3H-1,2-benzoxathiepine 2,2-dioxides: a new class of isoform-selective carbonic anhydrase inhibitors

Aleksands Pustenko,a, b Dmitrijs Stepanovs,a Raivis Žalubovskis,a Daniela Vullo,c Andris Kazaks,d Janis Leitans,d Kaspars Tarsf and Claudiu T. Supuranf

Latvian Institute of Organic Synthesis, Riga, Latvia; Institute of Technology of Organic Chemistry, Faculty of Materials Science and Applied Chemistry, Riga Technical University, Riga, Latvia; Dipartimento di Chimica, Laboratorio di Chimica Bioinorganica, Polo Scientifico, Università degli Studi di Firenze, Sesto Fiorentino, Florence, Italy; Dipartimento di Chimica, Laboratorio di Chimica Bioinorganica, Polo Scientifico, Università degli Studi di Firenze, Sesto Fiorentino, Florence, Italy; Latvian Institute of Organic Synthesis, Riga, Latvia; Latvian Biomedical Research and Study Centre, Riga, Latvia; Faculty of Biology, Department of Molecular Biology, University of Latvia, Riga, Latvia; Faculty of Biology, Department of Molecular Biology, University of Latvia, Riga, Latvia; Dipartimento Neurofarba, Sezione di Scienze Farmaceutiche e Nutraceutiche, Università degli Studi di Firenze, Sesto Fiorentino, Florence, Italy

ABSTRACT

A new chemotype with carbonic anhydrase (CA, EC 4.2.1.1) inhibitory action has been discovered, the homo-sulfocoumarins (3H-1,2-benzoxathiepine 2,2-dioxides) which have been designed considering the (sulfo)coumarins as lead molecules. An original synthetic strategy of a panel of such derivatives led to compounds with a unique inhibitory profile and very high selectivity for the inhibition of the tumour associated (CA IX/XII) over the cytosolic (CA I/II) isoforms. Although the CA inhibition mechanism with these new compounds is unknown for the moment, we hypothesize that it may be similar to that of the sulfocoumarins, i.e. hydrolysis to the corresponding sulfonic acids which thereafter anchor to the zinc-coordinated water molecule within the enzyme active site.

Introduction

Sulfocoumarins (1,2-benzoxathiane 2,2-dioxides) such as derivatives of type A were discovered by our groups to act as inhibitors of the metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1)1–2. A large series of sulfocoumarins derivatives, among which compounds of type B, were thereafter reported, by using click chemistry or other conventional drug design approaches (Figure 1)3–6. A salient feature of this type of CA inhibitor (CAI) was the fact that they showed a very pronounced isoform selectivity for inhibiting tumour-associated CA isoforms (CA IX and XII) over the widespread, cytosolic ones CA I and II1–3. This has been explained when the mechanism of CA inhibition with sulfocoumarins was elucidated, by using kinetic and X-ray crystallographic experiments7. Indeed, in the X-ray crystal structure of the adduct of a CA II/IX mimic complexed with the 6-bromosulfocoumarin A2 (A, R = Br) (Figure 1), the 2-dihydroxy-5-bromophenyl-vinyl sulfonic acid D was observed within the enzyme active site, probably due to the CA-mediated hydrolysis of A2 to the cis-sulfonic acid C which was thereafter isomerized to the more stable trans-derivative D (Scheme 1)7.

This inhibition mechanism is similar to the one observed earlier for coumarins8–10 the class of CAIs which constituted the lead compounds for the discovery of sulfocoumarins. Finding isoform-selective CAIs for the 15 different human CA isoforms is a challenging task11,12, but coumarins and sulfocoumarins (and several families of sulphonamides) do show such properties, which make them of great interest for the design of pharmaceutical agents useful as diuretics, antiglaucoma, anticonvulsant and/or antitumor drugs13–15.

Here, we report the homo-sulfocoumarins or 3H-1,2-benzoxathiepine 2,2-dioxides, which can be considered as homologs of sulfocoumarins or 1,2-benzoxathiepine 2,2-dioxides1, where oxathiene ring was expanded by one carbon to form an oxathiepine ring. To the best of our knowledge, there is no reported method for the synthesis of 3H-1,2-benzoxathiepine 2,2-dioxides in the literature. The general strategy for the formation of oxathiepine ring reported in this paper involves a ruthenium-catalysed olefin metathesis as a key step.

