Bioorganic Chemistry 82 (2019) 117–122

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Design, synthesis and biological evaluation of novel ureido benzenesulfonamides incorporating 1,3,5-triazine moieties as potent carbonic anhydrase IX inhibitors

Nabih Lolak[a], Suleyman Akocak[a,*], Silvia Bua[b], Claudiu T. Supuran[b,*]

[a] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Adiyaman University, 02040 Adiyaman, Turkey
[b] Università degli Studi di Firenze, NEUROFARBA Dept., Sezione di Scienze Farmaceutiche, Via Ugo Schiff 6, 50019 Sesto Fiorentino, Florence, Italy

ARTICLE INFO

Keywords:
Ureido benzenesulfonamides
1,3,5-Triazine moiety
Carbonic anhydrase
Isoforms
Isoform-selective inhibitor
Cancer

ABSTRACT

A series of novel ureido benzenesulfonamides incorporating 1,3,5-triazine moieties were obtained by reacting 4-isocyanato-benzenesulfonamide (**2**) with 2-amino-4,6-dicholoro-1,3,5-triazine (**4**). The 4-(3-(4,6-dichloro-1,3,5-triazin-2-yl)ureido) benzenesulfonamide (**5**) was subsequently derivatized by reaction with various nucleophiles such as, morpholine, ammonia, methyl amine, dimethyl amine, and piperidine. The ureido benzenesulfonamides incorporating triazinyl moieties were investigated as inhibitors of four selected physiologically relevant human carbonic anhydrase (hCA, EC 4.2.1.1) isoforms, namely, hCA I, II, IX, and XII which are involved in various diseases such as glaucoma, epilepsy, obesity and cancer. The membrane-bound tumor-associated isoform hCA IX was potently inhibited with these compounds with K_is in the range of 0.91–126.2 nM. Specifically, compound **7j** showed great potency against hCA IX with sub-nanomolar K_i of 0.91 nM. Since hCA IX is a validated drug target for anticancer agents, these isoform-selective and potent inhibitors may be considered of interest for further medicinal/pharmacologic studies.

1. Introduction

Cancer is the second most common disease causing death, after cardiovascular diseases across the world, and its incidence is expected to increase dramatically in the near future. The high incidence and mortality ratio of cancer is due to the fact that there are > 200 types of cancers and it is rather difficult to detect most of them in the early stage. For all these reasons, the research in the anti-cancer drug discovery focused on cancer treatment with more effective and less toxic agents [1–3].

Carbonic anhydrase (CA, EC 4.2.1.1) IX and XII (h, human isozymes, hCA IX and XII) are well-known transmembrane CA isoforms which are highly expressed in different tumor types and present a rather limited expression in most normal cells [4–7]. These tumor-associated proteins play important roles in tumor survival, acidification and proliferation under hypoxic conditions of primary tumors and metastases [4–7]. The extracellular localization of these isoforms, allows their efficient targeting by antibodies and small molecule inhibitors. Recently, one of the small molecule CA IX/XII inhibitors, **SLC-0111** advanced into Phase I/II clinical trials for the treatment of hypoxic, metastatic tumors over-expressing these proteins [8–10]. SLC-0111 belongs to the class of ureido-sulfonamides, which show a very good selectivity for inhibiting CA IX/XII over CA I and II [8–10], cytosolic isoforms which are off-targets when considering the antitumor applications of the CA inhibitors.

The 1,3,5-triazine scaffold, also known as *s*-triazine, is an interesting core for medicinal chemistry applications due to the broad biological activities and wide variety of applications of compounds incorporating it, such as antimicrobial, diuretics, antiviral, anti-inflammatory, and more anti-cancer agents [11–13]. In recent years, sulfonamides incorporating 1,3,5-triazine moieties were discovered as potent and highly selective hCA IX inhibitors [14–16]. These compounds showed one of the best selectivity ratio for hCA IX over the widespread, off-target hCA II, between 166 and 706 fold. The high selectivity ratio of these compounds makes them good lead compounds for designing other types of selective inhibitors targeting the tumor-associated isoform hCA IX [14].

In the current work, we combined these two powerful scaffolds (1,3,5-triazine and ureido substituted benzenesulfonamides) to obtain potent and selective hCA IX and XII inhibitors by using the tail approach, as described in Fig. 1. For this reason, novel ureido benzenesulfonamides incorporating 1,3,5-triazine moieties were synthesized and investigated as inhibitors of four physiologically and

* Corresponding authors.
E-mail addresses: sakocak@adiyaman.edu.tr (S. Akocak), claudiu.supuran@unifi.it (C.T. Supuran).

https://doi.org/10.1016/j.bioorg.2018.10.005
Received 20 August 2018; Received in revised form 26 September 2018; Accepted 4 October 2018
Available online 05 October 2018
0045-2068/

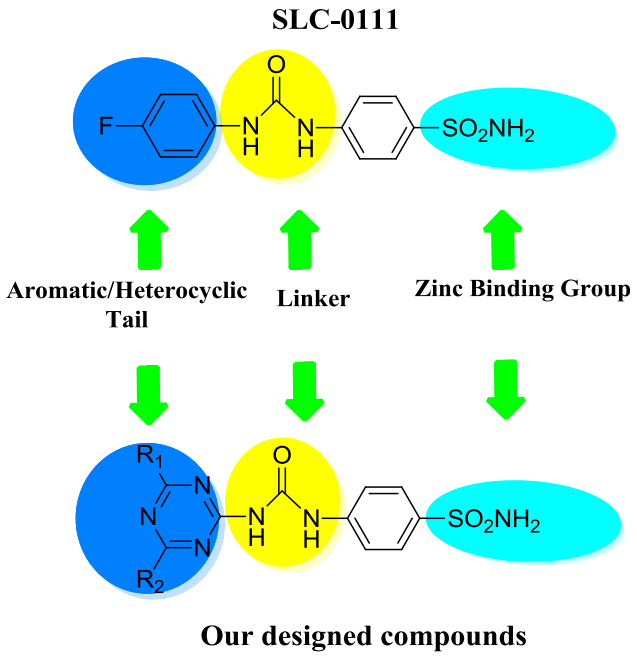


Fig. 1. The design strategy for the ureido benzensulfonamides incorporating 1,3,5-triazine moieties, starting from SLC-0111 as lead, by using tail approach.

pharmacologically relevant isoforms, which are the cytosolic isozymes hCA I and II, as well as tumor-associated membrane-bound isoforms hCA IX and XII.

