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

Marwa G. El-Gazzara, Nessma H. Nafiea, Alessio Nocentinib, Mostafa M. Ghoraba, Helmi I. Heibaa and Claudiu T. Supuranb, c

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

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\(^+\). In humans (h), 15 CA isoforms are known differing in tissue expression patterns, kinetic properties and subcellular localisation. 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. 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. 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. 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. Moreover, several 1,2,3-triazole containing compounds have proved considerable biological activities including antibacterial, antifungal and anticancer activities.

In view of these facts, and in continuation of an ongoing project aiming to develop new biologically active sulfonamide derivatives, 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). \(^1\)H NMR and 13C NMR spectra were recorded on a Varian Mercury Plus Oxford (300 MHz for \(^1\)H-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 ±0.4% of the theoretical.

CONTACT Marwa G. El-Gazzar marwagalalgazzar@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.
values. Purity of the compounds was checked by TLC on precoated 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, $^1$H NMR, 13C 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, 3130, 1566 (CN), 1610 (C=O), 137.48, 139.36, 150.62. MS, m/z (%) = 340(M$^+$). Anal. Calc. For C$_{16}$H$_{11}$ClN$_6$O$_2$S: C, 49.81; H, 3.01; N, 21.82.

4-(4-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, 1302 (SO$_2$). $^1$H-NMR (300 MHz, DMSO-d$_6$): $\delta$ 7.53 (d, 2H, Ar-H, $J = 8.8$ Hz), 6.85 (s, 2H, NH$_2$), 3.58 (s, 2H, CH$_2$), 7.69 (s, 2H, NH$_2$), D$_2$O exh). 7.89 (d, 2H, Ar-H, $J = 8.8$ Hz), 7.52 (d, 2H, Ar-H, $J = 8.8$ Hz). IR(cm$^{-1}$): 3337, 3187 (NH, NH$_3$), 3099 (CH aliph.), 2231 (CN), 1668 (C=O), 1345, 1112 (SO$_2$). $^1$H-NMR (300 MHz, DMSO-d$_6$): $\delta$ 4.26 (s, 2H, CH$_2$(CN)), 7.52 (d, 2H, Ar-H, $J = 8.6$ Hz), 7.69 (s, 2H, NH$_2$), D$_2$O exh). 7.90 (d, 2H, Ar-H, $J = 8.6$ Hz), 9.5 (s, 1H, NH, D$_2$O exh). 13C-NMR (75 MHz, DMSO-d$_6$): $\delta$ 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. Calc. For C$_{11}$H$_5$ClN$_6$O$_3$S: C, 48.83; H, 3.47; N, 21.73; Found: C, 48.81; H, 3.46; N, 21.77.

General procedure for the synthesis of compounds 8–10

(E)-4-(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.

2-chloro-N-(4-cyano-4-(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.

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 acetooacetate (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, 3245 (NH$_3$), 3088 (CH arom.), 2188 (CN), 1334, 1120 (SO$_2$). $^1$H-NMR (300 MHz, DMSO-d$_6$): $\delta$ 7.38–7.60 (m, 4H, Ar-H), 7.72 (s, 2H, NH$_2$), D$_2$O exh). 7.96 (d, 2H, Ar-H, $J = 8.4$ Hz), 7.99 (d, 2H, Ar-H, $J = 8.3$ Hz), 8.87 (s, 1H, CH). 13C-NMR (75 MHz, DMSO-d$_6$): $\delta$ 113.97, 118.12 (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. Calc. For C$_{11}$H$_5$ClN$_6$O$_3$S: 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), 3087 (CH amiph.), 2197 (C=N). 1H-NMR (300 MHz, DMSO-d₆): 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 (2H, NH₂, D₂O exch.), 7.96 (d, 2H, Ar-H, J = 8.4 Hz), 8.86 (s, 1H, CH). 13C-NMR (75 MHz, DMSO-d₆): δ 119.1 (2), 113.97, 128.55 (2), 129.26 (2), 129.45 (2), 134.64, 135.68, 137.42, 138.34, 151.50, 159.86. MS, m/z (%): 366 (M⁺). Anal. Calc. For C₁₈H₁₂ClN₇O₂S: C, 45.93; H, 3.37; N, 20.08.

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-1-yl)benzenesulfonamide 11. Yield = 78%; m.p.: >300°C. IR( cm⁻¹): 3319, 3231 (NH, NH₂), 3089 (CH amiph.), 2210 (CN), 1321, 1114 (SO₂). 1H-NMR (300 MHz, DMSO-d₆): δ 7.74 (d, 2H, Ar-H, J = 8.5 Hz), 7.49–7.68 (m, 5H, Ar-H), 7.14 (d, 2H, Ar-H, J = 8.5 Hz), 7.90 (s, 2H, NH₂, D₂O exch.), 8.21 (s, 1H, NH, D₂O exch.). 13C-NMR (75 MHz, DMSO-d₆): δ 119.17, 118.10 (2), 127.00 (2), 125.55 (2), 129.20 (2), 131.44, 135.14, 137.48, 138.24, 139.36, 150.62. MS, m/z (%): 404 (M⁺). Anal. Calc. For C₁₈H₁₃N₇O₂S: C, 44.77; H, 2.78; N, 20.78.

