Journal of Enzyme Inhibition and Medicinal Chemistry

ISSN: 1475-6366 (Print) 1475-6374 (Online) Journal homepage: http://www.tandfonline.com/loi/ienz20

Isoform-selective inhibitory profile of 2-imidazoline-substituted benzene sulfonamides against a panel of human carbonic anhydrases

Claudiu T. Supuran, Stanislav Kalinin, Muhammet Tanç, Pakornwit Sarnpitak, Prashant Mujumdar, Sally-Ann Poulsen & Mikhail Krasavin

To cite this article: Claudiu T. Supuran, Stanislav Kalinin, Muhammet Tanç, Pakornwit Sarnpitak, Prashant Mujumdar, Sally-Ann Poulsen & Mikhail Krasavin (2016) Isoform-selective inhibitory profile of 2-imidazoline-substituted benzene sulfonamides against a panel of human carbonic anhydrases, Journal of Enzyme Inhibition and Medicinal Chemistry, 31:sup1, 197-202, DOI: 10.1080/14756366.2016.1178248

To link to this article: https://doi.org/10.1080/14756366.2016.1178248

Journal of Enzyme Inhibition and Medicinal Chemistry

www.tandfonline.com/ienz
ISSN: 1475-6366 (print), 1475-6374 (electronic)

J Enzyme Inhib Med Chem, 2016; 31(S1): 197–202
 DOI: 10.1080/14756366.2016.1178248

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

Isoform-selective inhibitory profile of 2-imidazoline-substituted benzene sulfonamides against a panel of human carbonic anhydrases

Claudiu T. Supuran[1], Stanislav Kalinin[2], Muhammet Tanç[1], Pakornwit Sarnpitak[3], Prashant Mujumdar[3], Sally-Ann Poulsen[3], and Mikhail Krasavin[4]

[1]Neurofarba Department, Universita Degli Studi Di Firenze, Florence, Italy, [2]The Ushinsky Yaroslavl State Pedagogical University, Yaroslavl, Russian Federation, [3]Eskitis Institute for Drug Discovery, Griffith University, Brisbane, Queensland, Australia, and [4]Institutes of Chemistry and Translational Biomedicine, Saint Petersburg State University, Saint Petersburg, Russian Federation

Abstract

A series of novel benzene sulfonamides (previously evaluated as selective cyclooxygenase-2 inhibitors) has been profiled against human carbonic anhydrases I, II, IV and VII in an attempt to observe the manifestation of the well established "tail" approach for designing potent, isoform-selective inhibitors of carbonic anhydrases (CAs, EC 4.2.1.1). The compounds displayed an excellent (pKi 7–8) inhibitory profile against CA II (a cytosolic anti-glaucoma and anti-edema biological target) and CA VII (also a cytosolic target believed to be involved in epilepsy and neuropathic pain) and a marked (1–2 orders of magnitude) selectivity against cytosolic isoform CA I and membrane-bound isoform CA IV. The separation of the CA II and CA IV (both of which are catalytically active isoforms, highly sensitive to sulfonamide-type inhibitors) is particularly remarkable and is adding significantly to the global body of data on the chemical biology of carbonic anhydrases.

Keywords

Carbonic anhydrases, cyclooxygenase-2 inhibitors, dual active site architecture, 2-imidazolines, isoform selectivity, lipophilic appendages, tail approach

History

Received 24 March 2016
Revised 9 April 2016
Accepted 11 April 2016
Published online 3 May 2016

Introduction

The goal of developing isoform-selective inhibitors of human carbonic anhydrases (*h*CA) has stimulated extensive medicinal chemistry activity worldwide[1]. Selective inhibition of a particular isoform (in contrast to nonselective carbonic anhydrase inhibitors (CAIs), such as acetazolamide[2]) is likely to improve therapeutic benefit over side-effects and toxicity for a well-established *h*CA-disease relationship (e.g. *h*CA II for glaucoma)[3]. Enhancing the selectivity of CAIs may even validate CAs in fundamentally new therapeutic areas. For instance, selective targeting of membrane-bound isoforms *h*CA IX and XII has been proposed as a novel approach to halt the growth of solid tumors by suppressing tumor survival mechanism in hypoxic environment[4]. A significant body of pre-clinical proof-of-concept data[5] as well as one front-runner compound (SLC-0111) in clinical trials for cancer[6] offers much hope that selective *h*CA isoform targeting will receive clinical validation in the future.

A well-established, rational approach to designing isoform-selective CAIs exists in perhaps the most investigated class of inhibitors, namely, benzenesulfonamides. Anchoring of the sulfonamide group in this class of compounds to the prosthetic zinc ion defines the position of the organic portion of the molecule within the CA binding cavity[7] and offers significant opportunity to build, somewhat serendipitously, additional interactions with the protein environment where two distinct parts have been delineated[8]. By grafting appendages of various nature (hydrophilic and lipophilic alike) onto the benzenesulfonamide scaffold, it has become possible to impart significant potency and selectivity to the resulting CAIs. The effectiveness of building contacts with the lipophilic side of the CA active site has been shown to correlate with the potency of the CAIs (exemplified by compounds **1**–**3**)[9]. A well-designed and informative comparison of hydrophilic versus lipophilic (as well as mixed) appendage design by the so-called ''tail approach''[10] (illustrated by compounds **4**–**6**) has been recently published by Poulsen and colleagues[11].

Inspired by these findings, we became interested in assessing CAI profile of a series of benzene sulfonamides decorated with 2-aryl-2-imidazoline appendages (**7**). These compounds have been recently described in the context of selective cyclooxygenase-2 inhibition[12]. We were interested to see if these compounds (somewhat akin to ureido compounds **1**–**3**)[9] would be effective as CAIs in principle (Figure 1). Furthermore, assessing their isoform selectivity profile against CAs with COX-2 inhibition data[12] would offer an opportunity to consider developing dual-action therapeutic agents (e.g. endowed with antiglaucoma and anti-inflammatory activity manifested via inhibition of CAs and COX-2, respectively)[13]. This article describes the details of our findings in this area.