2. Result and discussion

2.1. Chemistry

In the design of novel and possibly isoform-selective CA inhibitors, the investigation of hybrid molecules through the combination of different scaffolds and pharmacophores in one structure may lead to improved potency and selectivity. Considering the versatile chemistry of cyanuric chloride (2,4,6-trichloro-1,3,5-triazine) and the ureido substituted benzene-sulfonamide scaffold present in SLC-0111, we have designed and synthesized novel ureido benzenesulfonamides incorporating the 1,3,5-triazine moiety as CA inhibitors.

The synthesis of this series of ureido benzenesulfonamides was performed according to the general synthetic route described in Scheme 1. The starting compounds, 4-isocyanato-benzenesulfonamide (**2**) and 4,6-dicholoro-1,3,5-triazine-2-amine (**4**) were synthesized as previously described [17,18]. The key intermediate of this work, 4-(3-(4,6-dichloro-1,3,5-triazin-2-yl)ureido) benzenesulfonamide (**5**) was obtained as shown in Scheme 1 by the reaction of compounds **2** and **4**. Subsequently, the chlorine atoms of the key intermediate **5** were substituted by using morpholine, piperidine, ammonia, methyl amine, and dimethyl amine as nucleophiles, in order to generate chemical diversity.

2.2. Carbonic anhydrase inhibition

The novel ureido benzenesulfonamides incorporating 1,3,5-triazine moieties obtained here were tested as inhibitors of four physiologically and pharmacologically relevant isoforms, namely, the cytosolic hCA I and II, as well as the transmembrane tumor-associated isoforms hCA IX and XII, by a stopped-flow CO_2 hydrase assay [19]. Acetazolamide (AAZ), a clinically employed sulfonamide CAI and SLC-0111 (Phase I/II clinical trials for the treatment of advanced, metastatic breast cancer) were also included in the assays as standard drugs. The following structure-activity relationship (SAR) may be drawn regarding the inhibition data of Table 1 for this series of ureido benzenesulfonamides incorporating the 1,3,5-triazine scaffold, **5**, **6 (a–e),** and **7 (a–k)**:

i. The widely abundant slow cytosolic isoform hCA I was moderately inhibited by all the novel inhibitors that are presented in this work, with the inhibition constants in the range of 91.7–8374.8 nM. Only one compound (**6e**) showed efficient inhibition, being more effective than the clinically used drug acetazolamide (AAZ), with a K_i of 91.7 nM. The least potent inhibitors from the series were compound **7a** (R_1 = morpholine, R_2 = NH_2), compound **7e** (R_1, R_2 = morpholine) and **7i** (R_1 = $N(Me)_2$, R_2 = NH_2), with K_is of 8343.1, 4186.7 and 8374.8 nM, respectively.
ii. All compounds reported here were more efficient as hCA II inhibitors compared to **SLC-0111**, which is a weak inhibitor of this isoform with a K_i of 960 nM. In general, all the new compounds reported here showed low nanomolar to subnanomolar inhibition of hCA II, with K_is ranging between 0.69 nM and 420.9 nM. The best hCA II inhibitors were derivatives **6d** (R_1 = morpholine, R_2 = Cl), **6e** (R_1 = piperidine, R_2 = Cl) and **7j** (R_1 = $N(Me)_2$, R_2 = NHMe), with K_is of 1.5, 0.69, and 3.1 nM.
iii. The transmembrane tumor-associated isoform hCA IX was efficiently inhibited by most of the compounds reported in this work. Only three compounds from the series were less potent than SLC-0111, which is an effective hCA IX inhibitor, namely compound **6a**, **7a**, and **7e** with K_is of 48.5, 126.2, 46.5 nM, respectively. One of the most important findings of the current work is that compound **7j** showed subnanomolar activity (K_i, 0.91 nM) against hCA IX, with good selectivity over hCA I and hCA XII, and reasonable selectivity over hCA II. Other hCA IX potent inhibitors were derivatives **5** (R_1, R_2 = Cl), **6c** (R_1 = $N(Me)_2$, R_2 = Cl), **6e** (R_1 = piperidine, R_2 = Cl), and **7f** (R_1 = piperidine, R_2 = NHMe) with K_is of 4.4, 4.5, 2.3, and 2.7 nM, respectively.
iv. The other tumor-associated membrane bound isoform hCA XII was moderately inhibited by most of the ureido benzenesulfonamides incorporating 1,3,5-triazine moieties reported here, with K_is ranging from 80.5 to 901.3 nM, except derivatives **7a** and **7e**, which did not inhibit the enzyme up to 10,000 nM. Among this series, two compounds showed potent inhibition against hCA XII, i.e., compound **5** (R_1 = R_2 = Cl) and compound **7f** (R_1 = piperidin, R_2 = NHMe) with K_is of 84.2 and 80.5 nM, respectively.