Materials and methods

Chemistry

Reagents, starting materials and solvents were obtained from commercial sources and used as received. Thin-layer chromatography was performed on silica gel, spots were visualized with UV light (254 and 365 nm). Melting points were determined on an OptiMelt automated melting point system. IR spectra were measured on Shimadzu FTIR IR Prestige-21 spectrometer. NMR spectra were recorded on Varian Mercury (400 MHz) spectrometer with chemical shifts values (δ) in ppm relative to TMS using the residual DMSO-d6 signal (1H 2.50; 13C 39.52) or CDCl3 signal (1H 7.26; 13C 77.16) as an internal standard. HRMS data were obtained with a Q-TOF micro high resolution mass spectrometer with ESI (ESI+/-/ESI-). Elemental analyses were performed on a CARLO ERBA ELEMENTAL ANALYZER EA 1108.

General procedure for the synthesis of 4-substituted 2-ethenylphenoles (2a–c)14

To a stirred solution of methyltriphenylphosphonium bromide (2.64 eq) in dry THF (5 ml/1 mmol of corresponding aldehyde),...
was added tBuOK (2.86–3.12 eq.) in several portions over 20 min. Reaction mixture was stirred for 1 h at RT. Corresponding 2-hydroxy benzaldehyde (1 eq.) was added and stirring continued at room temperature for 24 h. Reaction mixture was diluted with CH₂Cl₂ (5 ml/1 mmol aldehyde). Organic layer was collected and washed with water (2 × 20 ml) and brine (2 × 20 ml), dried over Na₂SO₄, solvent was driven off in vacuum. The crude product was purified by column chromatography (silica gel, EtOAc/PhMe 1:5).

2-Ethenylphenol (2a)

Compound 2a was prepared according to the general procedure from methyltriphenylphosphonium bromide (18.88 g, 52.9 mmol), tBuOK (6.42 g, 57.2 mmol) and 2-hydroxy benzaldehyde (2.44 g, 20.0 mmol) as yellow at room temperature melting solid (3.23 g, 70%). ¹H NMR (400 MHz, CDCl₃) δ = 4.98 (s, 1H), 5.40 (dd, 1H, J = 11.3, 1.0 Hz), 5.74 (dd, 1H, J = 17.8, 1.0 Hz), 6.68 (d, 1H, J = 8.6 Hz), 6.85 (dd, 1H, J = 17.8, 8.6 Hz), 7.23 (dd, 1H, J = 8.6, 2.4 Hz), 7.49 (dd, 1H, J = 7.7, 1.7 Hz).

4-Bromo-2-ethenylphenol (2b)

Compound 2b was prepared according to the general procedure from methyltriphenylphosphonium bromide (13.22 g, 37.0 mmol), tBuOK (2.86 g, 14.0 mmol) and 2-hydroxy benzaldehyde (1.48 g, 14.0 mmol) as yellowish at room temperature melting solid (2.81 g, 59%). ¹H NMR (400 MHz, CDCl₃) δ = 5.41 (m, 2H), 5.48 (d, 1H, J = 11.3, 1.1 Hz), 5.87 (dd, 1H, J = 17.8, 1.1 Hz), 6.92–7.00 (m, 2H), 7.96 (dd, 1H, J = 8.9, 2.6 Hz), 8.31 (d, 1H, J = 2.6 Hz), 8.82 (s, 1H).

2-Ethenyl-4-nitrophenol (2c)

Compound 2c was prepared according to the general procedure from methyltriphenylphosphonium bromide (28.31 g, 79.3 mmol), tBuOK (9.60 g, 85.6 mmol) and 5-nitro-2-hydroxybenzaldehyde (5.0 g, 30 mmol) as yellow at room temperature melting solid (3.23 g, 65%). ¹H NMR (400 MHz, CDCl₃) δ = 5.43 (dd, 1H, J = 11.3, 1.1 Hz), 5.87 (dd, 1H, J = 17.8, 1.1 Hz), 6.92–7.00 (m, 2H), 7.96 (dd, 1H, J = 8.9, 2.6 Hz), 8.31 (d, 1H, J = 2.6 Hz), 8.82 (s, 1H).

Prop-2-ene-1-sulfonyl chloride (3)¹⁵

To a solution of 3-bromoprop-1-ene (24.2 g, 0.20 mol) in water (140 ml) was added Na₂SO₃ (30 g, 0.24 mol) and the reaction mixture was refluxed overnight. After cooling to room temperature, reaction mixture was washed with Et₂O (3 × 35 ml). Aqueous phase was concentrated. Crude white solid was dried under high vacuum at 110 °C for 4 h. To the white solid at 0 °C POCl₃ (80 ml) was added, and mixture was refluxed for 4 h. After cooling to room temperature dry THF (60 ml) was added and reaction mixture was vigorously stirred for 10 min and filtered. Filter cake was suspended in dry THF (60 ml), suspension was vigorously stirred for 10 min and filtered. Filtrates were combined and solvent was carefully driven off on rotary evaporator. Residue was distilled in vacuum (10 mbar) and fraction with boiling point 38–42 °C was collected, to give prop-2-ene-1-sulfonil chloride (3) as colourless oil (18.8 g, 67%).