Address for correspondence: Claudiu T. Supuran, Neurofarba Department, Universita Degli Studi Di Firenze, Florence, Italy. E-mail: claudiu.supuran@unifi.it; Mikhail Krasavin, Institutes of Chemistry and Translational Biomedicine, Saint Petersburg State University, Saint Petersburg 199034, Russia. E-mail: m.krasavin@spbu.ru

Materials and methods

Chemical syntheses – general

All chemicals and solvents were used as received from the suppliers. The crude reaction mixtures were concentrated under

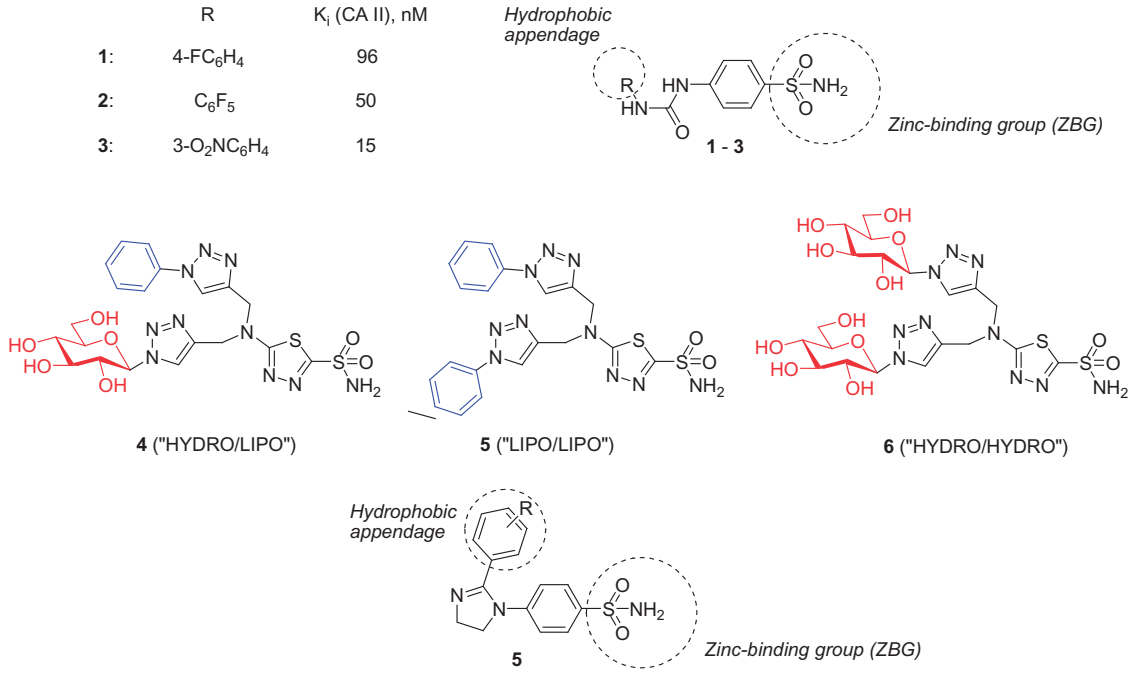


Figure 1. Structures of benzenesulfonamide CAIs containing hydrophobic ureido tails (**1**–**3**), the ''dual-tail'' compounds (**4**–**6**) and the design rationale for 2-imidazoline-substituted compounds **7**.

reduced pressure by removing organic solvents on rotary evaporator. Column chromatography was performed using silica gel 60 (particle size 0.040–0.063 mm, 230–400 mesh ASTM). Analytical thin-layer chromatography (TLC) was performed with silica gel 60 F_{254} aluminum sheets. Proton and carbon-13 nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were recorded on a Varian Unity INOVA 500 MHz spectrometer (NMR Laboratory, Urbana, IL). Chemical shifts for nuclear magnetic resonance (NMR) spectra were reported in parts per million (ppm, δ) using the residual solvent peak(s) as internal standard. Splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br) and doublets of doublet (dd). High-resolution mass spectra (HRMS) were acquired using a Bruker Daltonics Fourier Transform Ion Cyclotron Resonance Spectrometer (Bruker Daltonics Inc., Billerica, MA) fitted with an electrospray source operating in positive ion mode.

*General procedure 1 for preparation of 2-imidazolines **6a–o**[14]*

Ethylenediamine (10.5 mmol, 1.05 equiv.) was added to a stirred solution of aldehyde (10.0 mmol, 1.0 equiv.) in CH_2Cl_2 (50 mL) at 0 °C, and the resulting solution stirred for 20 min. *N*-bromosuccinimide (10.5 mmol, 1.05 equiv.) was then added in portions over 10 min. The reaction mixture was warmed to room temperature and stirred for an additional 12 h, during which time a precipitate formed. A solution of KOH (1.0 M, 50 mL, 50 mmol, 5.0 equiv.) was added causing the precipitate to disappear and a clear biphasic solution was formed. The organic layer was separated, dried over anhydrous $MgSO_4$, filtered, and concentrated at reduced pressure to afford the crude product. The analytically pure imidazolines **6** were obtained by crystallization from EtOAc/hexane. All imidazolines **6a–o** used in subsequent Pd-catalyzed *N*-arylation are known compounds[12].