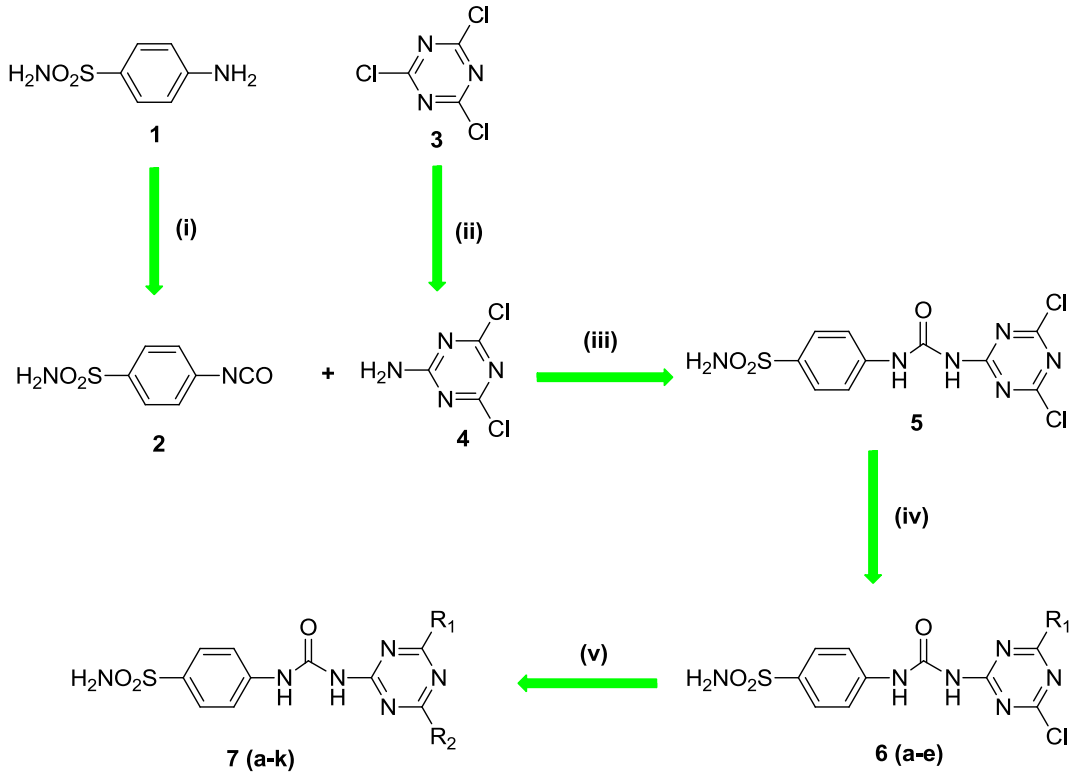
3. Conclusions

In conclusion, we report here a novel series of ureido benzenesulfonamides incorporating 1,3,5-triazine moieties, which also contain with morpholine, piperidine, ammonia, methyl amine, and dimethyl amine moieties in their molecules. The novel compounds were investigated as inhibitors of four physiologically and pharmacologically relevant isoforms, the cytosolic isoforms hCA I and II, as well as tumor-associated membrane-bound isoforms hCA IX and XII. All compounds showed potent inhibition against the tumor-associated isozyme hCA IX with low nanomolar to subnanomolar potency, with K_is in the range of 0.91–126.2 nM. For other isoforms, distinct inhibition profiles and interesting structure-activity relationship were observed, depending on the nature of the amine that was appended on the 1,3,5-triazine scaffold. As hCA IX is a validated drug target for metastatic hypoxic tumors and SLC-0111 advanced to Phase I/II clinical trials for the treatment of breast cancer, these hCA IX potent ureido benzenesulfonamides incorporating 1,3,5-triazine moieties might be of interest for further medicinal/pharmacologic studies.

4. Experimental

4.1. Chemistry

All chemicals and anhydrous solvents were purchased from Sigma-Aldrich, Merck, Alfa Aesar and TCI and used without further



Scheme 1. General synthetic route for the synthesis of benzenesulfonamides incorporating 1,3,5-triazine moieties. Reagents and conditions: (i) nitrobenzene, phosgene, −10 to 90 °C slowly, 6 h, (ii) Acetone, crushed ice, 25 wt% aqueous ammonia solution, 0 to 5 °C, 30 min, yield 95%. (iii) THF, 48 h, 40 °C, yield 45% (iv) R_1H, DMF, 0 to 5 °C, 1 h, then R.T. 4 h, yields 54–88% (v) R_2H, DMF, room temperature, 1 h, then 90 °C, 2 h, yields 45–88%.

purification. FT-IR spectra were obtained by using Perkin Elmer Spectrum 100 FT-IR spectrometer. Nuclear Magnetic Resonance (^{1}H NMR and ^{13}C NMR) spectra of compounds were recorded using a Bruker Advance III 300 MHz spectrometer in DMSO-d_6 and TMS as an internal standard operating at 300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR. Thin layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} plates.

*4.1.1. Synthesis of 4-(3-(4,6-dichloro-1,3,5-triazin-2-yl)ureido) benzenesulfonamide (**5**)*

The starting compounds, 4-isocyanato-benzenesulfonamide (**2**) and 4,6-dicholoro-1,3,5-triazine-2-amine (**4**) were synthesized as previously described [15,16]. A solution of **2** (10 mmol) in 5 mL of THF was slowly added over the solution of **4** (10 mmol) under stirring at room temperature. The solution was left under stirring for 48 h at 40 °C (TLC monitoring). After that, the solvent was evaporated and crude residue was purified by column chromatography (ethyl acetate/ petroleum ether) to give title compound **5**. The obtained product was dried under vacuum and fully characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, and melting point.

Yield: 25%; Color: white solid; mp: 244–246 °C; FT-IR (cm^{-1}): 3213, 1653, 1535 (asymmetric), 1342, 1164 (symmetric) (S═O), 1090; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 10.65 (s, 1H, −NH−), 9.10 (s, 1H, −NH−), 8.00 (d, 2H, J = 8.4, Ar-H), 7.93 (d, 2H, J = 6.9, Ar-H), 7.58 (s, 2H, $-SO_2NH_2$): ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 165.3, 156.1, 150.4, 147.1, 135.5, 129.2, 126.0;

*4.1.2. General procedure for the synthesis of compounds **6(a–e)**.*

At 0 °C, a 10 mmol solution of R_1-H (morpholine, piperidine, 25 wt% ammonia, 40 wt% methyl amine, dimethyl amine) was added to 5 mmol of **5** in DMF under stirring. After complete addition, the mixture was allowed to warm to room temperature for 4 h. Then, the product was filtered off washed with water and dried under vacuum at 40 °C. The obtained final pure products were fully characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, and melting points.

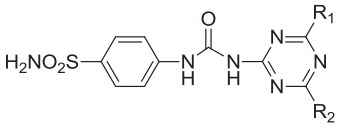
*4.1.2.1. 4-(3-(4-amino-6-chloro-1,3,5-triazin-2-yl)ureido) benzenesulfonamide **(6a)**.* Yield: 54%; Color: white solid; mp: 288–290 °C; FT-IR (cm^{-1}): 3304, 3148, 1643, 1547 (asymmetric), 1413, 1155 (symmetric) (S═O), 1093; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 10.60 (s, 1H, −NH−), 9.15 (s, 1H, −NH−), 8.11 (d, 2H, J = 7.2, Ar-H), 7.95 (d, 2H, J = 7.5, Ar-H), 7.56 (s, 2H, $-SO_2NH_2$), 6.62 (s, 2H, $-NH_2$): ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 165.7, 164.1, 156.4, 150.2, 147.3, 136.1, 129.1, 126.3.

