


RESEARCH PAPER



Carbonic anhydrase inhibition with a series of novel benzenesulfonamide-triazole conjugates

Marwa G. El-Gazzar^a, Nessma H. Nafie^a, Alessio Nocentini^b, Mostafa M. Ghorab^a, Helmi I. Heiba^a and Claudiu T. Supuran^b 

^aDepartment of Drug Radiation Research, National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), Cairo, Egypt; ^bNEUROFARBA Department, Pharmaceutical and Nutraceutical Sciences Section, University of Florence, Firenze, Italy

ABSTRACT

We report the synthesis and characterisation of a novel series of triazole benzenesulfonamide derivatives, which incorporate the general pharmacophore associated with carbonic anhydrase (CA, EC 4.2.1.1) inhibitors. The synthesised compounds were tested *in vitro* against four human carbonic anhydrase (hCA, EC 4.2.1.1) isozymes, hCA I, hCA II, hCA IV and hCA IX. The obtained results showed that the tumour-associated hCA IX was the most sensitive to inhibition with the synthesised derivatives, with the triazole-pyridine benzenesulfonamides **14**, **16** and **17** being the most effective inhibitors. Some selected compounds were chosen for a single dose anti-proliferative activity testing against a panel of 57 human tumour cell lines and show some anti-proliferative activity *ex vivo*.

ARTICLE HISTORY

Received 27 July 2018
Revised 16 August 2018
Accepted 16 August 2018

KEYWORDS

Carbonic anhydrase; benzenesulfonamide; 1,2,3-triazole; hCA IX; anti-proliferative

1. Introduction

Carbonic anhydrases (CA, EC 4.2.1.1) are a large family of zinc-containing metallo-enzymes that catalyse the reversible hydration of carbon dioxide to hydrogen carbonate and $H^{+1,2}$. In humans (h), 15 CA isoforms are known differing in tissue expression patterns, kinetic properties and subcellular localisation³. Their physiological roles are typically associated with acid–base homeostasis and the transport of CO_2 and hydrogen carbonate. Human (h) isoform hCA IX is a transmembrane protein with an extracellular active site, and is poorly expressed in healthy tissues (as GIT, bile duct and gall bladder), being instead over-expressed in many solid tumours as a result of hypoxia^{4,5}. The function of hCA IX in tumour cell is to maintain acid–base homeostasis under hypoxic conditions and to facilitate the diffusion of H^{+} through the entire solid tumour leading to low extracellular pH that produces matrix breakdown, invasion, immune suppression and multi-drug resistance leading to more tumour aggression and resistance^{6,7}. These findings led to great interest for new therapeutics targeting hCA IX. Inhibition of hCA IX with small molecules has emerged as a novel anticancer strategy^{8–10}. The most important and widely studied class of CA inhibitors are the aromatic sulfonamides which are capable to coordinate the catalytic Zn^{2+} from the enzyme active site, thus blocking the catalytic process^{11–16}. Moreover, several 1,2,3-triazole containing compounds have proved considerable biological activities including antibacterial, antifungal and anticancer activities^{17–22}.

In view of these facts, and in continuation of an ongoing project aiming to develop new biologically active sulfonamide derivatives^{23–30}, we report herein a new set of triazole-





benzenesulfonamides designed in agreement with the general pharmacophoric requirements for hCA IX inhibition: an aromatic sulfonamide moiety is used as base unit for the synthesis of the target compounds since necessary to coordinate with the Zn atom and bind to pivotal amino-acids in the active site pocket. An 1,2,3-triazole ring is appended at the aromatic scaffold and used as hydrophilic linker to incorporate several substitution patterns planned to increase the hydrophobic interactions within the active site cavity (Figure 1).

The synthesised compounds were tested for their inhibitory activity assay against four CA isoforms (hCA I, hCA II, hCA IV and hCA IX). Moreover, they were further evaluated against a panel of 57 human cell lines at National Cancer Institute (NCI, Bethesda, MD).

2. Materials and methods

2.1. Instruments

Melting points were taken in an open capillary tube on a Stuart melting point apparatus (Stuart Scientific, Redhill, UK) and were uncorrected. The IR spectra of the compounds were recorded on FTIR Shimadzu spectrometer (Shimadzu, Tokyo, Japan). ¹H NMR and ¹³C NMR spectra were recorded on a Varian Mercury Plus Oxford (300 MHz for ¹H-NMR and 75 MHz for ¹³C-NMR) spectrometer (Varian Inc., Palo Alto, CA) using TMS as an internal Standard and DMSO- d_6 as solvent. Mass spectra were run on HP Model MS-5988 (Hewlett Packard, Palo, Alto, CA). Microanalyses were obtained on a Carlo Erba 1108 Elemental Analyzer (Heraeus, Hanau, Germany). All values were within $\pm 0.4\%$ of the theoretical

CONTACT Marwa G. El-Gazzar  marwagalazzar@yahoo.com  Department of Drug Radiation Research, National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), Nasr City, Cairo P.O. Box 29, Egypt; Claudiu T. Supuran  claudiu.supuran@unifi.it  NEUROFARBA Department, Pharmaceutical and Nutraceutical Sciences Section, University of Florence, Via Ugo Schiff 6, Sesto Fiorentino, Firenze, I-50019, Italy.

© 2018 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

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

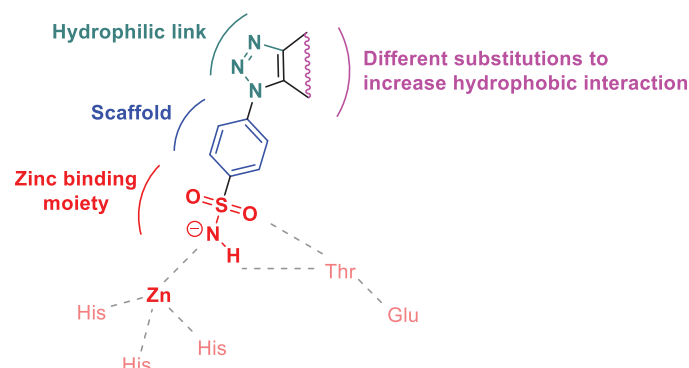


Figure 1. Design of the synthesised compounds.

values. Purity of the compounds was checked by TLC on pre-coated SiO_2 gel (HF254, 200 mesh) aluminium plates (Merk, Darmstadt, Germany). A developing solvent system of chloroform/methanol (8:2) was used and the spots were visualised under UV light. IR, ^1H NMR, ^{13}C NMR, Mass and elemental analysis were consistent with the assigned structures. Starting sulfanilamide and all reagents used were of analytical grade and were purchased from Sigma (St. Louis, MO).

2.2. Chemistry

4-(5-amino-4-cyano-1H-1,2,3-triazol-1-yl)benzenesulfonamide 3

A mixture of **2** (1 g, 0.005 mol) and malononitrile (0.33 g, 0.005 mol) was stirred in ethanol containing sodium ethoxide (0.11 g, 0.005 mol) at room temperature overnight and the precipitated solid was filtered off and crystallised from acetic acid to give **3**. Yield = 85%; m.p.: 100–101 °C. IR(cm^{-1}): 3335, 3205 (NH_2), 3096 (CH arom.), 2231 (CN), 1315, 1163 (SO_2). ^1H -NMR (300 MHz, DMSO-d_6): δ 7.59 (d, 2H, Ar-H, $J=8.8$ Hz), 7.63 (s, 2H, NH_2 , D_2O exch.), 7.89 (d, 2H, Ar-H, $J=8.8$ Hz), 8.5 (s, 2H, NH_2 , D_2O exch.). ^{13}C -NMR (75 MHz, DMSO-d_6): δ 113.97, 118.1 (2), 128.55(2), 137.48, 138.24, 139.36, 150.62. MS, m/z (%): 264(M^+). Anal. Calcd. For $\text{C}_9\text{H}_8\text{N}_6\text{O}_2\text{S}$ (264): C, 40.91; H, 3.05; N, 31.80; Found: C, 40.69; H, 3.35; N, 31.65.

4-(5-amino-4-(4,5-dihydro-1H-imidazol-2-yl)-1H-1,2,3-triazol-1-yl)benzenesulfonamide 4

A mixture of **3** (0.3 g, 0.001 mol) in ethylenediamine (7 ml) and carbon disulfide (7 ml) was heated under reflux for 6 h. After cooling, the reaction mixture was poured onto cold water and the formed solid was filtered off and crystallised from ethanol to give **4**. Yield = 80%; m.p.: 278–280 °C. IR(cm^{-1}): 3372, 3183 (NH_2), 3002 (CH arom.), 1610 (C=N), 1312, 1116 (SO_2). ^1H -NMR (300 MHz, DMSO-d_6): δ 3.33 (d, 2H, CH imidazole, $J=13.0$ Hz), 3.61 (d, 2H, CH imidazole, $J=13.2$ Hz), 7.49 (d, 2H, Ar-H, $J=8.8$ Hz), 7.65 (s, 2H, NH_2 , D_2O exch.), 7.86 (d, 2H, Ar-H, $J=8.8$ Hz), 8.2 (s, 2H, NH_2 , D_2O exch.). ^{13}C -NMR (75 MHz, DMSO-d_6): δ 49.41 (2), 118.10 (2), 128.55 (2), 137.48, 139.36, 138.24, 150.62, 152.89. MS, m/z (%): 307(M^+). Anal. Calcd. For $\text{C}_{11}\text{H}_{13}\text{N}_7\text{O}_2\text{S}$ (307): C, 42.99; H, 4.26; N, 31.90; Found: C, 42.75; H, 4.34; N, 31.77.

2-chloro-N-(4-cyano-1-(4-sulfamoylphenyl)-1H-1,2,3-triazol-5-yl)acetamide 5

A mixture of **3** (0.3 g, 0.001 mol) and chloroacetyl chloride (0.15 g, 0.001 mol) was stirred in DMF for 2 h, the reaction mixture was poured onto cold water and the formed solid was filtered off and crystallised from ethanol to give **5**. Yield = 91%; m.p.: 165–167 °C.