*General procedure 2 for preparation of compounds **5a–o**[12]*

A thick-glass screw-capped pressure tube (50 mL) was charged with a suspension of starting imidazoline (3.0 mmol), 4-bromo-*N,N-bis*(2,4-dimethoxybenzyl)benzenesulfonamide[15] (3.0 mmol), Cs_2CO_3 (3.0 mmol), and toluene (15 mL). $Pd(OAc)_2$ (0.12 mmol) and BINAP (0.24 mmol) were weighed as solids into a 10 mL screw-capped vial and toluene (8 mL) was added. The resulting suspension was heated at 110 °C until a clear purple solution was evident. The solution was rapidly transferred, while still hot, to the screw-capped pressure tube using a Pasteur pipette. The reaction vessel was filled with argon, sealed, and the reaction mixture vigorously stirred at 110 °C for 20 h. The reaction mixture was cooled to ambient temperature and filtered through a Celite pad (Beaver Chemicals, Burlington Ltd., Ontario, Canada), washed with copious amounts of EtOAc, and the combined filtrate and washings evaporated to dryness. The crude material was fractionated on silica (CH_2Cl_2:MeOH, 95:5) and the fractions containing the *N*-aryl 2-imidazolines were collected and evaporated to dryness. The residue was taken forward to the next step without further purification.

TFA (3 mL) was added drop wise to a solution of DMB-protected sulfonamides (obtained as described above) in CH_2Cl_2 (10 mL) at 0 °C and stirring continued at the same temperature for 30–60 min. The excess TFA was neutralized with sat. aq. $NaHCO_3$, the organic layer separated and dried over anhydrous $MgSO_4$. The solution was filtered and concentrated *in vacuo*. The resulting crude product was purified by column chromatography on silica using an appropriate gradient of MeOH in CH_2Cl_2 as eluent to provide sulfonamide products **5a–o**.

*4-(2-(4-Fluorophenyl)-4,5-dihydroimidazol-1-yl)benzenesulfonamide (**5a**).* Prepared according to General Procedure 2 from 2-(4-fluorophenyl)-4,5-dihydro-1*H*-imidazole. Yield 700 mg (73%), light yellow solid, m. p. 94–96 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 (d, $J = 8.8$ Hz, 2 H), 7.50–7.45 (m, 2 H), 7.07–7.02 (m, 2 H), 6.75 (d, $J = 8.8$ Hz, 2 H), 4.91 (br s, 2 H), 4.15–4.03 (m, 4 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.9 (d, $J_{C\text{-}F} = 251.4$ Hz), 160.1, 146.2, 134.9, 130.6 (d, $J_{C\text{-}F} = 8.6$ Hz), 127.4, 126.8 (d, $J_{C\text{-}F} = 3.4$ Hz), 120.5, 115.8 (d, $J_{C\text{-}F} = 21.9$ Hz), 53.13, 53.11; HRMS *m/z* calcd for $C_{15}H_{15}FN_3O_2S$ $(M + H)^+$ 320.0864 found 320.0860.

*4-(2-(4-Fluorophenyl)-4-methyl-4,5-dihydroimidazol-1-yl)benzenesulfonamide (**5b**).* Prepared according to General Procedure 2 from 2-(4-fluorophenyl)-4-methyl-4,5-dihydro-1*H*-imidazole. Yield 360 mg (36%), yellow solid, m. p. 157–159 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.86–7.82 (m, 2 H), 7.57–7.53 (m, 2 H), 7.26–7.19 (m, 4 H), 4.63–4.53 (m 2 H), 4.14–4.06 (m 1 H), 1.56–1.51 (m, 3 H), NH_2 protons in exchange. ^{13}C NMR (125 MHz, CD_3OD) δ 166.7 (d, $J_{C\text{-}F} = 254.0$ Hz), 164.2, 143.3, 142.8, 133.1 (d, $J_{C\text{-}F} = 9.4$ Hz), 128.6, 125.5, 117.5 (d, $J_{C\text{-}F} = 22.6$ Hz), 61.3, 55.8, 20.9. HRMS *m/z* calcd for $C_{16}H_{17}FN_3O_2S$ $(M+H)^+$ 334.1020 found 334.1016.

*4-(2-(4-Chlorophenyl)-4,5-dihydroimidazol-1-yl)benzenesulfonamide (**5c**).* Prepared according to General Procedure 2 from 2-(4-chlorophenyl)-4,5-dihydro-1*H*-imidazole. Yield 663 mg (66%), yellow solid, m. p. 141–143 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.81 (d, $J = 8.8$ Hz, 2 H), 7.54–7.46 (m, 4 H), 7.08 (d, $J = 8.8$ Hz, 2 H), 4.36 (t, $J = 9.9$ Hz, 2 H), 4.14 (t, $J = 9.9$ Hz, 2 H), NH_2 protons in exchange; ^{13}C NMR (125 MHz, CD_3OD) δ 163.9, 145.0, 140.7, 1387, 131.5, 130.1, 128.4, 128.3, 123.7, 54.3, 51.1; HRMS *m/z* calcd for $C_{15}H_{15}ClN_3O_2S$ $(M+H)^+$ 336.0568 found 336.0564.

*4-(2-(4-Chlorophenyl)-4-methyl-4,5-dihydroimidazol-1-yl)benzenesulfonamide (**5d**).* Prepared according to General Procedure 2 from 2-(4-chlorophenyl)-4-methyl-4,5-dihydro-1*H*-imidazole. Yield 534 mg (51%), light brown solid, m. p. 160–162 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.85 (d, $J = 8.7$ Hz, 2 H), 7.53–7.47 (m, 4 H), 7.26 (d, $J = 8.7$ Hz, 2 H), 4.67–4.58 (m, 2 H), 4.17–4.09 (m, 1 H), 1.58–1.50 (m, 3 H), NH_2 protons in exchange; ^{13}C NMR (125 MHz, CD_3OD) δ 164.3, 143.5, 142.5, 140.5, 132.0, 130.6, 128.6, 125.7, 124.5, 61.3, 55.6, 20.8; HRMS *m/z* calcd for $C_{16}H_{17}ClN_3O_2S$ $(M+H)^+$ 350.0724 found 350.0719.

