological screening and evaluation summarize that most of the tested compounds have good cytotoxic activity, with compounds **4a-d** and **6a-d** showing the best cytotoxic activity, comparable to 5-fluorouracil as a reference drug **(**Fig. 1**)**, where compounds **4a-c** have shown more cytotoxic activity against breast cancer cell line (MCF-7) than 5-fluorouracil, while compounds **4d** and **6a-d** showed slightly less cytotoxic activity against breast cancer cell line (MCF-7) than 5-fluorouracil. Accordingly, 5-phenylhydrazonothiazol-2-yl-aminobenzenesulfonamide derivatives **4a-d** and 4-phenylthiazol-2-yl-aminobenzenesulfonamide derivatives **6a-d** could be considered to be useful building blocks for future research aiming at the synthesis of effective cytotoxic agents.

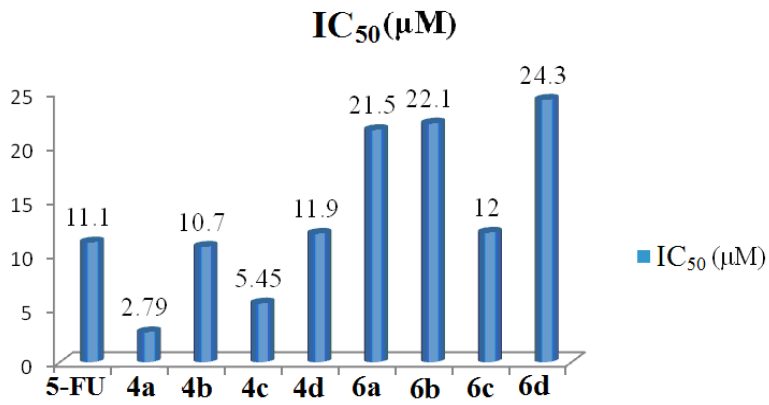


Fig. 1. The order of potency for the most active compounds **4a-d** and **6a-d,** compared to 5-fluorouracil as a reference drug, showing the *in- vitro* screening IC_{50} values.

3. Conclusions

A series of 4-(thiazol-2-ylamino)-benzenesulfonamides was synthesized and screened for their CA inhibitory and cytotoxic activity on human breast cancer cell line MCF-7. hCA isoforms I, II and IX were included in the study. The new sulfonamides showed excellent inhibition of all three isoforms, with K_Is in the range of 0.84-702 nM against hCA I, of 0.41 – 288 nM against hCA II and of 5.6 – 29.2 nM against the tumor-associated hCA IX, a validated anti-tumor target, with a sulfonamide (SLC-0111) in phase I clinical trials for the treatment of hypoxic, metastatic solid tumors over expressing CA IX. The new compounds showed micromolar inhibitory of growth efficacy against breast cancer MCF-7 cell lines.

4. Experimental

4.1. Chemistry

All chemicals were purchased from VWR International Merck, Germany or Sigma-Aldrich and used without further purification. Melting Points were carried out by open capillary tube method using IA 9100MK-Digital Melting Point Griffin Apparatus and they are uncorrected. Elemental Microanalysis was carried out at the Regional Micro Analytical Center for Mycology and Biotechnology, Al-Azhar University. Infrared spectra were recorded on BRUKER Vector 22 (Japan) and expressed in wave number (cm^{-1}) using potassium bromide discs at the microanalytical Center, Faculty of Science, Cairo University. ^{1}H-NMR and ^{13}C-NMR Spectra were recorded on a Bruker Avance III 400 MHz for ^{1}H and 100 MHz for ^{13}C (Bruker AG, Switzerland) with BBFO Smart Probe and Bruker 400 AEON Nitrogen-Free at the microanalytical Center, Faculty of Pharmacy, Beni Suef University. Chemical shifts were expressed in δ units and were related to that of the solvents. As for the proton magnetic resonance, D_2O was carried out for NH and OH exchangeable protons. Mass Spectra were recorded using Fennigan MAT, SSQ 7000, Mass spectrometer, at 70 eV (EI) at the microanalytical Center, Faculty of Science, Cairo University and Waters Micromass Q-Tof Micro mass spectrometer (ESI) and Waters Acquity Ultra Performance LC with ZQ detector in ESI mode. All the compounds were named according to the IUPAC system using CS Chem. Draw Ultra version 12.0. Compounds **2,**[18] **3**,[18] **5,**[19] **9**,[20] **10a-f,**[20] **13a-c,**[21] were prepared according to reported methods, while

compounds **14a-c** and **15a-c** were prepared according to modified procedures from reported methods.[22]

General procedure for the synthesis of compounds 4a-d.

A solution of sodium nitrite (0.18 g, 2.56 mmol) in water (3 mL) was added drop wise to a stirred and previously cooled solution of the appropriate amino derivative (2.56 mmol) in 2N hydrochloric acid (3 mL) in an ice bath. The mixture was stirred for one hour at 0-5°C. Then a solution of **3** (0.7 g, 2.56 mmol) in 2N hydrochloric acid (6 mL) was added and pH 7 was adjusted with sodium acetate and left to stir for 18 h. at room temperature. The product was then filtered, dried, and recrystallized from dioxane.

4-(2-(4-oxo-2-((4-sulfamoylphenyl)amino)thiazol-5(4H)-ylidene)hydrazinyl) benzenesulfonamide ***(4a).*** Yield 70%; m.p. 260-262 °C; IR (KBr) (υ_{max},/ cm^{-1}): 3468, 3280, 3109 ($2NH_2$, 2NHs), 3025 (CH aromatic), 1696 (C=O), 1396, 1145 (SO_2); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.17 (2H, s, ex, NH_2), 7.35-7.88 (4H, m, Ar-Hs), 7.46 (2H, s, ex, NH_2), 7.98 (2H, d, Ar-Hs, *J* = 8.2 Hz), 8.11 (d, 2H, Ar-Hs, *J* = 8.2 Hz), 10.05, 10.73 (2H, 2s, ex, 2 NH); ^{13}C NMR (DMSO- d_6, 100 MHz) δ (ppm): 113.8, 126.8, 127.7, 127.9, 129.8, 137.1, 137.9 and 144.3 (aromatic Cs), 146.87 (thiazole C5), 150.5 (thiazole C2), 162.1 (C=O). MS *m/z*: 454 (M^+). Anal. Calcd. For $C_{15}H_{14}N_6O_5S_3$ (454.50): C, 39.64; H, 3.10; N, 18.49. Found: C, 39.89; H, 3.17; N, 18.19.