IR(cm^{-1}): 3337, 3187 (NH , NH_2), 3099 (CH arom.), 2933, 2858 (CH aliph.), 2231 (CN), 1668 (C=O), 1345, 1112 (SO_2). ^1H -NMR (300 MHz, DMSO-d_6): δ 4.26 (s, 2H, CH_2Cl), 7.52 (d, 2H, Ar-H, $J=8.6$ Hz), 7.69 (s, 2H, NH_2 , D_2O exch.), 7.90 (d, 2H, Ar-H, $J=8.6$ Hz), 9.5 (s, 1H, NH, D_2O exch.). ^{13}C -NMR (75 MHz, DMSO-d_6): δ 42.64, 113.97, 118.10 (2), 128.55 (2), 137.48, 138.24, 139.36, 150.62, 164.79. MS, m/z (%): 340(M^+). Anal. Calcd. For $\text{C}_{11}\text{H}_9\text{ClN}_6\text{O}_3\text{S}$ (340): C, 38.77; H, 2.66; N, 24.66; Found: C, 38.85; H, 2.46; N, 24.45.

N-(4-cyano-1-(4-sulfamoylphenyl)-1H-1,2,3-triazol-5-yl)-3-oxobutanamide 6

A mixture of **3** (0.3 g, 0.001 mol) and ethyl acetoacetate (0.13 g, 0.001 mol) was refluxed in ethanol for 5 h, the reaction mixture was cooled and the formed solid was filtered off and crystallised from ethanol to give **6**. Yield = 95%; m.p.: >300 °C. IR(cm^{-1}): 3342, 3270 (NH , NH_2), 3097 (CH arom.), 2970, 2890 (CH aliph.), 2205 (CN), 1707 (2C=O), 1342, 1125 (SO_2). ^1H -NMR (300 MHz, DMSO-d_6): δ 2.13 (s, 3H, CH_3), 3.58 (s, 2H, CH_2), 7.69 (s, 2H, NH_2 , D_2O exch.), 7.52 (d, 2H, Ar-H, $J=8.6$ Hz), 7.90 (d, 2H, Ar-H, $J=8.6$ Hz), 9.1 (s, 1H, NH, D_2O exch.). ^{13}C -NMR (75 MHz, DMSO-d_6): δ 30.82, 50.25, 113.97, 118.1 (2), 128.55 (2), 137.48, 138.24, 139.36, 150.62, 170.59, 204.29. MS, m/z (%): 348 (M^+). Anal. Calcd. For $\text{C}_{13}\text{H}_{12}\text{N}_6\text{O}_4\text{S}$ (348): C, 44.83; H, 3.47; N, 24.13; Found: C, 44.90; H, 3.51; N, 24.35.

4-(4-cyano-5-((2-oxo-2-phenylethyl)amino)-1H-1,2,3-triazol-1-yl)benzenesulfonamide 7

A mixture of **3** (0.3 g, 0.001 mol) and phenacyl bromide (0.2 g, 0.001 mol) was refluxed in ethanol for 3 h, the reaction mixture was cooled, poured onto ice water and the formed solid was filtered off and crystallised from acetone to give **7**. Yield = 89%; m.p. 216–218 °C. IR(cm^{-1}): 3339, 3206 (NH , NH_2), 3088 (CH arom.), 2925, 2889 (CH aliph.), 2224 (CN), 1677 (C=O), 1343, 1119 (SO_2). ^1H -NMR (300 MHz, DMSO-d_6): δ 4.03 (s, 2H, CH_2), 7.48–7.58 (m, 5H, Ar-H), 7.59 (d, 2H, Ar-H, $J=8.7$ Hz), 7.66 (d, 2H, Ar-H, $J=8.8$ Hz), 7.88 (s, 2H, NH_2 , D_2O exch.), 10.1 (s, 1H, NH, D_2O exch.). ^{13}C -NMR (75 MHz, DMSO-d_6): δ 50.35, 113.97, 118.10 (2), 128.47 (2), 128.55 (2), 128.89 (2), 128.92, 133.80, 137.48, 138.24, 139.36, 150.62, 191.85. MS, m/z (%): 382 (M^+). Anal. Calcd. For $\text{C}_{17}\text{H}_{14}\text{N}_6\text{O}_3\text{S}$ (382): C, 53.40; H, 3.69; N, 21.98; Found: C, 53.72; H, 3.61; N, 21.77.

General procedure for the synthesis of compounds 8–10

(E)-4-(5-((substituted-benzylidene)amino)-4-cyano-1H-1,2,3-triazol-1-yl)-benzenesulfonamide 8–10. A mixture of **3** (0.3 g, 0.001 mol) and the appropriate aromatic aldehyde (0.001 mol) was refluxed in acetic acid for 5 h and the precipitate formed while hot was filtered off and crystallised from ethanol to give **8–10**, respectively.

(E)-4-(5-((2-chlorobenzylidene)amino)-4-cyano-1H-1,2,3-triazol-1-yl)-benzenesulfonamide 8. Yield = 76%; m.p. 210–211 °C. IR(cm^{-1}): 3342, 3245 (NH_2), 3088 (CH arom.), 2188 (CN), 1334, 1120 (SO_2), 657 (C-Cl). ^1H -NMR (300 MHz, DMSO-d_6): δ 7.38–7.60 (m, 4H, Ar-H), 7.72 (s, 2H, NH_2 , D_2O exch.), 7.96 (d, 2H, Ar-H, $J=8.4$ Hz), 7.99 (d, 2H, Ar-H, $J=8.5$ Hz), 8.87 (s, 1H, CH). ^{13}C -NMR (75 MHz, DMSO-d_6): δ 113.97, 118.1 (2), 127.10, 128.70, 128.55 (2), 130.00, 130.55, 133.39, 136.10, 137.48, 138.24, 139.36, 151.50, 159.86. MS, m/z (%): 386 (M^+). Anal. Calcd. For $\text{C}_{16}\text{H}_{11}\text{ClN}_6\text{O}_2\text{S}$ (386): C, 49.68; H, 2.87; N, 21.73; Found: C, 49.81; H, 3.01; N, 21.82.

(E)-4-(5-((4-chlorobenzylidene)amino)-4-cyano-1H-1,2,3-triazol-1-yl)benzenesulfonamide 9. Yield = 80%; m.p.: 270–272 °C. IR(cm^{-1}): 3335, 3213 (NH_2), 3087 (CH arom.), 2197 (CN), 1343, 1123 (SO_2), 659 (C-Cl). $^1\text{H-NMR}$ (300 MHz, DMSO-d_6): δ 7.49 (d, 2H, Ar-H, $J=8.4$ Hz), 7.55 (d, 2H, Ar-H, $J=8.4$ Hz), 7.97 (d, 2H, Ar-H, $J=8.4$ Hz), 7.72 (s, 2H, NH_2 , D_2O exch.), 7.96 (d, 2H, Ar-H, $J=8.4$ Hz), 8.86 (s, 1H, CH). $^{13}\text{C-NMR}$ (75 MHz, DMSO-d_6): δ 118.1 (2), 113.97, 128.55 (2), 129.26 (2), 129.45 (2), 134.64, 135.68, 137.48, 138.24, 139.36, 151.50, 159.86. MS, m/z (%): 386 (M^+). Anal. Calcd. For $\text{C}_{16}\text{H}_{11}\text{ClN}_6\text{O}_2\text{S}$ (386): C, 49.68; H, 2.87; N, 21.73; Found: C, 48.77; H, 2.99; N, 21.81.

(E)-4-(4-cyano-5-((4-(dimethylamino)benzylidene)amino)-1H-1,2,3-triazol-1-yl)benzenesulfonamide 10. Yield = 89%; m.p.: 200–201 °C. IR(cm^{-1}): 3336, 3233 (NH_2), 3090 (CH arom.), 2207 (CN), 1333, 1115 (SO_2). $^1\text{H-NMR}$ (300 MHz, DMSO-d_6): δ 2.92 (s, 6H, 2CH_3), 6.72 (d, 2H, Ar-H, $J=8.4$ Hz), 7.72 (d, 2H, Ar-H, $J=8.4$ Hz), 7.70 (s, 2H, NH_2 , D_2O exch.), 7.91 (d, 2H, Ar-H, $J=8.4$ Hz), 7.92 (d, 2H, Ar-H, $J=8.4$ Hz), 9.12 (s, 1H, CH). $^{13}\text{C-NMR}$ (75 MHz, DMSO-d_6): δ 40.30 (2), 111.54 (2), 113.97, 118.10 (2), 128.55 (2), 130.30 (2), 134.64, 137.48, 138.24, 139.36, 151.43, 151.50, 159.86. MS, m/z (%): 395 (M^+). Anal. Calcd. For $\text{C}_{18}\text{H}_{17}\text{N}_7\text{O}_2\text{S}$ (395): C, 54.67; H, 4.33; N, 24.79; Found: C, 54.81; H, 4.45; N, 24.59.

General procedure for the synthesis of compounds 11 and 10

A mixture of **3** (0.3 g, 0.001 mol) and benzene or toluene sulfonyl chloride (0.001 mol) was refluxed in pyridine for 8 h, the reaction mixture was cooled, poured onto ice water and the formed solid was filtered off and crystallised from ethanol to give **11** and **12**, respectively.

N-(4-cyano-1-(4-sulfamoylphenyl)-1H-1,2,3-triazol-5-yl)benzenesulfonamide 11. Yield = 78%; m.p.: >300 °C. IR(cm^{-1}): 3319, 3231 (NH , NH_2), 3089 (CH arom.), 2210 (CN), 1321, 1124 (SO_2). $^1\text{H-NMR}$ (300 MHz, DMSO-d_6): δ 7.44 (d, 2H, Ar-H, $J=8.5$ Hz), 7.49–7.68 (m, 5H, Ar-H), 7.74 (d, 2H, Ar-H, $J=8.5$ Hz), 7.90 (s, 2H, NH_2 , D_2O exch.), 8.21 (s, 1H, NH, D_2O exch.). $^{13}\text{C-NMR}$ (75 MHz, DMSO-d_6): δ 113.97, 118.10 (2), 127.00 (2), 128.55 (2), 129.20 (2), 131.44, 135.14, 137.48, 138.24, 139.36, 150.62. MS, m/z (%): 404 (M^+). Anal. Calcd. For $\text{C}_{15}\text{H}_{12}\text{N}_6\text{O}_4\text{S}_2$ (404): C, 44.55; H, 2.99; N, 20.78; Found: C, 44.77; H, 2.78; N, 20.58.