*4-(2-(3-Bromophenyl)-4,5-dihydroimidazol-1-yl)benzenesulfonamide (**5e**).* Prepared according to General Procedure 2 from 2-(3-bromophenyl)-4,5-dihydro-1*H*-imidazole. Yield 409 mg (36%), white solid, m. p. 194–194 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.73 (d, $J = 8.8$ Hz, 2 H), 7.68–7.64 (m, 2 H), 7.40–7.36 (m, 1 H), 7.31 (t, $J = 7.8$ Hz, 1 H), 6.98 (d, $J = 8.8$ Hz, 2 H), 4.20 (td, $J = 9.3$, 1.2 Hz, 2 H), 4.05 (td, $J = 9.3$, 1.2 Hz, 2 H), NH_2 protons in exchange; ^{13}C NMR (125 MHz, CD_3OD) δ 162.6, 146.2, 139.4, 134.8, 134.0, 132.3, 131.5, 128.5, 128.1, 123.5, 122.6, 54.1, 53.3; HRMS *m/z* calcd for $C_{15}H_{15}BrN_3O_2S$ $(M+H)^+$ 380.0063 found 380.0057.

*4-(2-(3-Bromophenyl)-4-methyl-4,5-dihydroimidazol-1-yl)benzenesulfonamide (**5f**).* Prepared according to General Procedure 2 from 2-(3-bromophenyl)-4-methyl-4,5-dihydro-1*H*-imidazole. Yield 755 mg (64%), white solid, m. p. 180 °C (decomp.); ^{1}H NMR (500 MHz, CD_3OD) δ 7.91 (d, $J = 8.7$ Hz, 2 H), 7.86–7.83 (m, 1 H), 7.81–7.78 (m, 1 H), 7.48–7.37 (m, 4 H), 4.75–4.68 (m, 2 H), 4.32–4.24 (m, 1 H), 1.63–1.60 (m, 3 H), NH_2 protons in exchange; ^{13}C NMR (125 MHz, CD_3OD) δ 164.7, 145.1, 141.0, 138.0, 133.2, 132.2, 129.3, 128.8, 126.7, 125.9, 124.0, 51.5, 54.0, 20.4; HRMS *m/z* calcd for $C_{16}H_{17}BrN_3O_2S$ $(M+H)^+$ 394.0219 found 394.0214.

*4-(2-(3-Fluorophenyl)-4,5-dihydroimidazol-1-yl)benzenesulfonamide (**5g**).* Prepared according to General Procedure 2 from 2–(3-fluorophenyl)-4,5-dihydro-1*H*-imidazole. Yield 584 mg (61%), white solid, m. p. 225–227 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.77–7.75 (m, 2 H), 7.48–7.44 (m, 1 H), 7.29 (t, $J = 7.4$ Hz, 2 H), 7.25 (d, $J = 8.0$ Hz, 1 H), 6.97–6.96 (m, 2 H), 4.24 (t, $J = 9.6$ Hz, 2 H), 4.09 (t, $J = 9.6$ Hz, 2 H), NH_2 protons in exchange; ^{13}C NMR (125 MHz, CD_3OD) δ 164.7 (d, $J_{C\text{-}F} = 244.6$ Hz), 163.6, 147.1, 140.2, 134.9 (d, $J_{C\text{-}F} = 7.8$ Hz), 132.6 (d, $J_{C\text{-}F} = 8.1$ Hz), 128.9, 126.5 (d, $J_{C\text{-}F} = 3.2$ Hz), 123.5, 117.4 (d, $J_{C\text{-}F} = 23.1$ Hz), 119.4 (d, $J_{C\text{-}F} = 21.1$ Hz), 54.9, 54.2; HRMS *m/z* calcd for $C_{15}H_{15}FN_3O_2S$ $(M+H)^+$ 320.0864 found 320.0854.

*4-(2-(3-Chlorophenyl)-4,5-dihydroimidazol-1-yl)benzenesulfonamide (**5h**).* Prepared according to General Procedure 2 from 2-(3-chlorophenyl)-4,5-dihydro-1*H*-imidazole. Yield 794 mg (79%), white solid, m. p. 188–190 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.78–7.76 (m, 2 H), 7.54–7.56 (m, 2 H), 7.44–7.38 (m, 2 H), 6.98–6.96 (m, 2 H), 4.25 (t, $J = 9.4$ Hz, 2 H), 4.10 (t, $J = 9.7$ Hz, 2 H), NH_2 protons in exchange; ^{13}C NMR (125 MHz, CD_3OD) δ 163.5, 147.1, 140.2, 136.5, 134.7, 132.6, 132.2, 130.5, 129.0, 128.9, 123.5, 55.0, 54.2; HRMS *m/z* calcd for $C_{15}H_{15}ClN_3O_2S$ $(M+H)^+$ 336.0568 found 336.0560.

*4-(2-m-Tolyl-4,5-dihydroimidazol-1-yl)benzenesulfonamide (**5i**).* Prepared according to General Procedure 2 from 2-(*m*-tolyl)-4,5-dihydro-1*H*-imidazole. Yield 350 mg (37%), cream solid, m. p. 244–246 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.73 (d, $J = 8.8$ Hz, 2 H), 7.37–7.26 (m, 3 H), 7.22–7.18 (m, 1 H), 6.99 (d, $J = 8.8$ Hz, 2 H), 4.28 (t, $J = 10.3$ Hz, 2 H), 4.07 (t, $J = 10.3$ Hz, 2 H), 2.33 (s, 3H), NH_2 protons in exchange; ^{13}C NMR (125 MHz, CD_3OD) δ 164.7, 145.5, 140.1, 140.0, 133.1, 130.3, 130.2, 129.8, 128.1, 126.9, 123.1, 54.1, 51.4, 21.3; HRMS *m/z* calcd for $C_{16}H_{18}N_3O_2S$ $(M+H)^+$ 316.1114 found 316.1107.