4-((5-(2-(4-chlorophenyl)hydrazono)-4-oxo-4,5-dihydrothiazol-2-yl)amino)benzenesulfonamide ***(4b).*** Yield 65%. m.p. 250-252 °C; IR (KBr) (υ_{max},/ cm^{-1}): 3308, 3211 (NH_2, 2NHs), 3090 (CH aromatic),1639 (C=O), 1326, 1155 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 7.08-7.78 (4H, m, Ar-Hs), 7.50 (2H, s, ex, NH_2), 7.86 (2H, d, Ar-Hs, *J* = 8.6 Hz), 8.01 (2H, d, Ar-Hs, *J* = 8.6 Hz), 10.01, 10.53 (2H, 2s, ex, 2NHs); MS *m/z*: 409 (M^+). Anal. Calcd. For $C_{15}H_{12}ClN_5O_3S_2$ (409.87): C, 43.96; H, 2.95; N, 17.09. Found: C, 44.19; H, 2.94; N, 17.22.

4- (2- (4- oxo- 2- ((4 -sulfamoylphenyl) amino)thiazol- 5(4H)- ylidene)hydrazinyl) benzoicacid ***(4c).*** Yield 60%. m.p. 248-250 °C; IR (KBr) (υ_{max},/ cm^{-1}): 3356 (OH), 3262, 3228 (NH_2, 2NHs), 3065 (CH aromatic), 1720,1 607 (2C=O), 1375, 1160 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 6.96-7.66 (4H, m, Ar-Hs), 7.20

(1H, s, ex, NH), 7.49 (2H, s, ex, NH_2), 7.77 (2H, d, Ar-Hs, *J* = 8.8 Hz), 7.96 (2H, d, Ar-Hs, *J* = 8.8 Hz), 10.06 (1H, s, ex, NH), 11.00 (s, 1H, ex, OH); MS *m/z*: 419 (M^+). Anal. Calcd. For $C_{16}H_{13}N_5O_5S_2$ (419.43): C, 45.82; H, 3.12; N, 16.70. Found: C, 46.03; H, 3.17; N, 16.87.

N-(diaminomethylene)-4-(2-(4-oxo-2-((4-sulfamoylphenyl)amino)thiazol-5(4H)-ylidene)hydrazinyl)benzenesulfonamide ***(4d***). Yield 75%; m.p. 252-254 °C. IR (KBr) (υ_{max},/ cm^{-1}): 3390, 3329, 3272,3141 ($3NH_2$, 2NHs), 3039 (CH aromatic), 1611 (C=O), 1341, 1163 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 6.67 (4H, s, ex, 2 NH_2), 7.33-7.75 (4H, m, Ar-Hs), 7.49 (2H, s, ex, NH_2), 7.85 (2H, d, Ar-Hs, *J* = 8.0 Hz), 7.95 (2H, d, Ar-Hs, *J* = 8.0 Hz), 10.04, 10.71 (2H, 2s, ex, 2NHs); MS *m/z:* 496.31 (M^+). Anal. Calcd. For $C_{16}H_{16}N_8O_5S_3$ (496.54): C, 38.70; H, 3.25; N, 22.57. Found: C, 38.88; H, 3.23; N, 22.31.

General procedure for the synthesis of compounds 6a-d. The appropriate amine or piperidine (2.1 mmol) and triethylamine (5 mL) were added to a solution of **5** (0.58 g, 2 mmol) in dry DMF (10 mL). The mixture was heated under reflux for 24 h. Then the solution was poured onto ice water; the product was collected, dried, and recrystallized from ethanol.

4, 4′-(Thiazole-2,4-diylbis(azanediyl))dibenzenesulfonamide ***(6a).*** Yield 50%; m.p. 240-242 °C; IR (KBr) (υ_{max},/ cm^{-1}): 3255-3098 ($2NH_2$, 2NHs), 3010 (CH aromatic), 1321, 1156 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 5.82 (1H, s, ex, NH), 6.89 (1H, s, thiazole CH), 6.98-7.82 (8H, m, Ar-Hs), 7.23 (2H, s, ex, NH_2), 10.87 (1H, s, ex, NH), 11.60 (2H, s, ex, NH_2); ^{13}C NMR (DMSO- d_6, 100 MHz) δ(ppm): 112.3 (thiazole C_5), 121.2, 126.7, 127.4, 127.7, 129.7, 133.1, 133.4, 135.2 and 140.1 (aromatic Cs), 152.8 (thiazole C_2); MS *m/z:* 425(M^+). Anal. Calcd. For $C1_5H1_5N_5O_4S_3$ (425.51): C, 42.34; H, 3.55; N, 16.46. Found: C, 42.49; H, 3.52; N, 16.62.

4-((2-((4-Sulfamoylphenyl)amino)thiazol-4-yl)amino)benzoic acid ***(6b).*** Yield 55%; m.p. 237-239 °C; IR (KBr) (υ_{max},/ cm^{-1}): 3294-3100 (OH, NH_2, 2NHs), 3045 (CH aromatic), 1710 (C=O), 1338, 1159 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 6.50 (1H, s, thiazole CH), 7.09-7.72 (8H, m, Ar-Hs), 7.10 (2H, s, ex, NH_2),

9.64 (2H, s, ex, 2NHs), 13.01 (1H, s, ex, OH); MS *m*/*z*: 390(M^+). Anal. Calcd. For $C_{16}H_{14}N_4O_4S_2$ (390.44): C, 49.22; H, 3.61; N, 14.35. Found: C, 49.51; H, 3.67; N, 14.51.