N-(4-cyano-1-(4-sulfamoylphenyl)-1H-1,2,3-triazol-5-yl)-4-methylbenzenesulfonamide 12. Yield = 89%; m.p.: >300 °C. IR(cm^{-1}): 3338, 3231 (NH , NH_2), 3066 (CH arom.), 2928, 2843 (CH aliph.), 2224 (CN), 1333, 1114 (SO_2). $^1\text{H-NMR}$ (300 MHz, DMSO-d_6): δ 2.33 (s, 3H, CH_3), 7.32 (d, 2H, Ar-H, $J=8.1$ Hz), 7.44 (d, 2H, Ar-H, $J=8.5$ Hz), 7.62 (d, 2H, Ar-H, $J=8.1$ Hz), 7.90 (d, 2H, Ar-H, $J=8.7$ Hz), 7.94 (s, 2H, NH_2 , D_2O exch.), 8.27 (s, 1H, NH, D_2O exch.). $^{13}\text{C-NMR}$ (75 MHz, DMSO-d_6): δ 21.26, 113.97, 118.10 (2), 127.50 (2), 128.55 (2), 129.69 (2), 135.14, 137.48, 138.24, 139.36, 144.26, 150.62. MS, m/z (%): 418 (M^+). Anal. Calcd. For $\text{C}_{16}\text{H}_{14}\text{N}_6\text{O}_4\text{S}_2$ (418): C, 45.93; H, 3.37; N, 20.08; Found: C, 45.68; H, 2.91; N, 20.48.

4-(7-amino-6-cyano-5-oxo-4,5-dihydro-3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl) benzenesulfonamide 13. Fusion of **3** (0.3 g, 0.001 mol) with ethyl cyanoacetate (0.1 g, 0.001 mol) was done for 10 min, the reaction mixture was cooled, triturated with diethyl ether and the formed solid was filtered off and crystallised from ethanol to give **13**. Yield = 78%; m.p.: >300 °C. IR(cm^{-1}): 3339–3195 (NH , 2NH_2), 3080 (CH arom.), 2210 (CN), 1698 (C=O), 1323, 1141

(SO_2). $^1\text{H-NMR}$ (300 MHz, DMSO-d_6): δ 7.55 (d, 2H, Ar-H, $J=8.5$ Hz), 7.71 (s, 2H, NH_2 , D_2O exch.), 7.89 (d, 2H, Ar-H, $J=8.5$ Hz), 8.02 (s, 2H, NH_2 , D_2O exch.), 8.89 (s, 1H, NH, D_2O exch.). $^{13}\text{C-NMR}$ (75 MHz, DMSO-d_6): δ 91.52, 115.02, 118.10 (2), 128.55 (2), 137.48, 138.24, 139.36, 150.62, 163.08, 163.15. MS, m/z (%): 331 (M^+). Anal. Calcd. For $\text{C}_{12}\text{H}_9\text{N}_7\text{O}_3\text{S}$ (331): C, 43.50; H, 2.74; N, 29.59; Found: C, 43.71; H, 2.55; N, 29.38.

General procedure for the synthesis of compounds 14–17

4-(7-amino-6-cyano-5-(substituted)-3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-benzenesulfonamide 14–17. A mixture of **3** (0.3 g, 0.001 mol) and the appropriate benzylidene derivative (0.001 mol) was refluxed in ethanol containing catalytic amount of TEA (0.01 ml) for 5 h, the reaction mixture was left to cool and the precipitate formed was filtered off and crystallised from dioxane to give **14–17**, respectively.

4-(7-amino-6-cyano-5-phenyl-3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)benzenesulfonamide 14. Yield = 70%; m.p.: >300 °C. IR(cm^{-1}): 3356–3252 (2NH_2), 3077 (CH arom.), 2192 (CN), 1313, 1125 (SO_2). $^1\text{H-NMR}$ (300 MHz, DMSO-d_6): δ 7.51 (s, 2H, NH_2 , D_2O exch.), 7.70–7.80 (m, 5H, Ar-H), 7.87 (d, 2H, Ar-H, $J=7.1$ Hz), 7.96 (d, 2H, Ar-H, $J=7.1$ Hz), 8.35 (s, 2H, NH_2 , D_2O exch.). $^{13}\text{C-NMR}$ (75 MHz, DMSO-d_6): δ 116.59, 118.10 (2), 128.55 (2), 128.86 (2), 128.87 (2), 128.92, 130.66, 136.57, 138.24, 139.36, 140.47, 141.02, 156.05, 159.95. MS, m/z (%): 391 (M^+). Anal. Calcd. For $\text{C}_{18}\text{H}_{13}\text{N}_7\text{O}_2\text{S}$ (391): C, 55.24; H, 3.35; N, 25.05; Found: C, 55.56; H, 3.15; N, 25.29.

4-(7-amino-5-(2-chlorophenyl)-6-cyano-3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)benzenesulfonamide 15. Yield = 87%; m.p.: 115–117 °C. IR (cm^{-1}): 3330–3223 (2NH_2), 3087 (CH arom.), 2195 (CN), 1333, 1121 (SO_2), 678 (C-Cl). $^1\text{H-NMR}$ (300 MHz, DMSO-d_6): δ 7.51 (s, 2H, NH_2 , D_2O exch.), 7.59–7.77 (m, 4H, Ar-H), 7.89 (d, 2H, Ar-H, $J=8.4$ Hz), 8.05 (d, 2H, Ar-H, $J=8.4$ Hz), 8.08 (s, 2H, NH_2 , D_2O exch.). $^{13}\text{C-NMR}$ (75 MHz, DMSO-d_6): δ 116.59, 118.10 (2), 127.58, 127.82, 128.55 (2), 128.71, 130.55, 130.66 (2), 133.16, 138.24, 139.36, 140.47, 141.02, 156.05, 159.95. MS, m/z (%): 425 (M^+). Anal. Calcd. For $\text{C}_{18}\text{H}_{12}\text{ClN}_7\text{O}_2\text{S}$ (425): C, 50.77; H, 2.84; N, 23.02; Found: C, 50.56; H, 3.02; N, 23.22.

4-(7-amino-5-(4-chlorophenyl)-6-cyano-3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)benzenesulfonamide 16. Yield = 87%; m.p.: >300 °C. IR(cm^{-1}): 3358–3254 (2NH_2), 3011 (CH arom.), 2191 (CN), 1333, 1121 (SO_2), 688 (C-Cl). $^1\text{H-NMR}$ (300 MHz, DMSO-d_6): δ 7.12 (s, 2H, NH_2 , D_2O exch.), 7.49 (d, 2H, Ar-H, $J=8.6$ Hz), 7.89 (d, 2H, Ar-H, $J=8.4$ Hz), 8.11 (d, 2H, Ar-H, $J=8.4$ Hz), 8.21 (d, 2H, Ar-H, $J=8.6$ Hz), 8.55 (s, 2H, NH_2 , D_2O exch.). $^{13}\text{C-NMR}$ (75 MHz, DMSO-d_6): δ 116.59, 118.10 (2), 128.20 (2), 128.55 (2), 130.66, 130.70 (2), 135.68, 136.57, 138.24, 139.36, 140.47, 141.02, 156.05, 159.95. MS, m/z (%): 425 (M^+). Anal. Calcd. For $\text{C}_{18}\text{H}_{12}\text{ClN}_7\text{O}_2\text{S}$ (425): C, 50.77; H, 2.84; N, 23.02; Found: C, 50.58; H, 3.11; N, 23.29.

4-(7-amino-6-cyano-5-(4-(dimethylamino)phenyl)-3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)benzenesulfonamide 17. Yield = 89%; m.p.: 130–132 °C. IR(cm^{-1}): 3343–3211 (2NH_2), 3081 (CH arom.), 2199 (CN), 1333, 1126 (SO_2). $^1\text{H-NMR}$ (300 MHz, DMSO-d_6): δ 3.02 (s, 6H, 2CH_3), 6.88 (s, 2H, NH_2 , D_2O exch.), 7.37 (d, 2H, Ar-H, $J=8.4$ Hz), 7.79 (d, 2H, Ar-H, $J=8.4$ Hz), 7.82 (d, 2H, Ar-H, $J=8.8$ Hz), 8.06 (d, 2H, Ar-H, $J=8.8$ Hz), 8.50 (s, 2H, NH_2 , D_2O exch.). $^{13}\text{C-NMR}$ (75 MHz, DMSO-d_6): δ 40.30 (2), 113.84 (2), 116.59, 118.10 (2), 127.82 (2), 128.55 (2), 130.66, 136.57, 138.24, 139.36, 140.47,

141.02, 151.43, 156.05, 159.95. MS, m/z (%): 434 (M^+). Anal. Calcd. For $C_{20}H_{18}N_6O_2S$ (434): C, 55.29; H, 4.18; N, 25.79; Found: C, 55.52; H, 3.89; N, 25.55.

4-(7-amino-5-thioxo-4,5-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)benzenesulfonamide 18. Fusion of **3** (0.3 g, 0.001 mol) with thiourea (0.1 g, 0.001 mol) was done for 10 min, the reaction mixture was cooled, triturated with diethyl ether and the formed solid was filtered off and crystallised from ethanol to give **18**.

Yield = 91%; m.p. 285–287 °C. IR(cm^{-1}): 3360–3198 (NH, 2NH₂), 3089 (CH arom.), 1623 (C=S), 1323, 1121 (SO₂). ¹H-NMR (300 MHz, DMSO- d_6): δ 7.18 (s, 2H, NH₂, D₂O exch.), 7.52 (d, 2H, Ar-H, J = 8.5 Hz), 7.90 (d, 2H, Ar-H, J = 8.5 Hz), 8.01 (s, 2H, NH₂, D₂O exch.), 8.55 (s, 1H, NH, D₂O exch.). ¹³C-NMR (75 MHz, DMSO- d_6): δ 118.10 (2), 128.55 (2), 137.48, 138.24, 139.36, 150.62, 152.89, 179.08. MS, m/z (%): 323 (M^+). Anal. Calcd. For $C_{10}H_9N_7O_2S_2$ (323): C, 37.15; H, 2.81; N, 30.32; Found: C, 37.43; H, 3.03; N, 30.10.