*4-(2-(3-Fluoro-4-methoxyphenyl)-4,5-dihydroimidazol-1-yl)benzenesulfonamide (**5j**).* Prepared according to General Procedure 2 from 2-(3-fluoro-4-methoxyphenyl)-4,5-dihydro-1*H*-imidazole. Yield 712 mg (68%), yellow solid, m. p. 195–197 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.78–7.76 (m, 2 H), 7.25 (t, $J = 7.3$ Hz, 2 H), 7.14 (t, $J = 7.6$ Hz, 1 H), 7.00–6.98 (m, 2 H), 4.22 (t, $J = 9.6$ Hz, 2 H), 4.06 (t, $J = 9.6$ Hz, 2 H), 3.95 (s, 3H), NH_2 protons in exchange; ^{13}C NMR (125 MHz, CD_3OD) δ 163.6, 153.9 (d, $J_{C\text{-}F} = 245.3$ Hz), 152.0 (d, $J_{C\text{-}F} = 10.6$ Hz), 147.5, 140.1, 128.9, 127.4 (d, $J_{C\text{-}F} = 3.5$ Hz), 124.8 (d, $J_{C\text{-}F} = 7.0$ Hz), 123.7, 118.1 (d, $J_{C\text{-}F} = 20.0$ Hz), 115.3, 57.6, 55.1, 53.8; HRMS *m/z* calcd for $C_{16}H_{17}FN_3O_3S$ $(M+H)^+$ 350.0969 found 350.0960.

*4-(2-(4-Fluoro-3-methoxyphenyl)-4,5-dihydroimidazol-1-yl)benzenesulfonamide (**5k**).* Prepared according to General Procedure 2 from 2-(4-fluoro-3-methoxyphenyl)-4,5-dihydro-1*H*-imidazole. Yield 691 mg (66%), white solid, m. p. 248–249 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.79–7.76 (m, 2 H), 7.24 (dd, $J = 8.1$, 1.8 Hz, 1 H), 7.14 (dd, $J = 13.7$, 8.3 Hz, 1 H), 7.01–7.04 (m, 1 H), 7.00 (d, $J = 8.8$ Hz, 2 H), 4.25 (t, $J = 9.4$ Hz, 2 H), 4.09 (t, $J = 10.3$ Hz, 2 H), 3.83 (s, 3 H), NH_2 protons in exchange; ^{13}C NMR (125 MHz, CD_3OD) δ 164.1, 155.8 (d, $J_{C\text{-}F} = 249.1$ Hz), 150.0 (d, $J_{C\text{-}F} = 11.3$ Hz), 147.3, 140.1, 130.7, 128.8, 123.7, 123.6, 118.0 (d, $J_{C\text{-}F} = 19.0$ Hz), 116.2 (d, $J_{C\text{-}F} = 1.8$ Hz), 57.6, 54.9, 53.8; HRMS *m/z* calcd for $C_{16}H_{17}FN_3O_3S$ $(M+H)^+$ 350.09693 found 350.0960.

*4-(2-(3-Chloro-4-methoxyphenyl)-4,5-dihydroimidazol-1-yl)benzenesulfonamide (**5l**).* Prepared according to General Procedure 2 from 2-(3-chloro-4-methoxyphenyl)-4,5-dihydro-1*H*-imidazole. Yield 712 mg (63%), white solid, m. p. 255–257 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.78 (d, $J = 8.6$ Hz, 2 H), 7.55 (s, 1 H), 7.38 (d, $J = 8.6$ Hz, 1 H), 7.11 (d, $J = 8.4$ Hz, 1 H), 6.99 (d, $J = 8.6$ Hz, 2 H), 4.23 (t, $J = 9.6$ Hz, 2 H), 4.06 (t, $J = 9.2$ Hz, 2 H), 3.97 (s, 3 H), NH_2 protons in exchange; ^{13}C NMR (125 MHz, CD_3OD) δ 163.5, 159.3, 147.5, 140.1, 132.3, 130.8, 129.0, 125.4, 124.6, 123.6, 114.0, 57.7, 55.1, 53.8; HRMS *m/z* calcd for $C_{16}H_{17}ClN_3O_3S$ $(M+H)^+$ 366.0674 found 366.0664.

*4-(2-(3,4-Difluorophenyl)-4,5-dihydroimidazol-1-yl)benzenesulfonamide (**5m**).* Prepared according to General Procedure 2 (alternatively, the recently reported microwave-assisted protocol was used from 2-(3,4-difluorophenyl)-4,5-dihydro-1*H*-imidazole. Yield 465 mg (46%), white solid, m. p. 177–179 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.78 (d, $J = 7.9$ Hz, 2 H), 7.46–7.44 (dd, $J = 10.6$, 8.9 Hz, 1 H), 7.38–7.30 (m, 2 H), 6.98 (d, $J = 8.0$ Hz, 2 H), 4.24 (t, $J = 9.8$ Hz, 2 H), 4.09 (t, $J = 9.7$ Hz, 2 H), NH_2 protons in exchange; ^{13}C NMR (125 MHz, CD_3OD) δ 162.9, 153.8 (dd, $J_{C\text{-}F} = 251.8$, 12.5 Hz), 152.2 (dd, $J_{C\text{-}F} = 248.6$, 12.8 Hz), 147.1, 140.3, 129.8 (dd, $J_{C\text{-}F} = 6.3$, 4.2 Hz), 129.0, 127.7 (dd, $J_{C\text{-}F} = 6.7$, 3.7 Hz), 123.7, 119.9 (d, $J_{C\text{-}F} = 19.0$ Hz), 119.7 (d, $J_{C\text{-}F} = 18.0$ Hz), 55.1, 54.2; HRMS *m/z* calcd for $C_{15}H_{14}F_2N_3O_2S$ $(M + H)^+$ 338.0769 found 338.0760. Alternatively, the recently reported microwave-assisted protocol[15] was used to obtain the same compound in 68% yield.