4-((4-((4-Fluorophenyl)amino)thiazol-2-yl)amino)benzenesulfonamide ***(6c).*** Yield 60%; m.p. 220-222 °C; IR (KBr) (υ_{max},/ cm^{-1}): 3425-3303(NH_2, 2NHs), 3077 (CH aromatic), 1308, 1154 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 7.10 (1H, s, thiazole CH), 7.15-8.03 (8H, m, Ar-Hs), 7.28 (2H, s, ex, NH_2), 8.79, 10.93 (2H, 2s, ex, 2NHs); MS *m*/*z*: 364 (M^+). Anal. Calcd. For $C_{15}H_{13}FN_4O_2S_2$ (364.42): C, 49.44; H, 3.60; N, 15.37. Found: C, 49.68; H, 3.71; N, 15.44.

4-((4-(Piperidin-1-yl)thiazol-2-yl)amino)benzenesulfonamide ***(6d).*** Yield 49%; m.p. 245-247 °C; IR (KBr) (υ_{max},/ cm^{-1}): 3335 (NH_2, NH), 3085 (CH aromatic),1315, 1150 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 1.54-1.68 (m, 2H, piperidine), 2.89-3.17 (m, 4H, piperidine), 3.49-3.62 (m, 4H, piperidine), 7.30 (2H, s, ex, NH_2), 7.75-8.15 (5H, m, Ar-Hs+ thiazole CH), 10.94 (1H, s, ex, NH); MS *m*/*z*: 338 (M^+). Anal. Calcd. For $C_{14}H_{18}N_4O_2S_2$ (338.45): C, 49.68; H, 5.36; N, 16.55. Found: C, 49.87; H, 5.44; N, 16.43.

General procedure for the synthesis of compound 7. Hydrazine hydrate (3.16 g, 3.1 mL, 100 mmol) was added to a suspension of **5** (2.9 g, 10 mmol) in ethanol (35 mL). The reaction mixture was heated under reflux for 24 h. Then the mixture was cooled, the product collected, washed with ethanol, dried and recrystallized from dioxane.

4-((4-Hydrazinylthiazol-2-yl)amino)benzenesulfonamide ***(7).*** Yield: 66%. m.p.: 290-292°C. IR (KBr) (υ, cm^{-1}): 3355-3058 ($2NH_2$, 2NHs), 3005 (CH aromatic), 1321, 1156 (SO_2). ^{1}H-NMR (DMSO-d_6- 400 MHz) δ (ppm): 7.09 (2H, s, ex, NH_2), 7.52-7.66 (m, 5H, Ar-H), 9.62, 13.00 (2H, s, ex, 2NHs), 13.25 (2H, s, ex, NH_2). MS *m/z*: 285 (M^+). Anal. Calcd. For $C_9H_{11}N_5O_2S_2$ (285.35): C, 37.88; H, 3.89; N, 24.54. Found: C, 37.66; H, 4.11; N, 24.43.

General procedure for the synthesis of compounds 11a-f. A mixture of equimolar amounts of compound **7** and the appropriate oxazolone **10a-f** (10 mmol) in glacial acetic acid (10 mL) containing few drops of dry DMF was heated in boiling

water bath at 100 °C for 10 h. Then the solution was poured onto ice water; the product was collected, dried, and recrystallized from dioxane.

4-((4-((4-benzylidene-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-1-yl)amino)thiazol-2-yl)amino) benzenesulfonamide ***(11a).*** Yield: 49%; m.p.: 258-260 °C; IR (KBr) (υ_{max},/ cm^{-1}): 3452, 3263, 3192 (NH_2, 2NHs), 3087 (CH aromatic), 1644 (C=O), 1338, 1167 (SO_2); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ (ppm): 7.11 (1H, s, thiazole CH), 7.17 (2H, s, ex, NH_2), 7.40-7.71 (7H, m, Ar-Hs+ methine H), 7.91 (2H, d, Ar-Hs, *J* = 8.2 Hz), 8.12 (2H, d, Ar-Hs, *J* = 8.2 Hz), 8.21 (2H, d, Ar-Hs, *J* = 8.6 Hz), 8.34 (2H, d, Ar-Hs, *J* = 8.6 Hz), 9.73, 10.05 (2H, 2s, ex, 2NHs); ^{13}C NMR (DMSO- d_6, 100 MHz) δ (ppm): 113.1 (thiazole C_5), 113.9 (methine C), 117.4- 150.7 (aromatic Cs), 153.5 (pyrazolone C_2), 162.1 (thiazole C_2), 168.1 (C=O); MS *m/z*: 516 (M^+). Anal. Calcd. For $C_{25}H_{20}N_6O_3S_2$ (516.59): C, 58.12; H, 3.90; N, 16.27. Found: C, 58.40; H, 3.94; N, 16.29.

4-((4-((4-(chlorobenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-1-yl)amino)thiazol-2-yl)amino)benzenesulfonamide ***(11b).*** Yield: 53%; m.p.: 262-264 °C; IR (KBr) (υ_{max},/ cm^{-1}): 3412, 3216 (NH_2, 2NHs), 3095 (CH aromatic), 1678 (C=O), 1389, 1138 (SO_2); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ (ppm): 7.08 (1H, s, thiazole CH), 7.11 (2H, s, ex, NH_2), 7.41-7.93 (10H, m, Ar-Hs+ methine H), 8.13 (2H, d, Ar-Hs, *J* = 8.0 Hz), 8.33 (2H, d, Ar-Hs, *J* = 8.0 Hz), 9.69, 10.03 (2H, 2s, ex, 2NHs); MS *m/z*: 551 (M^+). Anal. Calcd. For $C_{25}H_{19}ClN_6O_3S_2$ (551.04): C, 54.49 ; H, 3.48; N, 15.25. Found: C, 54.67; H, 3.51; N, 15.37.