*4.1.2.2. 4-(3-(4-chloro-6-(methylamino)-1,3,5-triazin-2-yl)ureido) benzenesulfonamide **(6b)**.* Yield: 78%; Color: white solid; mp: 282–285 °C; FT-IR (cm^{-1}): 3255, 3117, 1647, 1551 (asymmetric), 1319, 1155 (symmetric) (S═O), 1104; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 10.62 (s, 1H, −NH−), 9.18 (s, 1H, −NH−), 8.08–7.94 (m, 4H, Ar−H), 7.55 (s, 2H, $-SO_2NH_2$), 6.52 (s, 2H, $-\underline{NH}CH_3$), 2.81–2.75 (m, 3H, $-NH\underline{CH_3}$): ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 165.7, 164.4, 156.5, 150.7, 146.7, 136.1, 128.9, 126.2, 27.6.

*4.1.2.3. 4-(3-(4-chloro-6-(dimethylamino)-1,3,5-triazin-2-yl)ureido) benzenesulfonamide **(6c)**.* Yield: 83%; Color: white solid; mp: 249–251 °C; FT-IR (cm^{-1}): 3361, 3257, 1669, 1577 (asymmetric), 1345, 1166 (symmetric) (S═O), 1113; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 10.65 (s, 1H, −NH−), 9.15 (s, 1H, −NH−), 8.08 (d, 2H, J = 7.2, Ar−H), 7.92 (d, 2H, J = 6.9, Ar−H), 7.54 (s, 2H, $-SO_2NH_2$), 3.12 (s, 6H, $-CH_3$): ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 165.9, 164.7, 156.2, 150.5, 146.8, 136.3, 128.8, 126.1, 35.5.

*4.1.2.4. 4-(3-(4-chloro-6-morpholino-1,3,5-triazin-2-yl)ureido) benzenesulfonamide **(6d)**.* Yield: 88%; Color: white solid; mp: 265–267 °C; FT-IR (cm^{-1}): 3337, 3201, 1669, 1577 (asymmetric),

Table 1
Inhibition data of human CA isoforms hCA I, II, IX and XII with derivatives **5**, **6 (a–e),** and **7 (a–k)** reported here and the standard sulfonamide inhibitors acetazolamide (**AAZ**) and **SLC-0111** (phase I/II clinical trials for the treatment of advanced metastatic breast cancer) by a stopped flow CO_2 hydrase assay [19].



Comp.	R1	R2	K_I^* (nM) hCAI	hCA II	hCA IX	hCA XII
5	Cl	Cl	873.0	93.9	4.4	84.2
6a	$-NH_2$	Cl	816.8	178.6	48.5	901.3
6b	−NHMe	Cl	676.5	9.0	26.8	579.0
6c	$-N(Me)_2$	Cl	660.2	12.4	4.5	346.5
6d		Cl	548.1	1.5	31.6	301.4
6e		Cl	91.7	0.69	2.3	277.5
7a		$-NH_2$	8343.1	420.9	126.2	> 10000
7b		−NHMe	803.2	33.6	32.0	872.9
7c		$-N(Me)_2$	602.9	3.9	7.4	747.7
7d			625.9	33.2	28.3	831.8
7e			4186.7	6.9	46.5	> 10000
7f		−NHMe	427.7	5.2	2.7	80.5
7g		$-N(Me)_2$	551.3	8.5	4.9	743.7
7h			474.2	78.9	12.7	494.7
7i	$-N(Me)_2$	$-NH_2$	8374.8	299.4	23.8	692.9
7j	$-N(Me)_2$	−NHMe	394.9	3.1	0.91	554.7
7k	$-N(Me)_2$	$-N(Me)_2$	923.8	7.5	11.4	626.3
AAZ	–	–	250	12	25	5.7
SLC-0111	–	–	5080	960	45.1	4.5

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

1345, 1166 (symmetric) (S═O), 1113; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 10.68 (s, 1H, −NH−), 9.10 (s, 1H, −NH−), 8.12 (d, 2H, J = 6.9, Ar−H), 7.90 (d, 2H, J = 6.3, Ar-H), 7.51 (s, 2H, $-SO_2NH_2$), 3.80–3.45 (m, 8H, morpholine): ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 165.6, 164.3, 156.5, 150.2, 146.7, 136.2, 128.7, 126.3, 66.3, 43.4.

*4.1.2.5. 4-(3-(4-chloro-6-(piperidin-1-yl)-1,3,5-triazin-2-yl)ureido) benzenesulfonamide (**6e**).* Yield: 66%; Color: white solid; mp: 229–231 °C; FT-IR (cm^{-1}): 3341, 3240, 1673, 1558 (asymmetric), 1332, 1159 (symmetric) (S═O), 1084; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 10.66 (s, 1H, −NH−), 9.02 (s, 1H, −NH−), 8.08 (d, 2H, J = 7.2, Ar-H), 7.88 (d, 2H, J = 6.9, Ar−H), 7.53 (s, 2H, -SO_2NH_2), 3.45–3.21 (m, 4H, piperidine), 1.74–1.48 (m, 6H, piperidine): ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 165.9, 164.7, 156.4, 150.5, 146.8, 136.5, 128.4, 126.2, 43.6, 25.8, 24.3.

*4.1.3. General procedure for the synthesis of compounds **7(a–k)**.*

Under stirring, a 2 mmol solution of R_2-H (morpholine, piperidine, 25 wt% ammonia, 40 wt% methyl amine, dimethyl amine) was added to 1 mmol of **6(a–e)** in DMF at room temperature. Then, the reaction temperature was raised to 90 °C for 2 h. After cooling to room temperature, the mixture was filtered and the precipitate was washed with water and dried at 50 °C. The obtained final pure products **7(a–k)** were fully characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, and melting points.