General procedure for the synthesis of 4-substituted 2-ethenyl prop-2-ene-1-sulfonates (4a–c)

To a stirred solution of corresponding 2-ethenylphenol 2 (1 eq.) in CH₂Cl₂ (10 ml/20 mmol phenol) at 0 °C was added prop-2-ene-1-sulfonate (3) (1.6 eq.) and Et₃N (1.5 eq.). Reaction mixture was stirred overnight (20 h) at room temperature. Water (10 ml/20 mmol phenol) was added, reaction mixture was extracted with EtOAc (3 × 10 ml/20 mmol phenol), combined organic extracts were washed with brine (2 × 10 ml/20 mmol olefin), dried over Na₂SO₄, filtered and solvent was driven off in vacuum. The crude product was purified by column chromatography (silica gel, CH₂Cl₂/PhMe 1:2).

2-Ethenylphenol prop-2-ene-1-sulfonate (4a)

Compound 4a was prepared according to the general procedure from 2-ethenylphenol (2a) (0.50 g, 4.16 mmol), prop-2-ene-1-sulfonyl chloride (3) (0.94 g, 6.69 mmol) and Et₃N (0.87 ml, 6.23 mmol) as colourless oil (0.52 g, 56%). IR (film, cm⁻¹) νmax = 3062 (S = O), 1776 (S = O); ¹H NMR (400 MHz, CDCl₃) δ = 3.96–4.00 (m, 2H), 5.37–5.41 (m, 1H), 5.48–5.54 (m, 2H), 5.79 (dd, 1H, J = 17.6, 0.9 Hz), 5.90–6.01 (m, 1H), 6.99 (dd, 1H, J = 17.6, 11.0 Hz), 7.23–7.34 (m, 2H), 7.57–7.62 (m, 1H); ¹³C NMR (100 MHz, CDCl₃) δ = 55.6, 117.3, 122.8, 123.9, 125.4, 126.9, 127.4, 129.2, 130.3, 131.3, 146.5; HRMS (ESI) m/z [M + H⁺] calcd for C₁₁H₁₁O₃S: 223.0429, found 223.0435.

4-Bromo-2-ethenylphenol prop-2-ene-1-sulfonate (4b)

Compound 4b was prepared according to the general procedure from 4-bromo-2-ethenylphenol (2b) (0.50 g, 2.51 mmol), prop-2-ene-1-sulfonyl chloride (3) (0.57 g, 4.05 mmol) and Et₃N (0.52 ml, 3.76 mmol) as colourless oil (0.51 g, 67%). IR (film, cm⁻¹) νmax = 3062 (S = O), 1776 (S = O); ¹H NMR (400 MHz, CDCl₃) δ = 4.00 (dd, 2H, J = 7.4, 0.9 Hz), 5.46 (d, 1H, J = 11.0 Hz), 5.51–5.59 (m, 2H), 5.81 (d, 1H, J = 17.6 Hz), 5.91–6.03 (m, 1H), 6.92 (dd, 1H, J = 17.6, 11.0 Hz), 7.22 (d, 1H, J = 8.6 Hz), 7.41 (dd, 1H, J = 8.6, 2.4 Hz), 7.73 (d, 1H, J = 2.4 Hz); ¹³C NMR (100 MHz, CDCl₃) δ = 55.7, 118.6, 121.0, 123.7, 124.6, 125.7, 129.2, 129.8, 132.0, 133.3,
2-Ethenyl-4-nitrophenyl prop-2-ene-1-sulfonate (4c)

Compound 4c was prepared according to the general procedure from 2-ethenyl-4-nitrophenol (2c) (0.32 g, 1.94 mmol), prop-2-ene-1-sulfonyl chloride (3) (0.44 g, 3.13 mmol) and Et$_3$N (0.41 ml, 2.96 mmol) as yellowish oil (0.30 mmol, 57%). IR (film, cm$^{-1}$) $\nu_{\text{max}}$ = 1350 (S=O), 1159 (S=O); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ = 4.01 (dt, 2H, $J$= 7.2, 0.9 Hz), 5.54-5.63 (m, 3H), 5.93-6.05 (m, 2H), 6.99 (dd, 1H, $J$ = 17.6, 11.0 Hz), 7.53 (d, 1H, $J$ = 9.0 Hz), 8.16 (dd, 1H, $J$ = 9.0, 2.8 Hz), 8.48 (d, 1H, $J$ = 2.8 Hz); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ = 56.3, 120.2, 122.4, 123.4, 123.8, 124.0, 126.2, 128.6, 132.8, 146.5, 150.2; HRMS (ESI) m/z [M - 1]$^{-}$ calcld for C$_{11}$H$_{10}$NO$_5$S: 268.0280, found 268.0280.