4-((4-((4-(4-(dimethylamino)benzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-1-yl)amino)thiazol-2-yl)amino)benzenesulfonamide ***(11c).*** Yield: 55%; m.p.: 270-272 °C; IR (KBr) (υ_{max},/ cm^{-1}): 3417, 3262 (NH_2, 2NHs), 3060 (CH aromatic), 2929 (CH Aliphatic) 1634 (C=O), 1320, 1154 (SO_2); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ (ppm): 3.17 (6H, s, $2CH_3$), 6.58 (1H, s, thiazole CH), 7.03 (2H, s, ex, NH_2), 7.24-7.99 (10H, m, Ar-Hs+ methine H), 8.11 (2H, d, Ar-Hs, *J* = 8.6 Hz), 8.24 (2H, d, Ar-Hs, *J* = 8.6 Hz), 10.31, 10.46 (2H, 2s, ex, 2NHs); MS *m/z*: 559 (M^+). Anal. Calcd. For $C_{27}H_{25}N_7O_3S_2$ (559.66): C, 57.94; H, 4.50; N, 17.52. Found: C, 58.18 ; H, 4.40; N, 17.73.

4-((4-((4-benzylidene-2-(4-chlorophenyl)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)amino)thiazol-2-yl)amino)benzenesulfonamide ***(11d).*** Yield: 48%; m.p.: 249-251 °C; IR (KBr) (υ_{max},/ cm^{-1}): 3450 (NH_2, 2NHs), 3075 (CH aromatic), 1644 (C=O),

1340, 1171 (SO_2); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ (ppm): 6.65 (1H, s, thiazole CH), 7.23 (2H, s, ex, NH_2), 7.32-7.89 (10H, m, Ar-Hs+ methine H), 8.22 (2H, d, Ar-Hs, *J* = 8.8 Hz), 8.34 (2H, d, Ar-Hs, *J* = 8.8 Hz), 9.98, 10.42 (2H, 2s, ex, 2NHs); MS *m/z*: 551 (M^+). Anal. Calcd. For $C_{25}H_{19}ClN_6O_3S_2$ (551.04): C, 54.49; H, 3.48 ; N, 15.25. Found: C, 54.62; H, 3.46; N, 15.41.

4-((4-((4-(4-chlorobenzylidene)-2-(4-chlorophenyl)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)amino)thiazol-2-yl)amino)benzenesulfonamide ***(11e).*** Yield: 51%; m.p.: 268-270 °C; IR (KBr) (υ_{max},/ cm^{-1}): 3417 (NH_2, 2NHs), 3093 (CH aromatic), 1627 (C=O), 1323, 1151 (SO_2); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ (ppm): 7.08 (1H, s, thiazole CH), 7.13-8.07 (9H, m, Ar-Hs+ methine H), 7.55 (2H, s , ex, NH_2), 8.14 (2H, d, Ar-Hs, *J* = 8.2 Hz), 8.38 (2H, d, Ar-Hs, *J* = 8.2 Hz), 9.95, 12.66 (2H, 2s, ex, 2NHs); MS *m/z*: 585 (M^+). Anal. Calcd. For $C_{25}H_{18}Cl_2N_6O_3S_2$ (585.48): C, 51.29; H, 3.10; N, 14.35. Found: C, 51.57; H, 3.18; N, 14.52.

4-((4-((2-(4-chlorophenyl)-4-(4-(dimethylamino)benzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)amino)thiazol-2- yl)amino)benzenesulfonamide ***(11f).*** Yield: 45%; m.p.: 253-255 °C; IR (KBr) (υ_{max},/ cm^{-1}): 3427 (NH_2, 2NHs), 3065 (CH aromatic), 2933 (CH Aliphatic), 1628 (C=O), 1325, 1155 (SO_2); ^{1}H-NMR (DMSO-d_6- 400 MHz) δ (ppm): 3.08 (6H, s, $2CH_3$), 6.85 (1H, s, thiazole CH), 7.12 (2H, s, ex, NH_2), 7.24-8.00 (7H, m, Ar-Hs+ methine H), 8.11 (2H, d, Ar-Hs, *J* = 8.4 Hz), 8.23 (2H, d, Ar-Hs, *J* = 8.4 Hz), 8.34 (2H, d, Ar-Hs, *J* = 8.0 Hz), 9.38, 11.48 (2H, s, ex, 2NHs); MS *m/z*: 594 (M^+). Anal. Calcd. For $C_{27}H_{24}ClN_7O_3S_2$ (594.11): C, 54.58; H, 4.07; N, 16.50. Found: C, 54.89; H, 4.03; N, 16.78.

General procedure for the synthesis of compounds 14a-c. A mixture of equimolar amounts of compound **7** and the appropriate 2-thioxo-tetrahydropyrimidine derivative **13a-c** (1.6 mmol) in dry DMF (10 mL) containing few drops of triethylamine was heated under reflux for 24 h. Then the solution was poured onto ice water; the product was collected, dried and recrystallized from DMF/water.

4-((4-(2-(5-Cyano-6-oxo-4-phenyl-1,6-dihydropyrimidin-2-yl)hydrazinyl)thiazol-2-yl)amino)benzenesulfonamide ***(14a).*** Yield 45%; m.p. 270-272 °C; IR (KBr) (υ, cm^{-1}): 3329, 3108 (NH_2, NHs), 3082 (CH aromatic), 2203 (C≡N), 1680 (C=O), 1324, 1156 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 7.07 (2H, s, ex, 2NHs), 7.20 (2H, s, ex, NH_2), 7.59-7.89 (6H, m, Ar-Hs+ thiazole CH), 8.00 (2H, d, Ar-Hs, *J* =

8.2 Hz), 8.16 (2H, d, Ar-Hs, *J* = 8.2 Hz), 9.52, 11.89 (2H, 2s, ex, 2NHs); MS *m/z:* 480 (M^+). Anal. Calcd. For $C_{20}H_{16}N_8O_3S_2$ (480.52): C, 49.99; H, 3.36; N, 23.32. Found: C, 50.12; H, 3.38; N, 23.67.