*4.1.3.1. 4-(3-(4-amino-6-morpholino-1,3,5-triazin-2-yl)ureido) benzenesulfonamide (**7a**).* Yield: 45%; Color: white solid; mp: 248–251 °C; FT-IR (cm^{-1}): 3273, 3205, 1635, 1529 (asymmetric), 1338, 1179 (symmetric) (S═O), 1091; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 10.55 (s, 1H, −NH−), 8.88 (s, 1H, −NH−), 8.15 (d, 2H, J = 7.2, Ar−H), 7.92 (d, 2H, J = 6.9, Ar−H), 7.53 (s, 2H, $-SO_2NH_2$), 6.48 (s, 2H, $-NH_2$), 3.79–3.65 (m, 4H, morpholine), 3.42–3.35 (m, 4H, morpholine): ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 165.2, 164.1, 156.2, 150.5, 146.8, 136.1, 128.3, 126.1, 66.5, 43.2.

*4.1.3.2. 4-(3-(4-(methylamino)-6-morpholino-1,3,5-triazin-2-yl)ureido) benzenesulfonamide (**7b**).* Yield: 85%; Color: white solid; mp: 248–251 °C; FT-IR (cm^{-1}): 3423, 3319, 3213, 1663, 1514 (asymmetric), 1330, 1157 (symmetric) (S═O), 1108; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 10.58 (s, 1H, −NH−), 8.85 (s, 1H, −NH−), 8.07 (d, 2H, J = 7.5, Ar−H), 7.88 (d, 2H, J = 7.2, Ar−H), 7.52 (s, 2H, $-SO_2NH_2$), 6.58 (s, 2H, $-\underline{NH}CH_3$), 3.75–3.62 (m, 4H, morpholine), 3.45–3.37 (m, 4H, morpholine), 2.99–2.85 (m, 3H, $-NH\underline{CH_3}$): ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 166.0, 164.7, 156.8, 150.7, 146.5, 136.4, 128.2, 126.6, 66.2, 42.8, 28.3.

*4.1.3.3. 4-(3-(4-(dimethylamino)-6-morpholino-1,3,5-triazin-2-yl)ureido) benzenesulfonamide (**7c**).* Yield: 88%; Color: white solid; mp: 262–265 °C; FT-IR (cm^{-1}): 3330, 3203, 1674, 1513 (asymmetric), 1306, 1167 (symmetric) (S═O), 1111; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 10.41 (s, 1H, −NH−), 8.76 (s, 1H, −NH−), 8.02 (d, 2H, J = 7.5, Ar−H), 7.93 (d, 2H, J = 7.2, Ar−H), 7.53 (s, 2H, $-SO_2NH_2$), 3.84–3.43 (m, 8H, morpholine), 3.05 (s, 6H, $-CH_3$): ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 166.8, 165.2, 156.9, 150.8, 146.3, 136.7, 128.5, 126.9, 66.6, 43.4, 35.8.

*4.1.3.4. 4-(3-(4-morpholino-6-(piperidin-1-yl)-1,3,5-triazin-2-yl)ureido) benzenesulfonamide (**7d**).* Yield: 82%; Color: white solid; mp: 222–225 °C; FT-IR (cm^{-1}): 3216, 3064, 1656, 1505 (asymmetric), 1331, 1162 (symmetric) (S═O), 1102; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 10.41 (s, 1H, −NH−), 8.75 (s, 1H, −NH−), 8.00 (d, 2H, J = 8.1, Ar−H), 7.93 (d, 2H, J = 8.4, Ar−H), 7.53 (s, 2H, $-SO_2NH_2$), 3.80–3.43 (m, 12H, morpholine and piperidine), 1.71–1.44 (m, 6H, piperidine): ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 166.7, 165.1, 156.7, 150.4, 146.6, 136.3, 128.4, 126.6, 66.5, 44.0, 43.7, 25.9, 24.8.

*4.1.3.5. 4-(3-(4,6-dimorpholino-1,3,5-triazin-2-yl)ureido) benzenesulfonamide (**7e**).* Yield: 87%; Color: white solid; mp: 298–300 °C; FT-IR (cm^{-1}): 3244, 3170, 1668, 1501 (asymmetric), 1339, 1165 (symmetric) (S═O), 1067; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 10.45 (s, 1H, −NH−), 8.72 (s, 1H, −NH−), 8.00 (d, 2H, J = 7.5, Ar-H), 7.91 (d, 2H, J = 8.1, Ar-H), 7.52 (s, 2H, $-SO_2NH_2$), 3.79–3.65 (m, 8H, morpholine), 3.54–3.41 (m, 8H, morpholine): ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 166.4, 156.8, 150.2, 146.3, 136.6, 128.7, 126.2, 66.8, 43.5.

*4.1.3.6. 4-(3-(4-(methylamino)-6-(piperidin-1-yl)-1,3,5-triazin-2-yl) ureido) benzenesulfonamide (**7f**).* Yield: 78%; Color: white solid; mp: 239–241 °C; FT-IR (cm^{-1}): 3404, 3334, 1667, 1515 (asymmetric), 1329, 1160 (symmetric) (S═O), 1088; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 10.65 (s, 1H, −NH−), 9.08 (s, 1H, −NH−), 8.05 (d, 2H, J = 7.5, Ar−H), 7.85 (d, 2H, J = 7.2, Ar−H), 7.54 (s, 2H, $-SO_2NH_2$), 6.55 (s, 2H, $-\underline{NH}CH_3$), 3.42–3.29 (m, 4H, piperidine), 2.79–2.72 (m, 3H, $-NH\underline{CH_3}$), 1.72–1.52 (m, 6H, piperidine): ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 166.1, 164.5, 156.7, 150.8, 146.9, 136.2, 128.7,

126.5, 43.9, 28.1, 25.5, 24.2.

*4.1.3.7. 4-(3-(4-(dimethylamino)-6-(piperidin-1-yl)-1,3,5-triazin-2-yl) ureido) benzenesulfonamide (**7g**)*. Yield: 86%; Color: white solid; mp: 236–239 °C; FT-IR (cm^{-1}): 3322, 3170, 1695, 1503 (asymmetric), 1335, 1159 (symmetric) (S=O), 1094; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 10.69 (s, 1H, −NH−), 9.05 (s, 1H, −NH−), 8.10 (d, 2H, J = 8.1, Ar-−H), 7.83 (d, 2H, J = 7.2, Ar−H), 7.53 (s, 2H, $-SO_2NH_2$), 3.40–3.27 (m, 4H, piperidine), 3.10 (s, 6H, $-CH_3$), 1.75–1.52 (m, 6H, piperidine): ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 166.4, 164.7, 156.5, 150.4, 146.6, 136.4, 128.2, 126.3, 43.7, 35.4, 25.7, 24.3.