4-(7-imino-5-oxo-6-phenyl-4,5,6,7-tetrahydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)benzenesulfonamide 19. A mixture of **3** (0.3 g, 0.001 mol) and phenyl isocyanate (0.13 g, 0.001 mol) was refluxed in DMF containing catalytic amount of TEA (0.01 ml) for 18 h, the reaction mixture was cooled, poured onto ice water and the formed solid was filtered off and crystallised from ethanol to give **19**. Yield = 89%; m.p.: 180–182 °C. IR(cm^{-1}): 3320, 3292, 3198, 3133 (2NH, NH₂), 3064 (CH arom.), 1675 (C=O), 1343, 1123 (SO₂). ¹H-NMR (300 MHz, DMSO- d_6): δ 7.47–7.55 (m, 5H, Ar-H), 7.52 (d, 2H, Ar-H, J = 8.8 Hz), 7.62 (s, 2H, NH₂, D₂O exch.), 7.89 (d, 2H, Ar-H, J = 8.8 Hz), 8.01, 8.11 (2s, 2H, 2NH, D₂O exch.). ¹³C-NMR (75 MHz, DMSO- d_6): δ 118.10 (2), 122.11 (2), 124.72, 128.55 (2), 129.25 (2), 137.48, 138.24, 139.36, 144.73, 149.00, 150.62, 151.73. MS, m/z (%): 383 (M^+). Anal. Calcd. For $C_{16}H_{13}N_7O_3S$ (383): C, 50.13; H, 3.42; N, 25.57; Found: C, 50.37; H, 3.21; N, 25.38.

4-(6-ethyl-7-imino-5-thioxo-4,5,6,7-tetrahydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)benzenesulfonamide 20. A mixture of **3** (0.3 g, 0.001 mol) and ethyl isothiocyanate (0.13 g, 0.001 mol) was refluxed in pyridine for 10 h, the reaction mixture was cooled, poured onto ice water, acidified with diluted HCl and the formed solid was filtered off and crystallised from ethanol to give **20**. Yield = 90%; m.p. 278–279 °C. IR(cm^{-1}): 3340–3214 (2NH, NH₂), 3069 (CH arom.), 2929, 2843 (CH aliph.), 1601 (C=S), 1333, 1120 (SO₂). ¹H-NMR (300 MHz, DMSO- d_6): δ 1.25 (t, 3H, CH₃, J = 7.1 Hz), 3.85 (q, 2H, CH₂, J = 7.1 Hz), 7.51 (d, 2H, Ar-H, J = 8.8 Hz), 7.89 (d, 2H, Ar-H, J = 8.8 Hz), 7.92 (s, 2H, NH₂, D₂O exch.), 8.00, 8.10 (2s, 2H, 2NH, D₂O exch.). ¹³C-NMR (75 MHz, DMSO- d_6): δ 12.98, 44.37, 118.10 (2), 128.55 (2), 137.48, 138.24, 139.36, 149.00, 150.62, 177.16. MS, m/z (%): 351 (M^+). Anal. Calcd. For $C_{12}H_{13}N_7O_2S_2$ (351): C, 41.02; H, 3.73; N, 27.90; Found: C, 41.31; H, 3.51; N, 27.73.

7-imino-N-(quinoxalin-2-yl)-3-(4-sulfamoylphenyl)-5-thioxo-3,4,5,7-tetrahydro-6H-[1,2,3]triazolo[4,5-d]pyrimidine-6-sulfonamide 21. A mixture of **3** (0.3 g, 0.001 mol) and 4-isothiocyanato-N-(quinoxalin-2-yl) benzenesulfonamide 2930 (0.4 g, 0.001 mol) was refluxed in DMF containing catalytic amount of TEA (0.01 ml) for 6 h, the reaction mixture was cooled, poured onto ice water and the formed solid was filtered off and crystallised from ethanol to give **21**. Yield = 89%; m.p.: 212–215 °C. IR(cm^{-1}): 3435–3238 (3NH, NH₂), 3074 (CH arom.), 2924, 2846 (CH aliph.), 1599 (C=S), 1320, 1146 (SO₂). ¹H-NMR (300 MHz, DMSO- d_6): δ 7.51 (d, 2H, Ar-H, J = 8.7 Hz), 7.68–7.81 (m, 4H, Ar-H), 7.73 (d, 2H, Ar-H, J = 8.8 Hz), 7.91 (d, 2H, Ar-H, J = 7.9 Hz), 8.09 (s, 1H, CH quinoxaline), 8.10 (d, 2H, Ar-H,

J = 8.0 Hz), 8.22 (s, 2H, NH₂, D₂O exch.), 8.32, 8.36, 8.55 (3s, 3H, 3NH, D₂O exch.). ¹³C-NMR (75 MHz, DMSO- d_6): δ 117.29 (2), 118.10 (2), 126.14 (2), 128.08 (2), 128.55 (3), 128.71, 129.18, 133.28, 136.75, 137.48, 138.24, 139.36, 143.64, 144.73, 149.00, 150.62, 153.92, 177.16. MS, m/z (%): 530 (M^+). Anal. Calcd. For $C_{24}H_{18}N_{10}O_4S_2$ (606): C, 47.52; H, 2.99; N, 23.09; Found: C, 47.41; H, 2.72; N, 23.25.

2.3. Carbonic anhydrase inhibition

An Applied Photophysics stopped-flow instrument has been used for assaying the CA-catalysed CO₂ hydration activity³¹. Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5) as buffer, and 20 mM Na₂SO₄ (for maintaining constant the ionic strength), following the initial rates of the CA-catalysed CO₂ hydration reaction for a period of 10–100 s. The CO₂ concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The uncatalysed 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-deionised water and dilutions up to 0.01 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were pre-incubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E-I complex. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3 and the Cheng-Prusoff equation, as reported earlier^{32,33} and represent the mean from at least three different determinations. All CA isoforms were recombinant ones obtained in-house as reported earlier^{34–38}.

2.4. In vitro anti-proliferative activity

Ten of the newly synthesised 1,2,3-triazolo benzenesulfonamide derivatives were selected by NCI (Bethesda, MD) for

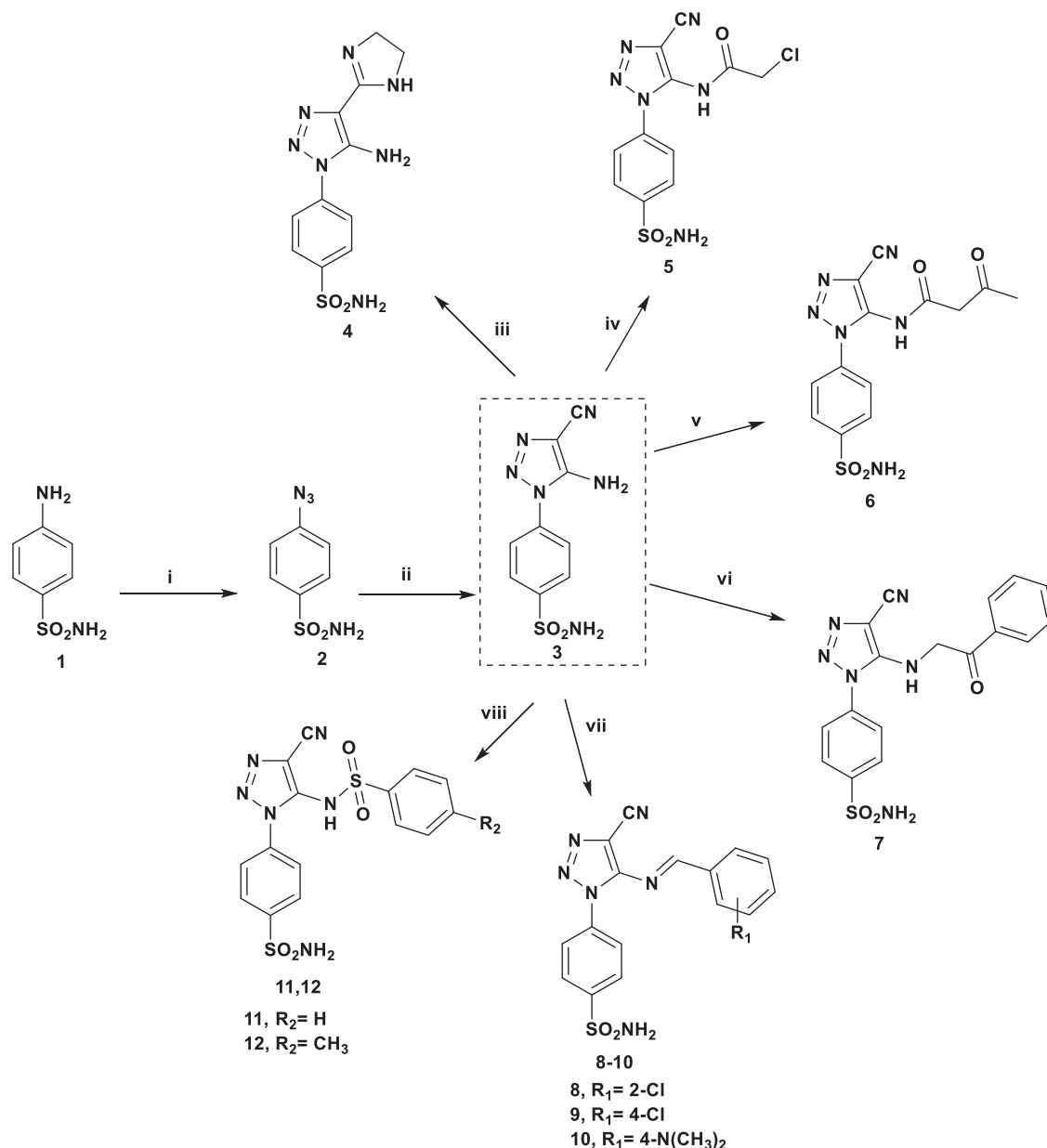
Table 1. Inhibition data of human CA isoforms hCA I, II, IV and IX with compounds 2–21 reported here and the standard sulfonamide inhibitor acetazolamide (AAZ) by a stopped flow CO₂ hydase assay.