4-((4-(2-(4-(4-Chlorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)hydrazinyl)thiazol-2-yl)amino)benzenesulfonamide ***(14b)***. Yield 40%; m.p. 260-262 °C; IR (KBr) (υ_{max},/ cm^{-1}): 3426 (NH_2, NHs), 3098 (CH aromatic), 2213 (C≡N), 1610 (C=O), 1324, 1154 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 7.25 (2H, s, ex, NH_2), 7.42-8.12 (5H, m, Ar-Hs+ thiazole CH), 8.25 (2H, d, Ar-Hs, *J* = 8.4 Hz), 8.33 (2H, d, Ar-Hs, *J* = 8.4 Hz), 10.28, 11.16 (2s, 4H, ex, 4NHs); ^{13}C NMR (DMSO- d_6, 100 MHz) δ (ppm): 110.1 (pyrimidinone C_5), 113.1 (thiazole C_5), 113.1-151.9 (aromatic Cs+CN), 152.3 (pyrimidinone C_2), 153.5 (thiazole C_2), 168.1 (C=O), 170.1 (of pyrimidinone C_4); MS *m/z*: 514 (M^+). Anal. Calcd. For $C_{20}H_{15}ClN_8O_3S_2$: (514.97): C, 46.65; H 2.94; N, 21.76. Found: C, 46.87; H, 2.92; N, 21.99.

4-((4-(2-(5-Cyano-4-(4-(dimethylamino)phenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)hydrazinyl) thiazol-2-yl)amino)benzenesulfonamide ***(14c).*** Yield 55%; m.p. 275-277 °C; IR (KBr) (υ_{max},/ cm^{-1}): 3429 (NH_2, NHs), 3090 (CH aromatic), 2932 (CH Aliphatic), 2206 (C≡N), 1623 (C=O), 1326, 1153 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 3.03 (6H, s, $2CH_3$), 6.81-7.95 (5H, m, Ar-Hs+ thiazole CH), 7.49 (2H, s, ex, NH_2), 8.05 (2H, d, Ar-Hs, *J* = 8.0 Hz), 8.17 (2H, d, Ar-Hs, *J* = 8.0 Hz), 10.02, 10.55, 12.95 (4H, 3s, ex, 4 NHs); MS *m/z*: 523 (M^+). Anal. Calcd. For $C_{22}H_{21}N_9O_3S_2$ (523.59): C, 50.47; H, 4.04; N, 24.08. Found: C, 50.62; H, 4.11; N, 24.46.

General procedure for the synthesis of compounds 15a-c. A mixture of compound **5** (2.9 g, 10 mmol), the appropriate 2-thioxo-tetrahydropyrimidine derivative **XIIIa-c** (10 mmol) and triethylamine (2 mL) was heated under reflux in dry DMF (10 mL) for 24 h. Then the solution was poured onto ice water; the product was collected, dried and recrystallized from DMF/water.

4-((4-((5-Cyano-6-oxo-4-phenyl-1,6-dihydropyrimidin-2-yl)thio)thiazol-2-yl)amino) benzenesulfonamide ***(15a)***. Yield 60%; m.p. 255-257 °C; IR (KBr) (υ_{max},/ cm^{-1}): 3477, 3382, 3318 (NH_2, 2NHs), 3075 (CH aromatic), 2236 (C≡N), 1626 (C=O), 1309, 1150 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 7.21 (1H, s,

thiazole CH), 7.39-8.09 (5H, m, Ar-Hs), 7.66 (2H, s, ex, NH_2), 8.18 (2H, d, Ar-Hs, *J* = 8.4 Hz), 8.35 (2H, d, Ar-Hs, *J* = 8.4 Hz), 11.95, 12.65 (2H, 2s, ex, 2NHs); MS *m*/*z*: 482 (M^+). Anal. Calcd. For $C_{20}H_{14}N_6O_3S_3$ (482.56): C, 49.78; H, 2.92; N, 17.42. Found: C, 49.97; H, 2.90; N, 17.58.

4-((4-((4-(4-Chlorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)thiazol-2-yl)amino)benzenesulfonamide ***(15b).*** Yield 65%; m.p. 260-262 °C; IR (KBr) (υ_{max},/ cm^{-1}): 3444, 3340, 3233 (NH_2, 2NHs), 3055 (CH aromatic), 2165 (C≡N), 1629 (C=O), 1374, 1158 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 7.11 (1H, s, thiazole CH), 7.22-8.06 (4H, m, Ar-Hs), 7.37 (2H, s, ex, NH_2), 8.24 (2H, d, Ar-Hs, *J* = 8.2 Hz), 8.37 (2H, d, Ar-Hs, *J* = 8.2 Hz),10.85, 11.20 (2H, 2s, ex, 2NHs); ^{13}C NMR (DMSO- d_6, 100 MHz) δ (ppm): 113.1 (pyrimidinone C_5), 113.8 (thiazole C_5), 117.37- 146.8 (aromatic Cs+ CN group), 150.6 (thiazole C_2), 153.5 (pyrimidinone C_2), 162.8 (C=O), 168.1 (pyrimidinone C_4); MS *m*/*z*: 517 (M^+). Anal. Calcd. For $C_{20}H_{13}ClN_6O_3S_3$ (517.00): C, 46.46; H, 2.53; N, 16.26. Found: C, 46.55; H, 2.56; N, 16.08.

4-((4-((5-Cyano-4-(4-(dimethylamino)phenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)thiazol-2-yl)amino)benzenesulfonamide ***(15c).*** Yield 50%; m.p. 245-247 °C; IR (KBr) (υ_{max},/ cm^{-1}): 3420 (NH_2, 2NHs), 3049 (CH aromatic), 2930 (CH Aliphatic), 2212 (C≡N), 1626 (C=O), 1321, 1153 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 3.17 (6H, s, $2CH_3$), 7.28 (2H, s, ex, NH_2), 7.34-7.95 (5H, m, Ar-Hs+ thiazole CH), 8.14 (2H, d, Ar-Hs, *J* = 8.0 Hz), 8.27 (2H, d, Ar-Hs, *J* = 8.0 Hz), 11.92 (2H, s, ex, 2NHs); MS *m*/*z:* 525 (M^+). Anal. Calcd. For $C_{22}H_{19}N_7O_3S_3$ (525.63): C, 50.27; H, 3.64; N, 18.65. Found: C, 50.53; H, 3.78; N, 18.26.