*4.1.3.8. 4-(3-(4,6-di(piperidin-1-yl)-1,3,5-triazin-2-yl)ureido) benzenesulfonamide (**7h**)*. Yield: 82%; Color: white solid; mp: 233–236 °C; FT-IR (cm^{-1}): 3339, 3225, 1664, 1501 (asymmetric), 1327, 1169 (symmetric) (S=O), 1103; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 10.60 (s, 1H, −NH−), 9.01 (s, 1H, −NH−), 8.01 (d, 2H, J = 7.5, Ar−H), 7.79 (d, 2H, J = 6.9, Ar−H), 7.52 (s, 2H, $-SO_2NH_2$), 3.41–3.29 (m, 8H, piperidine), 1.73–1.54 (m, 12H, piperidine): ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 166.3, 156.4, 150.2, 146.3, 136.5, 128.7, 126.4, 43.9, 25.3, 24.1.

*4.1.3.9. 4-(3-(4-amino-6-(dimethylamino)-1,3,5-triazin-2-yl)ureido) benzenesulfonamide (**7i**)*. Yield: 51%; Color: white solid; mp: 222–225 °C; FT-IR (cm^{-1}): 3273, 3103, 1639, 1528 (asymmetric), 1334, 1161 (symmetric) (S=O), 1091; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 10.45 (s, 1H, −NH−), 8.82 (s, 1H, −NH−), 8.01 (d, 2H, J = 7.2, Ar−H), 7.93 (d, 2H, J = 7.5, Ar−H), 7.53 (s, 2H, $-SO_2NH_2$), 6.60 (s, 2H, $-NH_2$), 3.08 (s, 6H, $-CH_3$): ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 166.1, 164.7, 156.6, 150.2, 147.5, 136.2, 129.3, 126.8, 35.6.

*4.1.3.10. 4-(3-(4-(dimethylamino)-6-(methylamino)-1,3,5-triazin-2-yl) ureido) benzenesulfonamide (**7j**)*. Yield: 82%; Color: white solid; mp: 245–248 °C; FT-IR (cm^{-1}): 3425, 3312, 3213, 1661, 1535 (asymmetric), 1331, 1158 (symmetric) (S=O), 1093; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 10.48 (s, 1H, −NH−), 8.80 (s, 1H, −NH−), 8.03 (d, 2H, J = 6.9, Ar−H), 7.94 (d, 2H, J = 7.2, Ar−H), 7.54 (s, 2H, $-SO_2NH_2$), 6.50 (s, 2H, $-\underline{NH}CH_3$), 3.12 (s, 6H, $-CH_3$), 2.75–2.69 (m, 3H, $-NH\underline{CH_3}$): ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 166.4, 164.5, 156.5, 150.2, 147.4, 136.8, 129.5, 126.3, 35.6, 28.7.

*4.1.3.11. 4-(3-(4,6-bis(dimethylamino)-1,3,5-triazin-2-yl)ureido) benzenesulfonamide (**7k**)*. Yield: 86%; Color: white solid; mp: 262–264 °C; FT-IR (cm^{-1}): 3317, 3270, 1678, 1517 (asymmetric), 1339, 1162 (symmetric) (S=O), 1089; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 10.47 (s, 1H, −NH−), 8.79 (s, 1H, −NH−), 8.05 (d, 2H, J = 6.9, Ar−H), 7.95 (d, 2H, J = 7.5, Ar-H), 7.53 (s, 2H, $-SO_2NH_2$), 3.09 (s, 12H, $-CH_3$): ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 166.5, 156.7, 150.1, 147.8, 136.4, 129.3, 126.2, 35.8.

4.1.4. CA inhibition

An SX.18MV-R Applied Photophysics (Oxford, UK) stopped-flow instrument has been used to assay the catalytic/inhibition of various CA isozymes [19]. Phenol Red (at a concentration of 0.2 mM) has been used as an indicator, working at the absorbance maximum of 557 nm, with 10 mM Hepes (pH 7.4) as a buffer, 0.1 M Na_2SO_4 or $NaClO_4$ (for maintaining constant the ionic strength; these anions are not inhibitory in the used concentration), following the CA-catalyzed CO_2 hydration reaction for a period of 5–10 s. Saturated CO_2 solutions in water at 25 °C were used as substrate. Stock solutions of inhibitors were prepared at a concentration of 10 mM (in DMSO-water 1:1, v/v) and dilutions up to 0.01 nM done with the assay buffer mentioned above. At least 7 different inhibitor concentrations have been used for measuring the inhibition constant. Inhibitor and enzyme solutions were pre-incubated together for 10 min at room temperature prior to assay, in order to allow for the formation of the E-I complex. Triplicate experiments were done for each inhibitor concentration, and the values reported throughout the paper is the mean of such results. The inhibition constants were obtained by nonlinear least-squares methods using the Cheng-Prusoff equation, as reported earlier, and represent the mean from at least three different determinations [20–22]. All CA isozymes used here were recombinant proteins obtained as reported earlier by our group.

Acknowledgement

The authors would like to thank to TUBITAK (The Scientific and Technological Research Council of Turkey) for financial support (grant no: 315S103).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2018.10.005.