*4-(2-(3,4-Dichlorophenyl)-4,5-dihydroimidazol-1-yl)benzenesulfonamide (**5n**).* Prepared according to General Procedure 2 from 2-(3,4-dichlorophenyl)-4,5-dihydro-1*H*-imidazole. Yield 886 mg (80%), white solid, m. p. 232–234 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.79–7.78 (m, 2 H), 7.70 (s, 1 H), 7.59–7.58 (m, 1 H), 7.36–7.38 (m, 1 H), 6.99–6.98 (m, 2 H), 4.29–4.19 (m, 2 H), 4.15–4.03 (m, 2 H), NH_2 protons in exchange; ^{13}C NMR (125 MHz, CD_3OD) δ 155.8, 147.0, 140.5, 136.7, 134.7, 133.0, 132.8, 132.6, 130.3, 129.1, 123.7, 55.2, 54.3; HRMS *m/z* calcd for $C_{15}H_{14}Cl_2N_3O_2S$ $(M + H)^+$ 370.0178 found 370.0169.

*4-(2-(4-Chloro-3-(trifluoromethyl)phenyl)-4,5-dihydroimidazol-1-yl)benzenesulfonamide (**5o**).* Prepared according to General Procedure 2 from 2-[4-chloro-3-(trifluoromethyl)phenyl]-4,5-dihydro-1*H*-imidazole. Yield 786 mg (65%), white solid, m. p. 215–217 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.98 (s, 1 H), 7.79 (d, $J = 8.6$ Hz, 2 H), 7.66–7.58 (m, 2 H), 7.00 (d, $J = 8.6$ Hz, 2 H), 4.27 (t, $J = 9.7$ Hz, 2 H), 4.12 (t, $J = 9.6$ Hz, 2 H), NH_2 protons in exchange; ^{13}C NMR (125 MHz, CD_3OD) δ 162.7, 147.0, 140.7, 136.2 (unresolved q, $J_{C\text{-}F} = 1.9$ Hz), 135.5, 133.9, 132.1, 130.5 (q, $J_{C\text{-}F} = 31.8$ Hz), 130.0 (q, $J_{C\text{-}F} = 5.1$ Hz), 129.1, 124.7 (q, $J_{C\text{-}F} = 271.1$ Hz), 123.9, 55.2, 54.4; HRMS *m/z* calcd for $C_{16}H_{14}ClF_3N_3O_2S$ $(M + H)^+$ 404.0442 found 404.0431.

Carbonic anhydrase inhibition assay

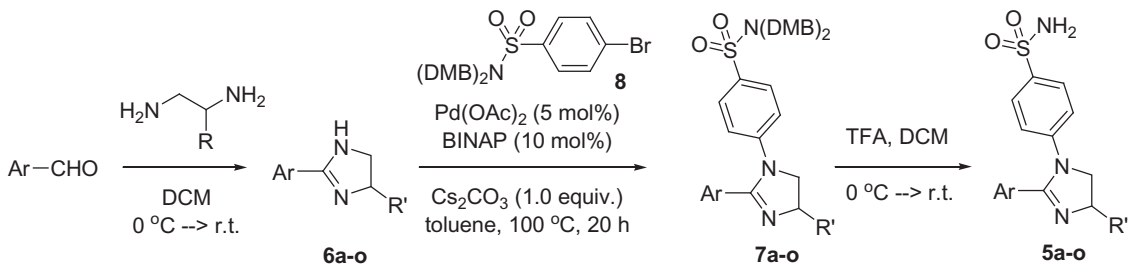
An Applied Photophysics Stopped-Flow instrument (Applied Photophysics Ltd., Leatherhead, Surrey, UK) has been used for assaying the CA catalyzed CO_2 hydration activity[16]. Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Tris (pH 8.3) as buffer, and 20 mM Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10–100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled-deionized water and dilutions up to 0.005 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, and represent the mean from at least three different determinations. All CA isoforms were recombinant ones obtained in-house[17–20].

Results and discussion

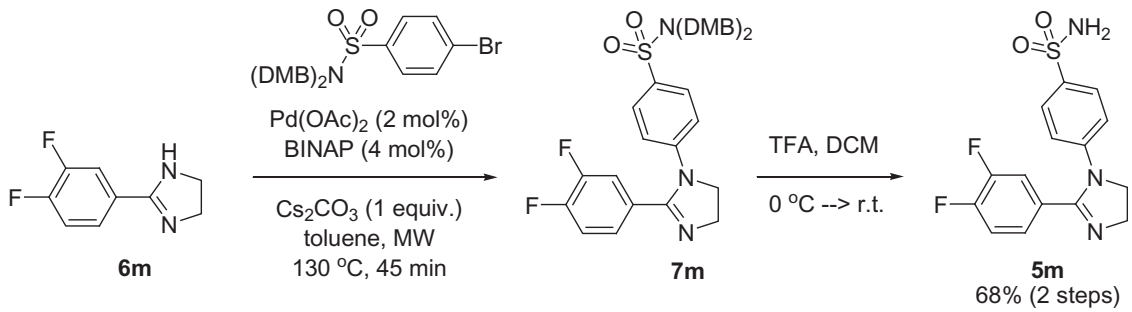
Compounds **5a–o** were synthesized as outlined in Scheme 1[12]. Imidazolines **6** were prepared by condensation of a series of benzaldehydes with ethylenediamine or 1,2-diaminopropane using a well-established protocol[13]. Pd-Catalyzed *N*-arylation of **6a–o** with **8** proved facile and, without further purification, the DMB (2,4-dimethoxybenzyl) protecting group from **7a–o** was removed with TFA and the target sulfonamides were obtained in fair to good yields (see Supplementary material).