| Compound | K_i (nM) | | | |
|----------|------------|--------|--------|--------|
| | hCA I | hCA II | hCA IV | hCA IX |
| 2 | 1267.5 | 700.4 | >10000 | 235.5 |
| 3 | 1940.7 | 16.7 | 2272.8 | 117.7 |
| 4 | 9938.3 | 6836.6 | 3186.2 | 3707.4 |
| 5 | 2545.5 | 518.1 | 1199.9 | 454.1 |
| 6 | 2855.9 | 683.9 | 471.7 | 410.1 |
| 7 | 1789.1 | 562.3 | 744.7 | 283.0 |
| 8 | 5139.6 | 6127.7 | >10000 | 1772.5 |
| 9 | 1601.2 | 94.5 | 4667.3 | 306.8 |
| 10 | 2922.9 | 178.7 | 3414.4 | 451.0 |
| 11 | 5549.1 | 356.4 | 4522.1 | 144.0 |
| 12 | 8101.8 | 90.1 | 2606.9 | 105.3 |
| 13 | 789.7 | 957.0 | 166.8 | 286.7 |
| 14 | 1247.8 | 57.0 | 675.5 | 46.4 |
| 15 | 2690.5 | 230.2 | 2577.4 | 407.1 |
| 16 | 604.8 | 168.6 | 788.1 | 42.6 |
| 17 | 682.6 | 56.8 | 473.3 | 35.1 |
| 18 | 955.4 | 732.3 | 489.1 | 272.3 |
| 19 | 2800.5 | 589.1 | 2270.2 | 158.2 |
| 20 | 908.5 | 929.5 | 874.6 | 241.8 |
| 21 | 5835.2 | 2374.7 | 5030.0 | 3809.6 |
| AAZ | 250 | 12 | 74 | 25 |

evaluating their anti-proliferative activity. The selected compounds were subjected to a primary *in vitro* one-dose (10 mM) anti-proliferative assay against 57 human tumour cell lines following the previously reported method³⁰ and their obtained growth inhibition percent (GI%) are presented in Table 2.

Table 2. Percentage growth inhibition (GI%) of *in vitro* human tumour cell lines at 10 μ M concentration for ten compounds.

| Panel/Cell line | Compound | | | | | | | | | |
|-----------------------------------|----------|------|------|------|------|------|------|------|------|------|
| | 3 | 5 | 7 | 9 | 11 | 13 | 16 | 17 | 18 | 20 |
| <i>Leukaemia</i> | | | | | | | | | | |
| CCRF-CEM | 15.3 | 11.3 | 16.4 | 15.9 | 6.7 | 13.8 | 16.4 | 11.4 | 17.8 | 20.9 |
| HL-60(TB) | 6.5 | 22.5 | 11.6 | 12.6 | 10.7 | 15.9 | 5.5 | 12.5 | 26.1 | 8.9 |
| MOLT-4 | 13.3 | 7.7 | 10.2 | 6.9 | 11.2 | 8.4 | 17.6 | 2.3 | 21.3 | 9.1 |
| RPMI-8226 | 8.5 | 8.7 | 12.5 | 12.3 | 7.1 | 7.5 | 8.4 | 12.5 | 12.3 | 12.1 |
| SR | 3.5 | 4.1 | 9.3 | 15.8 | 9.2 | 6.1 | 16.8 | 3.2 | 1.7 | 7.5 |
| <i>Non-small cell lung cancer</i> | | | | | | | | | | |
| A549/ATCC | 4.2 | 7.2 | 6.7 | 16.1 | 11.8 | 7.7 | 8.6 | 16.3 | 5.1 | 12.2 |
| EKVX | 8.7 | 8.6 | 9.2 | 4.2 | 2.0 | 8.3 | 39.5 | 17.2 | 3.7 | 3.1 |
| HOP-62 | 8.0 | 7.9 | 8.3 | 0.1 | 4.2 | 4.2 | 7.3 | 21.7 | 4.8 | – |
| HOP-92 | 8.2 | 3.9 | 13.5 | 12.8 | 4.2 | 12.5 | 35.4 | 18.5 | 10.6 | 18.0 |
| NCI-H226 | 5.2 | 4.6 | 11.8 | 4.8 | – | 4.2 | 9.8 | 16.9 | 9.8 | 6.9 |
| NCI-H23 | – | – | 2.1 | – | 4.1 | 5.9 | 11.2 | 9.8 | 4.7 | 9.4 |
| NCI-H322M | 0 | 6.6 | 8.8 | – | 2.6 | – | – | 3.9 | 2.2 | 3.2 |
| NCI-H460 | – | – | – | – | – | – | – | 0.9 | – | – |
| NCI-H522 | 9.7 | 5.1 | 16.1 | 10.5 | 10.3 | 3.6 | 9.8 | 14.8 | 13.7 | 7.8 |
| <i>Colon cancer</i> | | | | | | | | | | |
| COLO 205 | 5.1 | – | – | – | – | – | – | 17.0 | – | – |
| HCC-2998 | – | – | – | – | – | 3.3 | – | 3.9 | – | 2.2 |
| HCT-116 | 7.1 | 5.4 | 5.1 | 1.2 | 2.1 | 2.2 | 4.6 | 3.6 | – | – |
| HCT-15 | – | 4.1 | 1.7 | 4.3 | – | 1.9 | 1.6 | 1.2 | – | – |
| HT29 | 9.4 | – | 4.5 | 0.4 | 5.1 | – | 1.2 | 3.3 | 10.8 | – |
| KM12 | – | 5.2 | 0.6 | 0.5 | – | 2.6 | – | 5.9 | – | – |
| SW-620 | 5.1 | – | 2.8 | – | 2.3 | 0.9 | – | 2.7 | 4.3 | – |
| <i>CNS cancer</i> | | | | | | | | | | |
| SF-268 | 2.8 | 2.5 | 6.5 | – | – | – | 5.4 | 3.9 | 1.5 | – |
| SF-295 | – | 1.8 | – | 0.3 | – | 0.7 | 5.7 | 3.1 | – | – |
| SF-539 | 1.9 | 1.3 | – | – | 3.2 | 4.8 | – | 2.7 | – | – |
| SNB-19 | 2.9 | 3.8 | 7.8 | – | 2.8 | 0.9 | 3.4 | 0.8 | 1.7 | 1.9 |
| SNB-75 | 15.0 | 14.5 | 20.1 | 2.9 | 10.2 | 13.3 | 20.0 | 21.6 | 13.9 | 3.3 |
| U251 | 0.6 | 7.7 | 6.4 | 4.7 | 10.9 | 2.8 | 2.7 | 12.6 | – | 1.9 |
| <i>Melanoma</i> | | | | | | | | | | |
| LOX IMVI | 6.1 | 5.5 | 5.9 | – | – | 5.5 | 20.4 | 9.6 | 7.5 | 4.6 |
| MALME-3M | – | – | 3.6 | – | 1.2 | 2.7 | – | 0.4 | – | – |
| M14 | 2.7 | 4.7 | 2.6 | – | 3.3 | 1.6 | – | 3.5 | – | – |
| MDA-MB-435 | 3.1 | 0.4 | 8.3 | 3.4 | – | – | 2.9 | – | – | – |
| SK-MEL-2 | – | – | 2.2 | – | – | – | – | 2.3 | – | 8.2 |
| SK-MEL-28 | – | – | – | – | – | – | – | – | – | 0 |
| UACC-257 | 10.2 | 11.1 | 11.7 | 2.3 | 8.1 | 8.6 | 6.9 | 8.4 | 6.1 | 6.2 |
| UACC-62 | 5.5 | 3.4 | 4.0 | 6.0 | 5.9 | 8.4 | 15.5 | 11.6 | 5.6 | 4.3 |
| <i>Ovarian cancer</i> | | | | | | | | | | |
| IGROV1 | – | – | 0.5 | – | 3.3 | – | 16.4 | 12.1 | – | – |
| OVCAR-3 | – | – | – | – | – | – | – | – | – | – |
| OVCAR-4 | 4.1 | – | 10.2 | – | – | – | 21.6 | 0.8 | 7.9 | – |
| OVCAR-5 | – | 3.4 | – | – | – | – | – | 3.4 | – | – |
| OVCAR-8 | 4.6 | – | 2.4 | 7.4 | 2.9 | 4.1 | 3.8 | 11.4 | 3.7 | 4.8 |
| NCI/ADR-RES | – | – | – | 5.4 | 3.2 | 0.3 | 4.2 | – | – | 2.2 |
| SK-OV-3 | 4.6 | 3.5 | 8.2 | 9.9 | 15.1 | 6.7 | 9.7 | 12.3 | 11.6 | 3.6 |
| <i>Renal cancer</i> | | | | | | | | | | |
| 786-0 | – | 2.1 | – | 6.1 | – | 1.1 | – | – | – | – |
| A498 | 13.6 | 8.6 | – | 8.6 | – | 0.8 | 17.6 | 1.8 | 5.5 | 6.2 |
| ACHN | 1.3 | – | 0.6 | – | – | – | – | 9.3 | – | – |
| RXF 393 | – | – | – | – | – | – | 17.8 | – | – | – |
| SN12C | 1.1 | 11.8 | 2.9 | – | 1.6 | 2.3 | 8.9 | 4.7 | 1.2 | 0.9 |
| TK-10 | 9.2 | 3.6 | – | 7.6 | – | – | – | – | – | – |
| UO-31 | 14.5 | 13.5 | 21.1 | 0.3 | 13.4 | 12.7 | 25.0 | 29.9 | 13.8 | 7.3 |
| <i>Prostate cancer</i> | | | | | | | | | | |
| PC-3 | 8.3 | 10.3 | 15.9 | 9.3 | 9.7 | 13.0 | 22.2 | 13.8 | 7.8 | 9.7 |
| DU-145 | – | – | – | 1.0 | – | – | – | – | – | – |
| <i>Breast cancer</i> | | | | | | | | | | |
| MCF7 | 5.5 | 9.1 | 6.7 | 4.7 | 1.7 | 1.9 | 13.6 | 7.3 | 2.9 | 4.9 |
| MDA-MB-231/ATCC | – | – | 4.9 | – | – | 7.4 | 24.2 | 18.0 | 14.8 | – |
| HS 578T | 5.9 | 99.8 | 1.7 | – | – | 1.7 | 12.1 | 2.9 | 9.8 | 2.5 |
| BT-549 | – | 3.1 | 2.0 | 11.3 | – | 0.1 | – | 0.3 | – | – |
| T-47D | 5.1 | 4.8 | 4.6 | 11.5 | 9.6 | 4.8 | 5.6 | 5.2 | 5.8 | – |
| MDA-MB-468 | – | 1.3 | 3.3 | – | – | – | – | 3.2 | 3.5 | – |