References

[1] D. Hanahan, R.A. Winberg, The hallmarks of cancer, Cell 100 (2000) 57–70.
[2] A.L. Harris, Hypoxia-a key regulatory factor in tumour growth, Nat. Rev. Cancer 2 (2002) 38–47.
[3] G.L. Semenza, Targeting HIF-1 for cancer therapy, Nat. Rev. Cancer 3 (2003) 721–732.
[4] (a) C.T. Supuran, Carbonic anhydrases: novel therapeutic applications for inhibitors and activators, Nat. Rev. Drug Discov. 7 (2008) 168–181;
(b) D. Neri, C.T. Supuran, Interfering with pH regulation in tumours as a therapeutic strategy, Nat. Rev. Drug Discov. 10 (2011) 767–777;
(c) V. Alterio, A. Di Fiore, K. D'Ambrosio, C.T. Supuran, G. De Simone, Multiple binding modes of inhibitors to carbonic anhydrases: how to design specific drugs targeting 15 different isoforms? Chem. Rev. 112 (2012) 4421–4468;
(d) A.M. Shabana, U.K. Mondal, R. Alam, T. Spoon, C.A. Ross, M. Madesh, C.T. Supuran, M.A. Ilies, pH-Senstive multiligand gold nanoplatform targeting carbonic anhydrase IX enhances the delivery of Doxorubicin to hypoxic tumor spheroids and overcomes the hypoxia-induced chemoresistance, ACS Appl. Mater. Interfaces 10 (2018) 17792–17808.
[5] (a) C.T. Supuran, Advances in structure-based drug discovery of carbonic anhydrase inhibitors, Expert OpinDrug Discov. 12 (2017) 61–88;
(b) C.T. Supuran, How many carbonic anhydrase inhibition mechanisms exist? J. Enzyme Inhib. Med. Chem. 31 (2016) 345–360;
(c) C.T. Supuran, Carbon-versus sulphur-based zinc binding groups for carbonic anhydrase inhibitors? J. Enzyme Inhib. Med. Chem. 33 (2018) 485–495.
[6] (a) S. Akocak, M.A. Ilies, Next-generation primary sulfonamide carbonic anhydrase inhibitors, in: C.T. Supuran, C. Cappasso (Eds.), Targeting Carbonic Anhydrases, Future Science, London, 2014, pp. 35–51;
(b) S. Akocak, N. Lolak, A. Nocentini, G. Karakoc, A. Tufan, C.T. Supuran, Synthesis and biological evaluation of novel aromatic and heterocyclic bis-sulfonamide Schiff bases as carbonic anhydrase I, II, VII, and IX inhibitors, Bioorg. Med. Chem. 25 (2017) 3093–3097;
(c) S. Akocak, N. Lolak, S. Bua, I. Turel, C.T. Supuran, Synthesis and biological evaluation of novel N, N′-Diaryl Cyanoguanidines acting as potent and selective carbonic anhydrase II inhibitors, Bioorg. Chem. 77 (2018) 245–251;
(d) S. Akocak, M.R. Alam, A.M. Shabana, R.K. Sanku, D. Vullo, H. Thompson, E.R. Swenson, C.T. Supuran, M.A. Ilies, PEGylated bis-sulfonamide carbonic anhydrase inhibitors can efficiently control the growth of several carbonic anhydrase IX-expressing carcinomas, J. Med. Chem. 59 (2016) 5077–5088.
[7] (a) S. Pastorekova, S. Parkkila, J. Pastorek, C.T. Supuran, Carbonic anhydrases: current state of the art, therapeutic applications and future prospects, J. Enzyme Inhib. Med. Chem. 19 (2008) 199–229;
(b) S.M. Monti, C.T. Supuran, G. De Simone, Anticancer carbonic anhydrase inhibitors: a patent review (2008–2013), Expert Opin. Ther. Part. 23 (2013) 737–749;
(c) C.T. Supuran, Structure and function of carbonic anhydrases, Biochem. J. 473 (2016) 2023–2032;
(d) C.T. Supuran, Advances in structure-based drug discovery of carbonic anhydrase inhibitors, Expert Opin. Drug Discov. 12 (2017) 61–88;
(e) F. Carta, A. Scozzafava, C.T. Supuran, Sulfoanmides: a patent review (2008–2012), Expert Opin. Ther. Pat. 22 (2012) 747–758;
(f) F. Carta, C.T. Supuran, Diuretics with carbonic anhydrase inhibitory action: a patent and literature review (2005–2013), Expert Opin. Ther. Pat. 23 (2013) 681–691;
(g) A. Scozzafava, C.T. Supuran, F. Carta, Antiobesity carbonic anhydrase inhibitors: a literature and patent review, Expert Opin. Ther. Pat. 23 (2013) 725–735.
[8] Y. Lou, P.C. McDonald, A. Oloumi, S. Chia, C. Ostlund, A. Ahmadi, A. Kyle, U. Auf dem Keller, S. Leung, D. Huntsman, B. Clarke, B.W. Sutherland, D. Waterhouse, M. Bally, C. Roskelley, C.M. Overall, A. Minchinton, F. Pacchiano, F. Carta, A. Scozzafava, N. Touisni, J.Y. Winum, C.T. Supuran, S. Dedhar, Targeting tumor hypoxia: suppression of breast tumor growth and metastasis by novel carbonic