N-(4-cyano-1-(4-sulfamoylphenyl)-1H,1,2,3-triazol-1-yl)-4-methylbenzenesulfonamide 12. Yield = 89%; m.p.: >300°C. IR( cm⁻¹): 3338, 3231 (NH, NH₂), 3066 (CH amiph.), 2928, 2843 (CH aliph.), 2224 (CN), 1333, 1114 (SO₂). 1H-NMR (300 MHz, DMSO-d₆): δ 2.33 (s, 3H, CH₃), 7.32 (d, 2H, Ar-H, J = 8.1 Hz), 7.44 (d, 2H, Ar-H, J = 8.1 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, NH₂, D₂O exch.), 8.28 (s, 1H, NH, D₂O exch.). 13C-NMR (75 MHz, DMSO-d₆): 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. Calc. For C₁₆H₁₄N₇O₂S: C, 45.93; H, 3.37; N, 20.08. Found: C, 45.68; H, 2.91; N, 20.48.

(4-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, 2NH₂), 3080 (CH amiph.), 2210 (CN), 1698 (C = O), 1323, 1141 (SO₂). 1H-NMR (300 MHz, DMSO-d₆): δ 7.55 (d, 2H, Ar-H, J = 8.5 Hz), 7.71 (s, 2H, NH₂, D₂O exch.), 7.89 (d, 2H, Ar-H, J = 8.5 Hz), 8.02 (s, 2H, NH₂, D₂O exch.), 8.89 (s, 1H, NH, D₂O exch.). 13C-NMR (75 MHz, DMSO-d₆): δ 91.52, 110.02, 118.10 (2), 128.55 (2), 137.48, 138.24, 139.36, 150.62, 163.08, 163.15. MS, m/z (%): 331 (M⁺). Anal. Calc. For C₁₂H₁₂N₃O₂S: C, 43.50; H, 2.74; N, 29.59. Found: C, 43.71; H, 2.55; N, 29.38.
For C_{20}H_{18}N_{8}O_{2}S (434): C, 55.29; H, 4.18; N, 25.79; Found: C, 55.52; H, 3.89; N, 25.55.

2.3. Carbonic anhydrase inhibition

An Applied Photophysics stopped-flow instrument has been used for assaying the CA-catalysed CO₂ hydration activity. 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 mM 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 and represent the mean from at least three different determinations. All CA isoforms were recombinant ones obtained in-house as reported earlier.

### Table 1. Inhibition data of human CA isoforms

| Compound | hCA I | hCA II | hCA IV | hCA IX |
|----------|-------|--------|--------|--------|
| 250      | 564.8 | 789.7  | 2690.5 | 1178.1 |
| 750      | 5139.6| 4522.1 | 144.0  | 144.0  |
| 1000     | 1789.1| 789.7  | 2690.5 | 1178.1 |
| 1500     | 2545.5| 5139.6| 4522.1 | 144.0  |
| 2000     | 9938.3| 5139.6| 4522.1 | 144.0  |
| 2500     | 3435.1| 3360.0| 3292.0 | 3198.0 |
| 3000     | 1602.1| 4522.1| 144.0  | 144.0  |
| 3500     | 1789.1| 789.7  | 2690.5 | 1178.1 |
| 4000     | 2545.5| 5139.6| 4522.1 | 144.0  |
| 4500     | 9938.3| 5139.6| 4522.1 | 144.0  |
| 5000     | 3435.1| 3360.0| 3292.0 | 3198.0 |

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...
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 method\(^{19}\) and their obtained growth inhibition percent (GI\%) are presented in Table 2.