Scheme 1. Reagents and conditions: (i) $\text{NaN}_3/\text{H}_2\text{SO}_4/\text{NaNO}_2/\text{r.t.}$; (ii) $\text{CH}_2(\text{CN})_2/\text{EtONa}/\text{EtOH}/\text{r.t.}$; (iii) $\text{NH}_2\text{CH}_2\text{CH}_2\text{NH}_2/\text{CS}_2/\text{reflux } 6 \text{ h}$; (iv) $\text{ClCH}_2\text{COCl}/\text{DMF}/\text{r.t.}$; (v) $\text{CH}_3\text{COCH}_2\text{COOC}_2\text{H}_5/\text{reflux } 3 \text{ h}$; (vi) $\text{PhCOCH}_2\text{Br}/\text{EtOH}/\text{reflux } 3 \text{ h}$; (vii) $\text{Ar-CHO}/\text{AcOH}/\text{reflux } 5 \text{ h}$; (viii) $\text{Ar-SO}_2\text{Cl}/\text{pyridine}/8 \text{ h}$.

3. Results and discussion

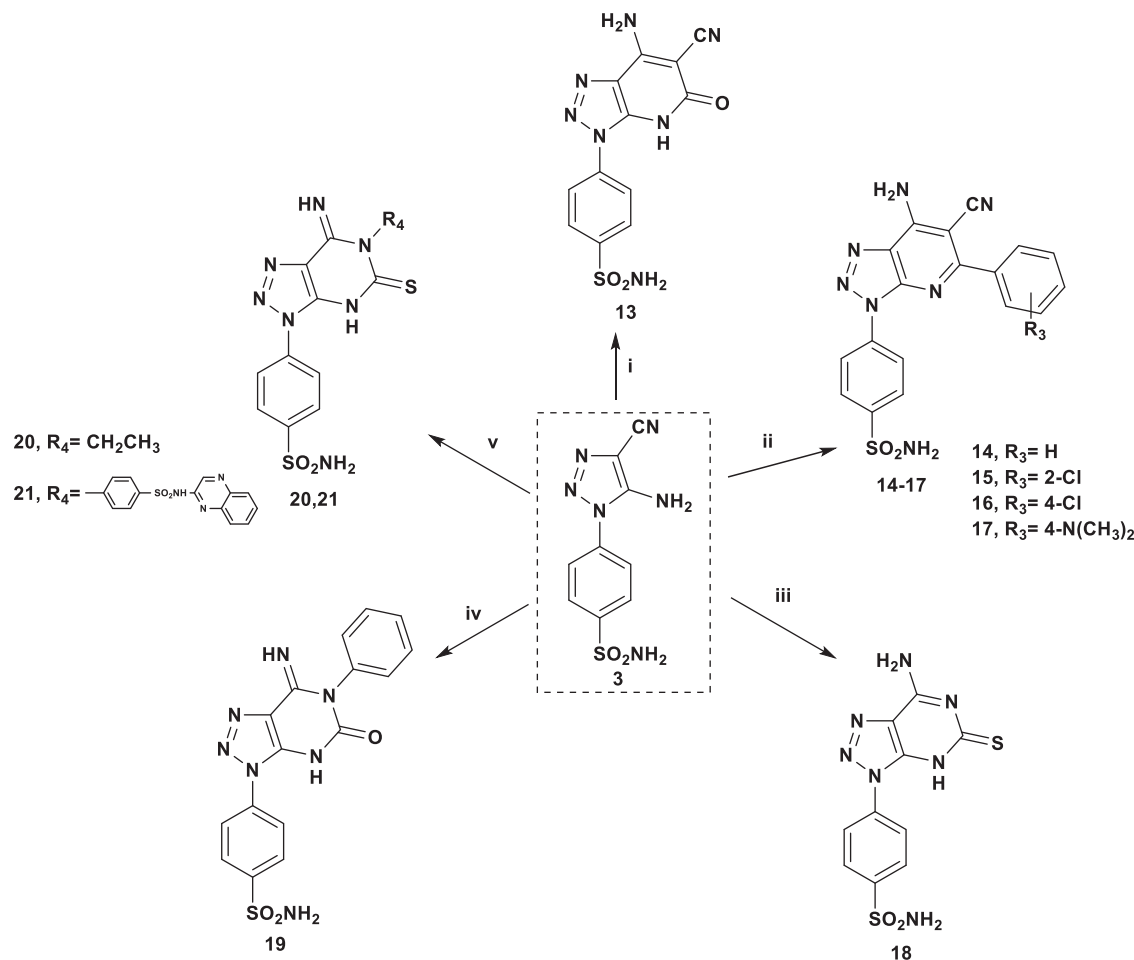
3.1. Chemistry

Synthesis of the series of 1,2,3-triazolo-benzensulfonamide derivatives **3–21** begins with 4-azido benzenesulfonamide³² which was subjected to reaction with malononitrile under stirring at room temperature to give 4-(5-amino-4-cyano-1H-1,2,3-triazol-1-yl)benzenesulfonamide **3**, which represents the key intermediate to produce the target compounds **4–21**. Reaction of **3** with ethylene diamine and carbon disulfide as catalyst^{32b} afforded the imidazoline derivative **4** whose structure was confirmed by disappearance of the carbonitrile band in IR and the presence of the corresponding protons and carbons of imidazoline ring in NMR spectra. Substitution on the amino group of **3** proceeded successfully via simple reactions with chloroacetyl chloride, ethyl acetoacetate and phenacyl bromide affording the corresponding compounds **5–7** in good yields. The Schiff's bases **8–10** were obtained by reaction of **3** with substituted aromatic aldehydes, and in compounds **11** and

12, a new sulfonamide moiety is introduced to the amino group of **3** through reaction with benzene/toluene sulfonyl chloride (Scheme 1). In compounds **5–12** the ¹H-NMR spectra showed the disappearance of NH₂ signals and the presence of the corresponding signals for the introduced groups as listed in the experimental section.

The triazole derivative **3**, bearing two active functional groups, was further cyclised by reaction with 3-ethylcyanoacetate and different 4-substituted benzylidene derivatives affording the corresponding triazolo-pyridine derivatives **13–17**. On the other hand, reaction of **3** with thiourea and phenyl thiocyanate afforded the triazolo-pyrimidines **18** and **19**. Similarly, the isothiocyanate derivatives reacted with **3** to give the triazolo-pyrimidine derivatives **20** and **21** bearing ethyl or sulfaquinoxaline moiety, respectively (Scheme 2).

The structures of the target compounds were proved by elemental and spectral data and were in consistency with assigned structures as presented in details in the experimental section.



Scheme 2. Reagents and conditions: (i) $CNCH_2COOC_2H_5/3\text{ h}$; (ii) $Ar-CH(CN)_2/EtOH/TEA/5\text{ h}$; (iii) $NH_2CSNH_2/15\text{ min}$; (iv) $PhNCO/DMF/TEA/reflux\ 18\text{ h}$; (v) CH_3CH_2 or $Ar-NCS/DMF/TEA/reflux\ 5\text{ h}$.

3.2. Carbonic anhydrase inhibition

The synthesised compounds were tested against the cytosolic hCA I, II the transmembrane IV and the tumour-associated membrane bound hCAIX by a stopped-flow CO_2 hydrase assay in comparison to acetazolamide (AAZ) as standard CAI³¹. The results presented in Table 1 allowed to depict the following SAR.

The cytosolic hCA I was moderately inhibited by all the tested compounds with inhibition constant (K_i) ranging from 604.8 to 9938.3 nM. The most active compounds were the triazolo-pyridine derivatives **13**, **16** and **17** (789.7, 604.8 and 682.6 nM, respectively).

The physiologically dominant isoform hCA II was moderately inhibited by all the synthesised compounds with different extents having inhibitory constants in the range of 16.7–6836.6 nM. The 4-(5-amino-4-cyano-1H-1,2,3-triazol-1-yl)benzenesulfonamide **3** showed the best activity (16.7 nM), very close to that of acetazolamide (12 nM). Moreover, a good activity was observed for the triazolo-pyridine **17** (56.8 nM).

A relatively poor affinity for the tested compounds was observed against hCA IV, with their inhibitory constant spanning between 116.8 and 5030.30 nM, whereas compounds **2** and **8** showed no activity.

The tumour-associated hCA IX was the most significantly inhibited by the tested compounds which showed inhibitory constants ranging from 35.1 to 3809.6 nM. The most active compounds were the triazolo-pyridine derivatives **14**, **16** and **17** (46.4, 42.6 and 35.1 nM, respectively).

Hence, *p*-substitution on benzenesulfonamide with triazolo-pyridine moiety was the most successful towards CA inhibition. Introduction of aryl group to position 5 of triazolo-pyridine led to high affinity towards hCA IX, whereas *p*-substitution on 5-aryl group increased activity especially for the *N*-dimethyl derivative **17**, which is the most potent candidate in this study.