Following our earlier reported synthesis of compounds **5a–o** and their evaluation as selective COX-2 inhibitors[12], we have since developed an operationally convenient protocol[21] for microwave-assisted, Pd-catalyzed *N*-arylation of 2-imidazolines which significantly shortened reaction times and, occasionally, improved isolated product yields. In this work, we demonstrated the same microwave-promoted procedure to be applicable to the synthesis of a representative compound (**5m**) and to lead to a noticeable improvement of the isolated yield (Scheme 2).

When tested for CA I, II, IV and VII inhibition, compounds **5a–o**, reassuringly, displayed an interesting inhibition profile (Table 1), which is likely a result of the sulfonamide moiety acting as a zinc-binding group (ZBG)[22]. A double-digit nanomolar inhibition was reliably achieved for CA II, which is a well-established biological target for anti-glaucoma and anti-edema drugs[2]. The same level of activity was also achieved against CA VII, while the lower activity against CA I is perhaps not very unusual considering the generally lower inhibitory potency of sulfonamides toward this isoform[1]. What constitutes an especially valuable finding in this work was, in our opinion, the marked selectivity (SI) between CA II and CA IV, which are both highly catalytically active CA isoforms and known to be very sensitive to sulfonamides. While the structural understanding of the observed separation of the CA II versus IV activity remains to be gained via crystallographic studies, the body of biological data found here contributes quite substantially to the global knowledge about isoform-selective CAIs.

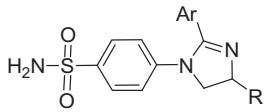


Scheme 1. Synthesis of 2-imidazoline-substituted benzene sulfonamides **5a–o**.



Scheme 2. Microwave-assisted synthesis of **5m**.

Table 1. Inhibitory activity data for compound **5a–o** obtained against *h*CA I, II, IV and VII.



Compound	Ar	R	K_i, nM				SI (*h*CAIV/*h*CAII)
			*h*CA I	*h*CA II	*h*CA IV	*h*CA VII	
5a	4-FC_6H_4	H	2 343.0	76.1	9 726.0	303.5	127.8
5b	4-FC_6H_4	Me	816.5	52.4	8 705.0	86.6	166.1
5c	4-ClC_6H_4	H	960.5	46.7	8 142.0	269.0	174.3
5d	4-ClC_6H_4	Me	897.9	54.1	6 785.0	69.6	125.4
5e	3-BrC_6H_4	H	1 844.0	53.0	5 010.0	204.7	94.5
5f	3-BrC_6H_4	Me	1 281.0	55.3	5 272.0	45.8	95.3
5g	3-FC_6H_4	H	3 230.0	75.9	>10 000	311.6	131.8
5h	3-ClC_6H_4	H	1 668.0	185.1	5 701.0	204.2	30.8
5i	3-MeC_6H_4	H	3 943.0	111.9	9 778.0	906.7	87.4
5j	3-F,4-$MeOC_6H_3$	H	1 231.0	80.7	8 266.0	709.4	102.4
5k	4-F,3-$MeOC_6H_3$	H	2 166.0	61.6	4 228.0	258.6	68.6
5l	3-Cl,4-$MeOC_6H_3$	H	3 987.0	227.1	>10 000	103.9	44.0
5m	3,4-diFC_6H_3	H	746.8	34.6	5 381.0	71.3	155.5
5n	3,4-diClC_6H_3	H	787.7	30.2	932.0	35.5	30.9
5o	4-Cl,3-$CF_3C_6H_3$	H	3 685.0	84.3	3 845.0	32.6	45.6
Acetazolamide (AAZ)			250.0	12.0	74.0	2.5	6.2

Currently, the absence of the crystallographic information on the potent 2-imidazoline CAIs described herein does not allow drawing substantiated conclusions on the role of the 2-aryl-2-imidazoline tails grafted onto benzenesulfonamide pharmacophore. However, in our view, it appears unlikely that the polar 2-imidazoline moiety participates in active interactions with the protein target. It is rather the lipophilic aromatic tail responsible for interacting with the hydrophobic half of the CA binding side which is likely to result in the observed potency and selectivity. Should the polar 2-imidazoline core be contributing significantly (e.g. by building hydrogen-bonding interactions with the hydrophilic half of the binding site), the introduction of a methyl substituent on the 2-imidazoline scaffold (as in **5b**, **5d** and **5f**) would be expected to disrupt this vital interaction. However, such a modification only seems to improve the CA VII affinity and virtually does not affect the CA I, II and IV inhibitory profile. This strongly argues for 2-imidazoline being a generally indifferent element of the periphery, responsible for a correct projection of the lipophilic periphery toward the target rather than building any specific interactions with the latter, in a direct analogy to 4-ureido benzenesulfonamides **1**–**3** (*vide supra*). The analogy is illustrated (Figure 2) quite well by the effective spatial superposition (in energy-minimized conformations) of compound **3**, a potent CA II inhibitor ($K_i = 96$ nM) with compound **5a** which inhibits CA II with roughly the same potency ($K_i = 76$ nM).

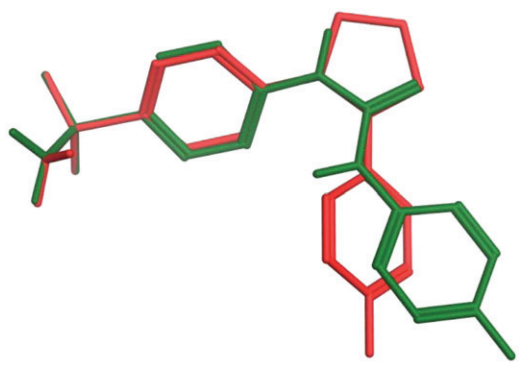


Figure 2. Spatial alignment of compound **5a** (green) with its ureido analog **3** (red).