4.2. Carbonic Anhydrase inhibition assay

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity.[56] 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 7.4) as buffer, and 20 mM Na_2SO_4 for maintaining constant the ionic strength (this anion is not inhibitory and has a K_I> 200 mM against these enzymes),[30] 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 measurement at least six traces of the initial 5-10% of the reaction have been used for determining the initial

velocity, working with 10-fold decreasing inhibitor concentrations ranging between0.1 nM and 10-100 µM (depending on the inhibitor potency, but at least 5 points at different inhibitor concentrations were employed for determining the inhibition constants). 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 0.1 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 the Cheng-Prusoff equation, and represent the mean from at least three different determinations. The human isoforms hCA I, II and IX were recombinant enzymes produced as described earlier in our laboratory. [25-28]

4.3. Cytotoxicity studies

In vitro screening was carried out in accordance with the Regional Center for Mycology and Biotechnology, Al-Azhar University, Egypt. All the targeted compounds were screened for their cytotoxic activity against human breast cancer cell line MCF-7, obtained from the American Type Culture Collection (ATCC, Rockville, MD), which has been known to be a good predictor of clinically useful drugs. IC_{50}, the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots, recorded and tabulated in table 2. In this protocol,[24] the cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50µg/ ml gentamycin. The cells were maintained at 37°C in a humidified atmosphere with 5% CO_2 and were subcultured two to three times a week. The cytotoxic activity was evaluated on tumor cells. The cells were grown as monolayers in growth RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50µg/ml gentamycin. The monolayers of 10,000 cells adhered at the bottom of the wells in a 96-well microtiter plate incubated for 24h at 37°C in a humidified incubator with 5% CO_2. The monolayers were then washed with sterile phosphate buffered saline (0.01 M pH 7.2) and simultaneously the cells were treated with 100 µl from different dilutions of tested sample in fresh maintenance medium and incubated at 37 °C. A control of untreated cells was made in the absence of tested sample. A positive control containing 5- Fluorouracil (5-FU) drug was also tested as reference drug for comparison. Six wells were used for each concentration of the test sample. Every 24h

the observation under the inverted microscope was made. The number of the surviving cells was determined by staining the cells with crystal violet,[24] followed by cell lysing using 33% glacial acetic acid and read the absorbance at 590nm using ELISA reader (SunRise, TECAN, Inc, USA) after well mixing. The absorbance values from untreated cells were considered as 100% proliferation. The number of viable cells was determined using ELISA reader as previously mentioned before and the percentage of viability was calculated as [1-(ODt/ODc)]x100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots.

Acknowledgements

The Authors would like to thank Prof. Dr. Hanan H. Georgey, Department of pharmaceutical Chemistry, Faculty of Pharmacy, Cairo University, Egypt**,** for giving time and effort to add fruitful scientific comments to the research and the analytical chemistry stuff at Regional microanalytical Center for Mycology & Biotechnology, Al-Azhar University for carrying out the *in vitro* screening. Work from Supuran laboratory was financed by two EU grants (Dynano and Metoxia).

References

1) a) McDonald, P.C.; Chafe, S.C.; Dedhar, S. *Front. Cell Developm. Biol*. **2016**, *4*, e27; b) Hanahan, D.; Weinberg, R. A. *Cell* **2011,** *144*, 646; c) Supuran, C.T. *World J. Clin. Oncol.* **2012**, *3*, 98.

2) a) Supuran, C.T.; Scozzafava, A. *Expert Opin. Ther. Pat.* **2004**, *14*, 25; b) Winum, J.Y.; Maresca, A.; Carta, F.; Scozzafava, A.; Supuran, C.T. *Chem. Commun.* **2012**, *48*, 8177; c) Casini, A; Scozzafava, A.; Supuran, C.T. *Expert Opin. Ther. Pat.* **2002,** *12*, 1307.

3) a) Ma, T.; A. Fuld, D.; Rigas, J. R.; Hagey, A. E.; Gordon, G. B.; Dmitrovsky, E.; Dragnev, K. H. *Chemotherapy* **2012**, *58*, 321; b) 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.

4) a) Supuran, C.T. *Biochem. J*. 2016, in press (doi: doi: 10.1042/BCJ20160115); b) Vander Heiden, M. G.; Cantley, L. C.; Thompson, C. B. *Science* **2009,** *324*, 1029.

5) a) Neri, D.; Supuran, C. T. *Nat. Rev. Drug Discov.* **2011,** *10*, 767; b) McDonald, P. C.; Winum, J. Y.; Supuran, C. T.; Dedhar, S. *Oncotarget* **2012,** *3*, 84; c) Ward, C.; Langdon, S.P.; Mullen, P.; Harris, A.L.; Harrison, D.J.; Supuran, C.T.; Kunkler, I. *Cancer Treatm. Rev*. **2013**, *39*, 171; d) 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; e) Ebbesen, P.; Pettersen, E.O.; Gorr, T.A.; Jobst, G.; Williams, K.; Kienninger, J.; Wenger, R.H.; Pastorekova, S.; Dubois, L.; Lambin, P.; Wouters, B.G.; Supuran, C.T.; Poellinger, L.; Ratcliffe, P.; Kanopka, A.; Görlach, A.; Gasmann, M.; Harris, A.L.; Maxwell, P.; Scozzafava, A. *J. Enzyme Inhib. Med. Chem.* **2009**, *24* (*S1)*, 1.

6) a) Supuran, C. T. *Nat. Rev. Drug Discov.***2008,** *7*, 168; b) Alterio, V.; Di Fiore, A.; D'Ambrosio, K.; Supuran, C. T.; De Simone, G. *Chem. Rev.* **2012,** *112*, 4421; c) Supuran, C.T. *J. Enzyme Inhib. Med. Chem*. **2016**, *31*, 345; d) De Simone, G.; Supuran, C.T. *Biochim. Biophys. Acta* **2010**, *1804*, 404.

7) a) Krall, N.; Pretto, F.; Decurtins, W.; Bernardes, G. J. L.; Supuran, C. T.; Neri, D. *Angew. Chem. Int. Ed. Engl.* **2014**, *53*, 4231; b) Scozzafava, A.; Menabuoni, L.; Mincione, F.; Supuran, C.T. *J. Med. Chem*. **2002**, *45*, 1466; c) De Simone, G.;

Alterio, V.; Supuran, C.T. *Expert Opin. Drug Discov*. **2013**, *8*, 793; d) Masini, E.; Carta, F.; Scozzafava, A.; Supuran, C.T. *Expert Opin. Ther. Pat*. **2013**, *23*, 705.