3.3. In vitro anti-proliferative activity

A subset of ten triazolo-benzenesulfonamides (**3**, **5**, **6**, **7**, **9**, **11**, **16**–**18**, and **20**) were selected and subjected to an *in vitro* anti-proliferative screening against a panel of 57 cancer cell lines at NCI at an initial high dose (10 mM). The human cell lines used were derived from nine cancer types: leukaemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancers. The mean percentages growth inhibition (GI%) of the tested compounds are shown in Table 2.

The tested compounds showed fair anti-proliferative activity over the different cell lines. They inhibited cell growth by different extents and their sensitivity varies as presented in the table. The tested compounds were active mostly against leukaemia and lung cancer cell lines. Compound **16** was sensitive against 9 cell lines showing the highest GI% on EKVX (39.5%) and HOP-92 (34.4%) lung cancer cell lines. Compound **17** was active against 12 cell lines with the highest GI% on SNB-75 CNS cancer cell line (21.6%). Compound **18** was mostly active on HL-60(TB) Leukaemia cell line with GI% = 26.1%. While, compound **20** showed highest activity

(20.9%) on CCRF-CEM leukaemia cell line. An exceptional activity was observed for compound **5** on HS 578T breast cancer cell line showing GI% of 99.8%.

4. Conclusions

The present work describes the design and synthesis of novel series of 1,2,3-triazolo benzenesulfonamide derivatives according to the general pharmacophoric requirements of the tumour-associated hCA IX. The synthesised compounds were tested *in vitro* against four human carbonic anhydrase isozymes (hCA I, hCA II, hCA IV and hCA IX). hCA IX was the most sensitive isozyme towards the tested compounds, with the most active inhibitors being the triazolo-pyridine benzenesulfonamides **14**, **16**, and **17**. In addition, they showed fair anti-proliferative activity against a panel of 57 human tumour cell lines. These results present a novel group of selective hCA IX inhibitors having triazolo-pyridine benzenesulfonamide moiety. Future work will focus on varying hydrophobic substitutions on the pyridine ring to enhance selectivity.

Acknowledgments

The authors are grateful to National Cancer Institute (NCI) Developmental Therapeutic Program (www.dtp.nci.nih.gov) for screening the anticancer activity of the newly synthesised compounds *in vitro*.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Claudiu T. Supuran  <http://orcid.org/0000-0003-4262-0323>

References

- Supuran CT. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. *Nat Rev Drug Discov* 2008;7:168–81.
- Supuran CT. Carbonic anhydrase inhibitors. *Bioorg Med Chem Lett* 2010;20:3467–74.
- (a) Supuran CT. Structure-based drug discovery of carbonic anhydrase inhibitors. *J Enzyme Inhib Med Chem* 2012;27:759–72; (b) Boztaş M, Çetinkaya Y, Topal M, et al. Synthesis and carbonic anhydrase isoenzymes I, II, IX, and XII inhibitory effects of dimethoxybromophenol derivatives incorporating cyclopropane moieties. *J Med Chem* 2015; 58:640–50.
- Swietach P, Patiar S, Supuran CT, et al. The role of carbonic anhydrase 9 in regulating extracellular and intracellular pH in three-dimensional tumor cell growths. *J Biol Chem* 2009; 284:20299–310.
- (a) Supuran CT. Advances in structure-based drug discovery of carbonic anhydrase inhibitors. *Expert Opin Drug Discov* 2017;12:61–88; (b) Supuran CT. Structure and function of carbonic anhydrases. *Biochem J* 2016;473:2023–32; (c) Neri D, Supuran CT. Interfering with pH regulation in tumours as a therapeutic strategy. *Nat Rev Drug Discov* 2011;10:767–77; (d) Supuran CT, Vullo D, Manole G, Casini A, Scozzafava A. Designing of novel carbonic anhydrase inhibitors and activators. *Curr Med Chem Cardiovasc Hematol Agents* 2004;2:49–68.
- Supuran CT, Alterio V, Di Fiore A, et al. Inhibition of carbonic anhydrase IX targets primary tumors, metastases, and cancer stem cells: three for the price of one. *Med Res Rev* 2018; in press.
- Ledaki I, McIntyre A, Wigfield S, et al. Carbonic anhydrase IX induction defines a heterogeneous cancer cell response to hypoxia and mediates stem cell-like properties and sensitivity to HDAC inhibition. *Oncotarget* 2015;6:19413–27.
- (a) Supuran CT. How many carbonic anhydrase inhibition mechanisms exist? *J Enzyme Inhib Med Chem* 2016;31:345–60; (b) Alterio V, Di Fiore A, D'Ambrosio K, Supuran CT, De Simone, G. Multiple binding modes of inhibitors to carbonic anhydrases: how to design specific drugs targeting 15 different isoforms? *Chem Rev* 2012;112:4421–68; (c) Abbate F, Winum JY, Potter BV, Casini A, Montero JL, Scozzafava A, Supuran CT. Carbonic anhydrase inhibitors: X-ray crystallographic structure of the adduct of human isozyme II with EMATE, a dual inhibitor of carbonic anhydrases and steroid sulfatase. *Bioorg Med Chem Lett* 2004;14:231–4; (d) Capasso C, Supuran CT. An overview of the alpha-, beta- and gamma-carbonic anhydrases from Bacteria: can bacterial carbonic anhydrases shed new light on evolution of bacteria? *J Enzyme Inhib Med Chem* 2015;30:325–32.
- (a) Wichert M, Krall N. Targeting carbonic anhydrase IX with small organic ligands. *Curr Opin Chem Biol* 2015;26:48–54; (b) Supuran CT. Carbon- versus sulphur-based zinc binding groups for carbonic anhydrase inhibitors? *J Enzyme Inhib Med Chem* 2018;33:485–95; (c) Di Fiore A, Maresca A, Supuran CT, De Simone G. Hydroxamate represents a versatile zinc binding group for the development of new carbonic anhydrase inhibitors. *Chem Commun (Camb)* 2012;48:8838–40; (d) Marques SM, Nuti E, Rossello A, Supuran CT, Tuccinardi T, Martinelli A, Santos MA. Dual inhibitors of matrix metalloproteinases and carbonic anhydrases: iminodiacetyl-based hydroxamate-benzenesulfonamide conjugates. *J Med Chem* 2008;51:7968–79.
- (a) Supuran CT. Carbonic anhydrase inhibition and the management of hypoxic tumors. *Metabolites* 2017;7:E48; (b) Ward C, Langdon SP, Mullen P, Harris AL, Harrison DJ, Supuran CT, Kunkler IH. New strategies for targeting the hypoxic tumour microenvironment in breast cancer. *Cancer Treat Rev* 2013;39:171–9; (c) Garaj V, Puccetti L, Fasolis G, et al. 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* 2005;15:3102–08; (d) Casey JR, Morgan PE, Vullo D, Scozzafava A, Mastrolorenzo A, Supuran CT. Carbonic anhydrase inhibitors. Design of selective, membrane-impermeant inhibitors targeting the human tumor-associated isozyme IX. *J Med Chem* 2004;47:2337–47.
- Gul HI, Yamali C, Sakagami H, et al. New anticancer drug candidates sulfonamides as selective hCA IX or hCA XII inhibitors. *Bioorg Chem* 2018;77:411–9.
- Havrankova E, Csollei J, Vullo D, et al. Novel sulfonamide incorporating piperazine, aminoalcohol and 1,3,5-triazine structural motifs with carbonic anhydrase I, II and IX inhibitory action. *Bioorg Chem* 2018;77:25–37.