Interestingly, compound **5n** which we had earlier identified as the lead selective COX-2 inhibitor[12], turned out to be a rather potent CA II and CA VII inhibitor which confirms the potential of using this series as a dual CA/COX-2 inhibitors.

Conclusion

The polypharmacology of primary aromatic sulfonamides prompted us to evaluate the profile of compounds **5a–o** (earlier

described in the context of selective cyclooxygenase-2 inhibition) against a panel of *h*CA (CA I, II, IV and VII). The compounds showed a strong inhibition of CA II (a well-established cytosolic glaucoma target) and CA VII (also a cytosolic isoform believed to be involved in the onset of epilepsy). The absence of activity against CA I is perhaps unsurprising (considering the documented lower affinity of CA I toward sulfonamides). However, the selectivity established for some of compounds **5a–o** with respect to CA II versus CA IV (both of which are highly active isoforms of the enzyme, strongly inhibited by sulfonamides in general) constitutes a very significant finding in light of isoform-selective CA targeting. For compound **5n**, strong inhibition of COX-2 and some CA isoforms could be a basis for designing dual-action therapeutic agents.

Acknowledgements

This research was supported by the Russian Scientific Fund (project grant 14-50-00069).

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

References

1. Alterio V, Di Fiore A, D'Ambrosio K, et al. Multiple binding modes of inhibitors to carbonic anhydrases: how to design specific drugs targeting 15 different isoforms? Chem Rev 2012;112:4421–68.
2. Supuran CT. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. Nat Rev Drug Discov 2008;7:168–181.
3. Neri D, Supuran CT. Interfering with pH regulation in tumors as a therapeutic strategy. Nat Rev Drug Discov 2011;10:767–77.
4. Wilson WR, Hay MP. Targeting hypoxia in cancer therapy. Nat Rev Cancer 2011;11:393–410.
5. McDonald PC, Winum JY, Supuran CT, Dedhar S. Recent developments in targeting carbonic anhydrase IX for cancer therapeutics. Oncotarget 2012;3:84–97.
6. Palmer C, Supuran CT. A Key Opinion Leader interview: insight into the research and career of Prof. Claudiu T Supuran. Expert Opin Ther Pat 2015;25:501–5.
7. Winum JY, Scozzafava A, Montero JL, Supuran CT. Design of zinc binding functions for carbonic anhydrase inhibitors. Curr Pharm Des 2008;14:615–21.
8. Krasavin M, Korsakov M, Dorogov M, et al. Probing the 'bipolar' nature of the carbonic anhydrase active site: aromatic sulfonamides containing 1,3-oxazol-5-yl moiety as picomolar inhibitors of cytosolic CA I and CA II isoforms. Eur J Med Chem 2015;101:334–47.
9. Pacchiano F, Aggarwal M, Avvaru BS, et al. Selective hydrophobic pocket binding observed within the carbonic anhydrase II active site accommodate different 4-substituted-ureidobenzenesulfonamides and correlate to inhibitor potency. Chem Commun 2010;46:8371–3.
10. Supuran CT. Structure-based drug discovery of carbonic anhydrase inhibitors. J Enzyme Inhib Med Chem 2012;27:759–72.
11. Tanpure RP, Ren B, Peat TS, et al. Carbonic anhydrase inhibitors with dual-tail moieties to match the hydrophobic and hydrophilic halves of the carbonic anhydrase active site. J Med Chem 2015;58:1494–501.
12. Sarnpitak P, Mujumdar P, Morisseau C, et al. Potent, orally available, selective COX-2 inhibitors based on 2-imidazoline core. Eur J Med Chem 2014;84:160–72.
13. De Monte C, Carradori S, Gentili A, et al. Dual cyclooxygenase and carbonic anhydrase inhibition by nonsteroidal anti-inflammatory drugs for the treatment of cancer. Curr Med Chem 2015;22:2812–18.
14. Krasavin M. Novel diversely substituted 1-heteroaryl-2-imidazolines for fragment-based drug discovery. Tetrahedron Lett 2012;53:2876–80.
15. Krasavin M. Pd-catalyzed N-arylation of 2-imidazolines provides convenient access to selective cyclooxygenase-2 inhibitors. Lett Org Chem 2013;10:235–9.
16. 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.
17. Maresca A, Carta F, Vullo D, Supuran CT. Dithiocarbamates strongly inhibit the β-class carbonic anhydrases from *Mycobacterium tuberculosis*. J Enzyme Inhib Med Chem 2013;28:407–11.
18. Ekinci D, Kurbanoglu NI, Salamci E, et al. Carbonic anhydrase inhibitors: inhibition of human and bovine isoenzymes by benzenesulphonamides, cyclitols and phenolic compounds. J Enzyme Inhib Med Chem 2012;27:845–8.
19. Ekinci D, Karagoz L, Ekinci D, et al. Carbonic anhydrase inhibitors: *in vitro* inhibition of α isoforms (hCA I, hCA II, bCA III, hCA IV) by flavonoids. J Enzyme Inhib Med Chem 2013;28:283–8.
20. Alp C, Maresca A, Alp NA, et al. Secondary/tertiary benzenesulfonamides with inhibitory action against the cytosolic human carbonic anhydrase isoforms I and II. J Enzyme Inhib Med Chem 2013;28:294–8.
21. Mujumdar P, Sarnpitak P, Shetnev A, et al. Pd-catalyzed amination of imidazolin-1-yl azines: toward a new kinase-inhibitory chemotype. Tetrahedron Lett 2015;56:2827–31.
22. Supuran CT. How many carbonic anhydrase inhibition mechanisms exist? J Enzyme Inhib Med Chem 2016;31:345–60.

Supplementary material available online