8) a) Dubois, L.; Douma, K.; Supuran, C.T.; Chiu, R.K.; van Zandvoort, M.A.M.; Pastoreková, S.; Scozzafava, A.; Wouters, B.G.; Lambin, P. *Radiother. Oncol.* **2007**, *83*, 367; b) Dubois, L.; Lieuwes, N.G.; Maresca, A.; Thiry, A.; Supuran, C.T.; Scozzafava, A.; Wouters, B.G.; Lambin, P. *Radiother. Oncol*. **2009**, *92*, 423; c) Akurathi, V.; Dubois, L.; Lieuwes, N.G.; Chitneni, S.K.; Cleynhens, B.J.; Vullo, D.; Supuran, C.T.; Verbruggen, A.M.; Lambin, P.; Bormans, G.M. *Nuclear Med. Biol*. **2010**, *37*, 557; d) Pan, J.; Lau, J.; Mesak, F.; Hundal, N.; Pourghiasian, M.; Liu, Z.; Benard, F.; Dedhar, S.; Supuran, C.T.; Lin, K.S. *J. Enzyme Inhib. Med. Chem*.**2014**, *29*, 249.

9) a) Bozdag, M.; Ferraroni, M.; Nuti, E.; Vullo, D.; Rossello, A.; Carta, F.; Scozzafava, A.; Supuran, C.T. *Bioorg Med. Chem.* **2014**, *22*, 334; b) Winum, J.-Y.;Temperini, C.; El Cheikh, K.; Innocenti, A.;Vullo, D.; Ciattini, S.; Montero, J.-L.; Scozzafava, A.; Supuran, C. T. *J. Med. Chem*. **2006**, 49, 7024; c) Vitale, R. M.; Pedone, C.; Amodeo, P.; Antel, J.; Wurl, M.; Scozzafava, A.; Supuran, C. T.; De Simone, G. *Bioorg. Med. Chem*. **2007**, 15, 4152; d) Di Fiore, A.; Scozzafava, A.; Winum, J.Y.; Montero, J.L.; Pedone, C.; Supuran, C. T.; De Simone, G. *Bioorg. Med. Chem. Lett*. **2007**, 17, 1726; e) Abbate, F.; Winum, J. Y.; Potter, B. V. L.; Casini, A.; Montero, J. L.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett*. **2004**, 14, 231.

10) a) Supuran, C. T. *Bioorg. Med. Chem. Lett.*, **2010**, *20*, 3467; b) Abbate, F.; Casini, A.; Owa, T.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 217; c) Nishimori, I.; Minakuchi, T.; Onishi, S.; Vullo, D.; Cecchi, A.; Scozzafava, A.; Supuran, C.T. *Bioorg. Med. Chem*. **2007**, *15*, 7229; d) Carta, F.; Supuran, C. T.; Scozzafava, A. *Future Med Chem.* **2014**, *6***,** 1149; e) Capasso, C.; Supuran, C.T. *Expert Opin. Ther. Pat*. **2013**, *23*, 693.

11) a) Supuran, C.T. *J. Enzyme Inhib. Med. Chem*. **2012**, *27*, 759; b) Pastorekova, S.; Parkkila, S.; Pastorek, J.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem*. **2004**, *19*, 199; c) Scozzafava, A.; Owa, T.; Mastrolorenzo, A.; Supuran, C. T. *Curr. Med. Chem.*, **2003**, 10, 925; d) Supuran, C. T. *J. Enzyme Inhib. Med. Chem.*, **2013,** *28*, 229.

12) a) Grandane, A.; Tanc, M.; Di Cesare Mannelli., L.; Carta, F.; Ghelardini, C.; Žalubovskis, R.; Supuran, C.T. *J. Med. Chem.* **2015**, *58,* 3975; b) Clare, B. W.; Scozzafava, A.; Supuran, C. T. *J. Med. Chem.*, **2001**, *44*, 2253; c) Capasso, C.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.***2014**, *29*, 379.

13) 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) Lou, Y.; McDonald, P. C.; Oloumi, A.; Chia, S.; Ostlund, C.; Ahmadi, A.; Kyle, A.; Auf dem Keller, U.; Leung, S.; Huntsman, D.; Clarke, B.; Sutherland, B. W.; Waterhouse, D.; Bally, M.; Roskelley, C.; Overall, C. M.; Minchinton, A.; Pacchiano, F.; Carta, F.; Scozzafava, A.; Touisni, N.; Winum, J. Y.; Supuran, C. T.; Dedhar, S. *Cancer Res* **2011,** *71*, 3364.

14) a) Supuran, C. T.; Scozzafava, A. *Bioorg. Med. Chem.*, **2007,** *15*, 4336; b) Thiry, A.; Ledecq, M.; Cecchi, A.; Dogne, J. M.; Wouters, J.; Supuran, C. T.; Masereel, B. *J. Med. Chem.*, 2006, **49**, 2743; c) Supuran, C. T.; Scozzafava, A.; Casini, A. *Med. Res. Reviews*, **2003**, *23*, 146; d) Supuran, C. T.; Scozzafava, A. *Eur. J. Med. Chem.*, **2000**, *35*, 867.

15) a) Şentürk, M.; Gülçin, I.; Daştan, A.; Küfrevioğlu, Ö. I.; Supuran, C.T. *Bioorg. Med. Chem.* **2009**, *17*, 3207; b) Supuran, C.T.; Casini, A.; Mastrolorenzo, A.; Scozzafava, A. *Mini. Rev. Med. Chem.* **2004**, *4*, 625; c) Groves, K.; Bao, B.; Zhang, J.; Handy, E.; Kennedy, P.; Cuneo, G.; Supuran, C.T.; Yared, W.; Peterson, J.D.; Rajopadhye, M. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 653; d) Supuran, C.T. *Expert Opin. Drug Metab. Toxicol*. **2016**, *12*, 423.

16) a) Gieling, R.G.; Parker, C.A.; De Costa, L.A.; Robertson, N.; Harris, A.L.; Stratford, I.J.; Williams, K.J. *J. Enzyme Inhib. Med. Chem*. **2013**, *28*, 360; b) Bayram, E.; Senturk, M.; Kufrevioglu, O.I.; Supuran, C.T. *Bioorg. Med. Chem.* **2008**, *16*, 9101.