13. Lolak N, Akocak S, Bua S, et al. Design and synthesis of novel 1,3-diaryltriazene-substituted sulfonamides as potent and selective carbonic anhydrase II inhibitors. *Bioorg Chem* 2018;77:542–7.
14. (a) Monti SM, Meccariello A, Ceruso M, Szafranski K, Slawinski J, Supuran CT. Inhibition studies of *Brucella suis* beta-carbonic anhydrases with a series of 4-substituted pyridine-3-sulphonamides. *J Enzyme Inhib Med Chem* 2018;33:255–9; (b) Modak JK, Liu YC, Supuran CT, Roujeinikova A. Structure-Activity relationship for sulfonamide inhibition of *helicobacter pylori* α -carbonic anhydrase. *J Med Chem* 2016;59:11098–109; (c) Buzás GM, Supuran CT. The history and rationale of using carbonic anhydrase inhibitors in the treatment of peptic ulcers. In *memoriam Ioan Pușcaș (1932–2015)*. *J Enzyme Inhib Med Chem* 2016;31:527–33; (d) Supuran CT. Bacterial carbonic anhydrases as drug targets: toward novel antibiotics? *Front Pharmacol*. 2011;2:34; (e) Nishimori I, Onishi S, Takeuchi H, Supuran CT. The alpha and beta classes carbonic anhydrases from *Helicobacter pylori* as novel drug targets. *Curr Pharm Des* 2008;14:622–30.
15. Nocentini A, Bua S, Lomelino CL, et al. Discovery of new sulfonamide carbonic anhydrase IX inhibitors incorporating nitrogenous bases. *ACS Med Chem Lett* 2017;8:1314–9.
16. Vullo D, Lehneck R, Poggeler S, Supuran CT. Sulfonamide inhibition studies of two beta-carbonic anhydrases from the ascomycete fungus *Sordaria macrospora*, CAS1 and CAS2. *J Enzyme Inhib Med Chem* 2018;33:390–6.
17. Chipoline IC, Alves E, Branco P, et al. Synthesis and cytotoxic evaluation of 1H-1,2,3-triazol-1-ylmethyl-2,3-dihydronaphtho[1,2-b]furan-4,5-diones. *An Acad Bras Cienc* 2018;90:1027–1033.
18. El-Sherief HAM, Youssif BGM, Bukhari SNA, et al. Novel 1,2,4-triazole derivatives as potential anticancer agents: Design, synthesis, molecular docking and mechanistic studies. *Bioorg Chem* 2018;76:314–25.
19. Lal K, Yadav P, Kumar A, et al. Design, synthesis, characterization, antimicrobial evaluation and molecular modeling studies of some dehydroacetic acid-chalcone-1,2,3-triazole hybrids. *Bioorg Chem* 2018;77:236–44.
20. Lopez-Rojas P, Janeczko M, Kubinski K, et al. Synthesis and antimicrobial activity of 4-substituted 1,2,3-triazole-coumarin derivatives. *Molecules* 2018;23:199.
21. Tsai YH, Borini Etichetti CM, Di Benedetto C, et al. Synthesis of triazole derivatives of levoglucosenone as promising anticancer agents. Effective exploration of the chemical space through retro-aza-Michael//aza-Michael isomerizations. *J Org Chem* 2018;83:3516–3528.
22. Paprocka R, Modzelewska-Banachiewicz B, Kutkowska J, et al. Antibacterial and central nervous system activity of (4,5-diaryl-4H-1,2,4-triazol-3-yl)methacrylic acid derivatives. *Acta Pol Pharm* 2017;74:289–92.
23. Ghorab MM, Alsaid MS, Ceruso M, et al. Carbonic anhydrase inhibitors: synthesis, molecular docking, cytotoxic and inhibition of the human carbonic anhydrase isoforms I, II, IX, XII with novel benzenesulfonamides incorporating pyrrole, pyrrolopyrimidine and fused pyrrolopyrimidine moieties. *Bioorg Med Chem* 2014;22:3684–95.
24. Ghorab MM, Ceruso M, Alsaid MS, et al. Novel sulfonamides bearing pyrrole and pyrrolopyrimidine moieties as carbonic anhydrase inhibitors: synthesis, cytotoxic activity and molecular modeling. *Eur J Med Chem* 2014;87:186–96.
25. Ghorab MM, Ragab FA, Heiba HI, et al. In vitro anticancer screening and radiosensitizing evaluation of some new quinolines and pyrimido[4,5-b]quinolines bearing a sulfonamide moiety. *Eur J Med Chem* 2010;45:3677–84.
26. Ghorab MM, Ragab FA, Heiba HI, et al. Synthesis of novel pyrrole and pyrrolo[2,3-d]pyrimidine derivatives bearing sulfonamide moiety for evaluation as anticancer and radiosensitizing agents. *Bioorg Med Chem Lett* 2010;20:6316–20.
27. Ghorab MM, Ragab FA, Heiba HI, et al. Synthesis, anticancer and radiosensitizing evaluation of some novel sulfonamide derivatives. *Eur J Med Chem* 2015;92:682–92.
28. Ghorab MM, Ragab FA, Heiba HI, Soliman AM. Design and synthesis of some novel 4-Chloro-N-(4-(1-(2-(2-cyanoacetyl)-hydrazono)ethyl)phenyl) benzenesulfonamide derivatives as anticancer and radiosensitizing agents. *Eur J Med Chem* 2016;117:8–18.
29. Ghorab M, Ragab F, Heiba H, et al. Synthesis, in vitro anticancer screening and radiosensitizing evaluation of some new 4-[3-(substituted)thioureido]-N-(quinoxalin-2-yl)-benzenesulfonamide derivatives. *Acta Pharm* 2011;61:415–25.
30. (a) Ghorab MM, Ragab FA, Heiba HI, El-Gazzar MG. Synthesis, in-vitro anticancer screening and radiosensitizing evaluation of some new N-(quinoxalin-2-yl)benzenesulfonamide derivatives. *Arzneimittelforschung* 2012;62:46–52; (b) Abou-Seri SM, Eldehna WM, Ali MM, Abou El Ella DA. 1-Piperazinylphthalazines as potential VEGFR-2 inhibitors and anticancer agents: Synthesis and in vitro biological evaluation. *Eur J Med Chem* 2016;107:165–79.
31. Khalifah RG. The carbon dioxide hydration activity of carbonic anhydrase: I. Stop-flow kinetic studies on the native human isoenzymes B and C. *J Biol Chem* 1971;246:2561–73.
32. (a) Singer M, Lopez M, Bornaghi LF, Innocenti A, Vullo D, Supuran CT, Poulsen SA. Inhibition of carbonic anhydrase isozymes with benzene sulfonamides incorporating thio, sulfinyl and sulfonyl glycoside moieties. *Bioorg Med Chem Lett* 2009;19:2273–6; (b) Radwan SM, El-Kashef HS. Synthesis and anti-microbial activity of some imidazo [1',2':5,6]pyrimido [4,5-c] pyridazines and related heterocycles. *II Farmaco* 1998;53:113–7.
33. (a) Angeli A, Del Prete S, Osman SM, et al. Activation studies of the α - and β -carbonic anhydrases from the pathogenic bacterium *Vibrio cholerae* with amines and amino acids. *J Enzyme Inhib Med Chem* 2018;33:227–33; (b) Bua S, Bozdog M, Del Prete S, et al. Mono- and di-thiocarbamate inhibition studies of the δ -carbonic anhydrase TweCA δ from the marine diatom *Thalassiosira weissflogii*. *J Enzyme Inhib Med Chem* 2018;33:707–13.
34. (a) Diaz JR, Fernández Baldo M, Echeverría G, et al. A substituted sulfonamide and its Co (II), Cu (II), and Zn (II) complexes as potential antifungal agents. *J Enzyme Inhib Med Chem* 2016;31(suppl. 2):51–62; (b) Menchise V, De Simone G, Alterio V, et al. Carbonic anhydrase inhibitors: stacking with Phe131 determines active site binding region of inhibitors as exemplified by the X-ray crystal structure of a membrane-impermeant antitumor sulfonamide complexed with isozyme II. *J Med Chem* 2005;48:5721–7; (c) Supuran CT, Mincione F, Scozzafava A, et al. Carbonic anhydrase inhibitors—part 52. Metal complexes of heterocyclic sulfonamides: a new class of strong topical intraocular pressure-lowering agents in rabbits. *Eur J Med Chem* 1998;33:247–54; (d) Garaj V, Puccetti L, Fasolis G, et al. 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*. 2005;15:3102–8; (e) Şentürk M, Gülçin İ, Beydemir Ş, et al. In

- vitro inhibition of human carbonic anhydrase I and II isozymes with natural phenolic compounds. *Chem Biol Drug Des.* 2011;77:494–9; (f) Fabrizi F, Mincione F, Somma T, et al. A new approach to antiglaucoma drugs: carbonic anhydrase inhibitors with or without NO donating moieties. Mechanism of action and preliminary pharmacology. *J Enzyme Inhib Med Chem* 2012;27:138–47.
35. (a) Krall N, Pretto F, Decurtins W, et al. A small-molecule drug conjugate for the treatment of carbonic anhydrase IX expressing tumors. *Angew Chem Int Ed Engl* 2014;53:4231–35; (b) Rehman SU, Chohan ZH, Gulnaz F, Supuran CT. In-vitro antibacterial, antifungal and cytotoxic activities of some coumarins and their metal complexes. *J Enzyme Inhib Med Chem* 2005;20:333–40; (c) Clare BW, Supuran CT. Carbonic anhydrase activators. 3: structure-activity correlations for a series of isozyme II activators. *J Pharm Sci* 1994;83:768–73; (d) Dubois L, Peeters S, Lieuwes NG, et al. Specific inhibition of carbonic anhydrase IX activity enhances the in vivo therapeutic effect of tumor irradiation. *Radiother Oncol* 2011;99:424–31; (e) Chohan ZH, Munawar A, Supuran CT. Transition metal ion complexes of Schiff-bases. Synthesis, characterization and antibacterial properties. *Met Based Drugs* 2001;8:137–43; (f) Zimmerman SA, Ferry JG, Supuran CT. Inhibition of the archaeal β -class (Cab) and γ -class (Cam) carbonic anhydrases. *Curr Top Med Chem* 2007;7:901–8.
 36. (a) Supuran CT, Nicolae A, Popescu A. Carbonic anhydrase inhibitors: part 35. Synthesis of Schiff bases derived from sulfanilamide and aromatic aldehydes: the first inhibitors with equally high affinity towards cytosolic and membrane-bound isozymes. *Eur J Med Chem* 1996;31:431–8; (b) Pacchiano F, Aggarwal M, Avvaru BS, et al. 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)* 2010;46:8371–3; (c) Ozensoy Guler O, Capasso C, Supuran CT. A magnificent enzyme superfamily: carbonic anhydrases, their purification and characterization. *J Enzyme Inhib Med Chem* 2016;31:689–94; (d) De Simone G, Langella E, Esposito D, et al. Insights into the binding mode of sulphamates and sulphamides to hCA II: crystallographic studies and binding free energy calculations. *J Enzyme Inhib Med Chem* 2017;32:1002–11.
 37. (a) Supuran CT. Carbonic anhydrase inhibition and the management of neuropathic pain. *Expert Rev Neurother* 2016;16:961–8; (b) Di Cesare Mannelli L, Micheli L, Carta F, Cozzi A, Ghelardini C, Supuran CT. Carbonic anhydrase inhibition for the management of cerebral ischemia: in vivo evaluation of sulfonamide and coumarin inhibitors. *J Enzyme Inhib Med Chem* 2016;31:894–9; (c) Margheri F, Ceruso M, Carta F, et al. Overexpression of the transmembrane carbonic anhydrase isoforms IX and XII in the inflamed synovium. *J Enzyme Inhib Med Chem* 2016;31(suppl. 4):60–3; (d) Bua S, Di Cesare Mannelli L, Vullo D, et al. Design and synthesis of novel nonsteroidal anti-inflammatory drugs and carbonic anhydrase inhibitors hybrids (NSAIDs-CAIs) for the treatment of rheumatoid arthritis. *J Med Chem* 2017;60:1159–70.
 38. (a) Alper Türkoğlu E, Şentürk M, Supuran CT, Ekinici D. Carbonic anhydrase inhibitory properties of some uracil derivatives. *J Enzyme Inhib Med Chem* 2017;32:74–7; (b) Soydan E, Güler A, Bıyık S, et al. Carbonic anhydrase from *Apis mellifera*: purification and inhibition by pesticides. *J Enzyme Inhib Med Chem* 2017;32:47–50.