anhydrase IX inhibitors, Cancer Res. 71 (2011) 3364–3376.
[9] (a) F. Pacchiano, F. Carta, P.C. McDonald, Y. Lou, D. Vullo, A. Scozzafava, S. Dedhar, C.T. Supuran, Ureido-substituted benzenesulfonamides potently inhibit carbonic anhydrase IX and show antimetastatic activity in a model of breast cancer metastasis, J. Med. Chem. 54 (2011) 1896–1902;
(b) F. Carta, D. Vullo, S.M. Osman, Z. AlOthman, C.T. Supuran, Synthesis and carbonic anhydrase inhibition of a series of SLC-0111, Bioorg. Med. Chem. 25 (2017) 2569–2576.
[10] F. Pacchiano, M. Aggarwal, B.S. Avvaru, A.H. Robbins, A. Scozzafava, R. McKenna, C.T. Supuran, Selective hydrophobic pocket binding observed within the carbonic anhydrase II active site accommodate different 4-substituted-ureido-benzenesulfonamides and correlate to inhibitor potency, Chem. Commun. (Camb) 46 (2010) 8371–8373.
[11] M. Zheng, C. Xu, J. Ma, Y. Sun, F. Du, H. Liu, L. Lin, C. Li, J. Ding, K. Chen, H. Jiang, Synthesis and antitumor evaluation of a novel series of triaminotriazine derivatives, Bioorg. Med. Chem. 15 (2007) 1815–1827.
[12] (a) R.V. Patel, Y.S. Keum, S.W. Park, Medicinal chemistry discoveries among 1,3,5-triazines: recent advances (2000–2013) as antimicrobial, anti-TB, anti-HIV and antimalarials, Mini Rev. Med. Chem. 14 (2014) 768–789;
(b) B. Liu, T. Sun, Z. Zhou, L. Du, A systematic review on antitumor agents with 1,3,5-triazines, Med. Chem. 5 (2015) 131–148.
[13] S. Cascioferro, B. Parrino, V. Spano, A. Carbone, A. Montalbano, P. Barraja, P. Diana, G. Cirrincione, 1,3,5-Triazines: a promising scaffald, for anticancer drugs development, Eur. J. Med. Chem. 142 (2017) 523–549.
[14] V. Garaj, L. Puccetti, G. Fasolis, J.Y. Winum, J.L. Montero, A. Scozzafava, D. Vullo, A. Innocenti, C.T. Supuran, Carbonic anhydrase inhibitors: synthesis and inhibition of cytosolic/tumor-associated carbonic anhydrase isozymes I, II, and IX with sulfonamides incorporating 1,2,4-triazine moieties, Bioorg. Med. Chem. Lett. 14 (2004) 5427–5433.
[15] V. Garaj, L. Puccetti, G. Fasolis, J.Y. Winum, J.L. Montero, A. Scozzafava, D. Vullo, A. Innocenti, C.T. Supuran, Carbonic anhydrase inhibitors: novel sulfonamides incorporating 1,3,5-triazine moieties as inhibitors of the cytosolic and tumour-associated carbonic anhydrase isozymes I, II, and IX, Bioorg. Med. Chem. Lett. 15 (2005) 3102–3108.
[16] (a) M. Ceruso, D. Vullo, A. Scozzafava, C.T. Supuran, Inhibition of human carbonic anhydrase isoforms I-XIV with sulfonamides incorporating fluorine and 1,3,5-triazine moieties, Bioorg. Med. Chem. 21 (2013) 6929–6936;
(b) F. Carta, V. Garaj, A. Maresca, J. Wagner, B.S. Avvaru, A.H. Robbins, A. Scozzafava, R. McKenna, C.T. Supuran, Sulfonamides incorporating 1,3,5-triazine moieties selectively and potently inhibit carbonic anhydrase transmembrane isoforms IX, XII, and XIV over cytosolic isoforms I and II: solution and X-ray crystallographic studies, Bioorg. Med. Chem. 19 (2011) 3105–3119.
[17] T.K. Brotherton, J.W. Lynn, J. Smith, Isocyanatophenyl sulfonyl isocyanates, US 3454606A, 1969.
[18] M. List, H. Puchinger, H. Gabriel, U. Monkowius, C. Schwarzinger, N-methylamines-synthesis, characterization and physical properties, J. Org. Chem. 10 (2016) 4066–4075.
[19] R.G. Khalifah, The carbon dioxide hydration activity of carbonic anhydrase. I. Stop-flow kinetic studies on the native human isoenzymes B and C, J. Biol. Chem. 246 (1971) 2561–2573.
[20] (a) B. Draghici, D. Vullo, S. Akocak, E.A. Walker, C.T. Supuran, M.A. Ilies, Ethylene bis-imidazoles are highly potent and selective activators for isozymes VA and VII of carbonic anhydrase, with a potential nootropic effect, Chem. Commun. 50 (2014) 5980–5983;
(b) S. Akocak, N. Lolak, D. Vullo, M. Durgun, C.T. Supuran, Synthesis and biological evaluation of histamine Schiff bases as carbonic anhydrase I, II, IV, VII and IX activators, J. Enzyme Inhib. Med. Chem. 32 (2017) 1305–1312;
(c) N. Lolak, S. Akocak, S. Bua, M. Koca, C.T. Supuran, Design and synthesis of novel 1,3-diaryltriazene-substituted sulfonamides as potent and selective carbonic anhydrase II inhibitors, Bioorg. Chem. 77 (2018) 542–547;
(d) S. Akocak, N. Lolak, S. Bua, C.T. Supuran, Discovery of novel 1,3-diaryltriazene sulfonamides as carbonic anhydrase I, II, VII and IX inhibitors, J. Enzyme Inhib. Med. Chem (2018), https://doi.org/10.1080/14756366.2018.1515933;
(e) Y. Tuluce, G. Gorgisen, I.M. Gulacar, et al., Antiproliferative and apoptotic role of novel synthesized Cu(II) complex with 3-(3-(4-fluorophenyl)Triaz-1-en-1-yl) benzenesulfonamide in common cancer models, Anticancer Res. 38 (2018) 5115–5120.
[21] (a) M.A. Ilies, D. Vullo, J. Pastorek, A. Scozzafava, M. Ilies, M.T. Caproiu, S. Pastorekova, C.T. Supuran, Carbonic anhydrase inhibitors. Inhibition of tumor-associated isozyme IX by halogenosulfanilamide and halogenophenylaminobenzolamide derivatives, J. Med. Chem. 46 (2003) 2187–2196;
(b) C.T. Supuran, A. Scozzafava, B.C. Jurca, M.A. Ilies, Carbonic anhydrase inhibitors – Part 49: synthesis of substituted ureido and thioureido derivatives of aromatic/heterocyclic sulfonamides with increased affinities for isozyme I, Eur. J. Med. Chem. 33 (1998) 83–93;
(c) H.I. Gul, C. Yamali, F. Yesilyurt, H. Sakagami, K. Kucukoglu, I. Gulcin, M. Gul, C.T. Supuran, Microwave-assisted synthesis and bioevaluation of new sulfonamides, J. Enzyme Inhib. Med., Chem. 32 (2017) 369–374.
[22] (a) A. Casini, A. Scozzafava, F. Mincione, L. Menabuoni, C.T. Supuran, Carbonic anhydrase inhibitors: synthesis of water soluble sulfonamides incorporating a 4-sulfamoylphenylmethylthiourea scaffold, with potent intraocular pressure lowering properties, J. Enzyme Inhib. Med. Chem. 17 (2002) 333–343;
(b) A. Casini, A. Scozzafava, F. Mincione, L. Menabuoni, M.A. Ilies, C.T. Supuran, Carbonic anhydrase inhibitors: water-soluble 4-sulfamoylphenylthioureas as topical intraocular pressure-lowering agents with long-lasting effects, J. Med. Chem. 43 (2000) 4884–4892;
(c) E. Svastová, A. Huliková, M. Rafajová, M. Zatovičová, A. Gibadulinová, A. Casini, et al., Hypoxia activates the capacity of tumor-associated carbonic anhydrase IX to acidify extracellular pH, FEBS Lett. 577 (2004) 439–445.