| Compound | Table 2: Percentage growth inhibition (GI\%) of in vitro human tumour cell lines at 10 \(\mu\)M concentration for ten compounds. |
|-----------|-------------------------------------------------------------------------------------------------------------------------------------|
| Panel/Cell line | 3 | 5 | 7 | 9 | 11 | 13 | 16 | 17 | 18 | 20 |
| **Leukaemia** | | | | | | | | | | |
| 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 |
| **Non-small cell lung cancer** | | | | | | | | | | |
| 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 | 9.0 | 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 |
| **Colon cancer** | | | | | | | | | | |
| 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 | – |
| **Ovarian cancer** | | | | | | | | | | |
| A498 | 13.6 | 8.6 | – | 8.6 | – | 0.8 | 17.6 | 1.8 | 5.5 | 6.2 |
| MDA-MB-435 | – | – | 3.6 | – | 1.2 | 2.7 | – | 0.4 | – | – |
| M14 | 2.7 | 4.7 | 2.6 | – | 3.3 | 1.6 | – | 3.5 | – | – |
| SK-MEL-2 | – | 0.4 | 8.3 | 3.4 | – | – | 2.9 | – | – | – |
| SK-MEL-2 | – | – | – | – | – | – | – | – | – | – |
| 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 |
| **Melanoma** | | | | | | | | | | |
| LOX IMVI | 6.1 | 5.5 | 5.9 | – | – | – | 5.5 | 20.4 | 9.6 | 7.5 |
| 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 | – | – | 0.4 | 8.3 | 3.4 | – | – | 2.9 | – | – |
| SK-MEL-2 | – | – | – | – | – | – | 2.3 | – | – | 8.2 |
| SK-MEL-28 | – | – | – | – | – | – | 0 | – | – | – |
| SR | 3.5 | 3.4 | 4.0 | 6.0 | 5.9 | 8.4 | 15.5 | 11.6 | 5.6 | 4.3 |
| **Breast cancer** | | | | | | | | | | |
| A498 | 13.6 | 8.6 | – | 8.6 | – | 0.8 | 17.6 | 1.8 | 5.5 | 6.2 |
| MDA-MB-231/ATCC | – | – | – | – | – | – | – | – | – | – |
| 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 | – | – |

**JOURNAL OF ENZYME INHIBITION AND MEDICINAL CHEMISTRY**
3. Results and discussion

3.1. Chemistry

Synthesis of the series of 1,2,3-triazolo-benzensulfonamide derivatives 3–21 begins with 4-azido benzenesulfonamide 3 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 afforded the imidazoline derivative 4 whose structure was confirmed by disappearance of the carbonotrile 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. 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.

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.
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 hCA IX by a stopped-flow CO2 hydrase assay in comparison to acetazolamide (AAZ) as standard CAI. 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).

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...
Designing of novel carbonic anhydrase inhibitors and activators. Curr Med Chem Cardiovasc Hematol Agents 2004;2:49–68.
6. Supuran CT, Altero 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.
7. 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 sensitiv-
yty to HDAC inhibition. Oncotarget 2015;6:19413–27.
8. (a) Supuran CT. How many carbonic anhydrase inhibition mechanisms exist? J Enzyme Inhib Med Chem 2016;31:345–60; (b) Altero V, Di Fiore A, D’Ambrosio K, Supuran CT, De Simone, G. Multiple binding modes of inhib-
itors 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 anhy-
drase 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 anhy-
drases from Bacteria: can bacterial carbonic anhydrases shed new light on evolution of bacteria? J Enzyme Inhib Med Chem 2015;30:325–32
9. (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 car-
bonic 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 anhy-
drases: iminodiacetyl-based hydroxamate-benzenesulfo-
amide conjugates. J Med Chem 2008;51:7968–79.
(a) Supuran CT. Carbonic anhydrase inhibition and the man-
gement 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 iso-
zymes 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 tar-
getting 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 inhibi-
tory action. Bioorg Chem 2018;77:25–37.
13. Lolak N, Akocak S, Bua S, et al. Design and synthesis of novel 1,3-diaryltriadiazine-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 z-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 Puşcaş (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;2:24; (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. Chipolina IC, Alves E, Branco P, et al. Synthesis and cytotoxic evaluation of 1H,1,2,3-triazol-1-ylmethyl-2,3-dihydropyrazot[h](1,2-b)furano-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, Janezcko 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 anti-cancer 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, Kutowska J, et al. Antibacterial and central nervous system activity of (4,5-diaryl-4H-1,2,4-triazol-3-YL)metacrylic acid derivatives. Acta Pol Pharm 2017;74:289–92.

23. Ghorab MM, Alsaied 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, Alsaied 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-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)thiourea]-N-(quinolin-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-(quinolin-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. Khalfah 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 isoforms 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, Bozdag M, Del Prete S, et al. Mono- and di-thiocarbamate inhibition studies of the δ-carbonic anhydrase TwaCAl from the marine diatom Thalassiosira weissflogii with amines and amino acids. J Enzyme Inhib Med Chem 2018;33:707–13.

34. (a) Díez JR, Fernández Baldo M, Echeverría G, et al. 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) Ménich 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;6:137–43; (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 sulphanamides 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 Türköğlu E, Şentürk M, Supuran CT, Ekinci D. Carbonic anhydrase inhibitory properties of some uracil derivatives. J Enzyme Inhib Med Chem 2017;32:74–7; (b) Soydan E, Gülér A, Byik S, et al. Carbonic anhydrase from Apis mellifera: purification and inhibition by pesticides. J Enzyme Inhib Med Chem 2017;32:47–50.