17) Lomelino, C.L.; Mahon, B.P.; McKenna, R.; Carta, F.; Supuran, C.T. *Bioorg. Med. Chem.* **2016**, *24*, 976.

18) a) Vicini, P.; Geronikaki, A.; Karioti, A.; Incerti, M.; Zani, F. *Bioorg. Med. Chem*. **2006**, *14*, 3859; b) Donkor, I. Q.; Abdel-Ghany, Y.S.; Kador, P.F.; Mizoguchi, T.; Bartoszko-Malik, A.; Miller, D. D. *Eur. J. Med. Chem.*, **1998**, *33*, 15; c)

Mahboobi, S.; Sellmer, A.; Höcher, H.; Eichhorn, E.; Bär, T.; Schmidt, M.; Maier, T.; Stadlwieser, J. F.; Beckers, T. L. *J. Med. Chem.*, 2006, **49**, 5769.

19) Nițu, S.V.; Bercean, V.N.; Badea, V.; Csunderlik, C. *Chem. Bull. Polytechn. Univ. Timisoara* **2008**, *53*, 1.

20) a) Crawford, M.; Little, W. T. *J. Chem. Soc*., **1959**, 729; b) Taile, V.; Hatzade, K.; Gaidhane, P.; Ingle, V. *Turk. J. Chem*. **2009**, *33*, 295.

21) Kambe, S.; Saito, K.; Kishi, H.; Sakurai, A.; Midorikawa, H. *Synthesis* **1979**, *4*, 287.

22) a) Ramesh, B.; Bhalgat, C.M. *Eur. J. Med. Chem.*, **2011**, *46*, 1882; b) Galal, S.A.; Abdelsamie, A.S.; Tokuda, H.; Suzuki, N.; Lida, A.; El Hefnawi, M.; Ramadan, R.A.; Atta, M. H.; El Diwani, H. I. *Eur. J. Med. Chem.*, **2011,** *46*, 327.

23) R.G. Khalifah, *J. Biol. Chem.* **1971**, *246*, 2561.

24) a)nAlafeefy, A.M.; Al-Obaid, O.; Abdulla, M.; Eldehna, W.M.; Al-Tamimi, A.M.; Abdel-Aziz, H.A.; Ahmad, R.; Carta, F.; Al-Kahtani, A.A.; Supuran, C.T. *Eur. J. Med. Chem*. **2016**,*109*, 247; b) Supuran, C.T.; Scozzafava, A.; Mastrolorenzo, A. *Expert Opin. Ther. Pat*. **2001**, *11*, 221.

25) a) Bonneau, A.; Maresca, A.; Winum, J.Y.; Supuran, C.T. *J.Enzyme Inhib. Med. Chem.* **2013**, *28*, 397-401; b) Sharma, A.; Tiwari, M.; Supuran,C.T. *J. Enzyme Inhib. Med. Chem.* **2014,** *29*, 292.

26) 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) Supuran, C.T.; Scozzafava, A.; Jurca, B.C.; Ilies, M.A. *Eur. J. Med. Chem.* **1998**, *33*, 83.

27) a) Avvaru, B.S.; Wagner, J.M.; Maresca, A.; Scozzafava, A.; Robbins, A.H.; Supuran, C.T.; McKenna, R. *Bioorg. Med. Chem.Lett.* **2010**, *20*, 4376; b) Wagner, J.; Avvaru, B.S.; Robbins, A.H.; Scozzafava, A.; Supuran, C.T.; McKenna, R. *Bioorg. Med. Chem.* **2010**, *18*, 4873; c) Carta, F.; Garaj, V.; Maresca, A.; Wagner, J.; Avvaru,

B.S.; Robbins, A.H.; Scozzafava, A.; McKenna, R.; Supuran, C.T. *Bioorg. Med. Chem*. **2011**, *19*, 3105; d) Hen, N.; Bialer, M.; Yagen, B.; Maresca, A.; Aggarwal, M.; Robbins, A.H.; McKenna, R.; Scozzafava, A.; Supuran, C.T. *J. Med. Chem*. **2011**, *54*, 3977; e) Biswas, S.; Carta, F.; Scozzafava, A.; McKenna, R.; Supuran, C.T. *Bioorg. Med. Chem*. **2013**, *21*, 2314; f) Di Fiore, A.; De Simone, G.; Menchise, V.; Pedone, C.; Casini, A.; Scozzafava, A.; Supuran, C.T. *Bioorg. Med. Chem. Lett*. **2005**, *15*, 1937.

28) a) Vitale, R. M.; Alterio, V.; Innocenti, A.; Winum, J.-Y.; Monti, S. M.; De Simone, G.; Supuran, C. T. *J. Med. Chem*. **2009**, 52, 5990; b) Temperini, C.; Cecchi, A.; Boyle, N. A.; Scozzafava, A.; Cabeza, J. E.; Wentworth, P.; Blackburn, G. M.; Supuran, C. T. *Bioorg. Med. Chem. Lett*. **2008**, 18, 999; c) De Simone, G.; Di Fiore, A.; Menchise, V.; Pedone, C.; Antel, J.; Casini, A.; Scozzafava, A.; Wurl, M.; Supuran, C. T. *Bioorg. Med. Chem. Lett*. **2005**, 15, 2315; d) Crocetti, L.; Maresca, A.; Temperini, C.; Hall, R. A.; Scozzafava, A.; Mühlschlegel, F. A.; Supuran, C. T. *Bioorg. Med. Chem. Lett*. **2009**, 19, 1371.

Synthesis of 4-(thiazol-2-yl)amino-benzenesulfonamides with potent carbonic anhydrase I, II and IX inhibitory activity and cytotoxic effects against breast cancer cell lines

Nagwa M. Abdel Gawad,[a] Noha H. Amin,*[b] Mohammed T. Elsaadi,[b] Fatma M.M. Mohamed,[b] Andrea Angeli,[c] Viviana De Luca,[d] Clemente Capasso,[d] and Claudiu T. Supuran*[c]