7-Nitro-3H-1,2-benzoxathiepine 2,2-dioxide (6c)

Compound 5c was prepared according to the general procedure from 2-ethenyl-4-nitrophenol prop-2-ene-1-sulfonate (4c) (100 mg, 0.37 mmol), catalyst 5 (18 mg, 0.019 mmol) as yellowish solid (86 mg, 96%). Mp 130–131 °C. IR (film, cm$^{-1}$) $\nu_{\text{max}}$ = 1375 (S=O), 1351 (S=O), 1170 (S=O), 1161 (S=O); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ = 4.18 (dd, 2H, $J$ = 5.8, 1.2 Hz), 6.05-6.12 (m, 1H), 6.89 (d, 1H, $J$ = 11.3 Hz), 7.48 (d, 1H, $J$ = 8.9 Hz), 8.24 (d, 1H, $J$ = 2.6 Hz), 8.28 (dd, 1H, $J$ = 8.9, 2.6 Hz); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ = 52.4, 121.6, 124.3, 125.6, 126.8, 129.4, 130.8, 151.3; Anal. Calcd for C$_{11}$H$_7$N$_2$O$_5$S (241.22); C 44.81, H 2.92, N 5.81, found C 44.70, H 2.95, N 5.79.

7-Azido-3H-1,2-benzoxathiepine 2,2-dioxide (7)

To a solution of 7-azido-3H-1,2-benzoxathiepine 2,2-dioxide (6c) (250 mg, 1.04 mmol) in EtOH (4.3 ml) and H$_2$O (2.8 ml) AcOH (0.06 ml, 1.04 mmol) was added following by iron powder (350 mg, 6.27 mmol) at room temperature. Resulting suspension was stirred at 75 °C for 1 h. It was cooled to room temperature, EtOAc (50 ml) was added and washed with sat. aq. NaHCO$_3$ (5 x 30 ml). Organic layer was dried over Na$_2$SO$_4$ and concentrated in vacuum. Re-crystallized of the crude product from EtOAc/Hex afforded 7 (220 mg, 98%) as yellowish solid. Mp 170–171 °C. IR (film, cm$^{-1}$) $\nu_{\text{max}}$ = 3465 (N=H), 3382 (N=H), 1358 (S=O), 1163 (S=O); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ = 3.72–3.85 (br s, 2H), 3.92 (dd, 2H, $J$ = 6.3, 1.0 Hz), 5.93–6.00 (m, 1H), 6.53 (d, 1H, $J$ = 2.9 Hz), 6.68 (d, 1H, $J$ = 8.8, 2.6 Hz), 6.80 (d, 1H, $J$ = 10.6 Hz), 7.12 (d, 1H, $J$ = 8.8 Hz); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ = 50.5, 115.0, 116.8, 119.8, 123.8, 133.4, 140.4, 145.5; HRMS (ESI) m/z [M+H]$^{+}$ calcld for C$_{14}$H$_{12}$N$_3$O$_5$S: 312.0381, found 312.0364.

7-Azido-3H-1,2-benzoxathiepine 2,2-dioxide (8)

To a solution of 7-amino-3H-1,2-benzoxathiepine 2,2-dioxide (7) (220 mg, 1.03 mmol) in trifluoroacetic acid (1.3 ml) at 0 °C, slowly was added NaNO$_2$ (80 mg, 1.12 mmol). After 30 min stirring at 0 °C, solution of Na$_2$S (67 mg, 1.03 mmol) in water (3 ml) was added. Mixture was stirring at 0 °C for 1 h. Collection of solid precipitate and drying in vacuum afforded 8 (170 mg, 69%) as brown solid. IR (film, cm$^{-1}$) $\nu_{\text{max}}$ = 2116 (Na), 1374 (S=O), 1369 (S=O), 1167 (S=O); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ = 4.01 (dd, 2H, $J$ = 6.3, 1.2 Hz), 5.99–6.07 (m, 1H), 6.83 (d, 1H, $J$ = 10.9 Hz), 6.94 (d, 1H, $J$ = 2.8 Hz), 7.06 (dd, 1H, $J$ = 8.9, 2.8 Hz), 7.32 (d, 1H, $J$ = 8.9 Hz); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ = 51.2, 120.5, 120.8, 120.9, 124.4, 132.4, 139.2, 144.5.
**1-(2,2-Dioxido-3H-1,2-benzoxathiepin-7-yl)-4-phenyl-1H-1,2,3-triazole (9)**

Compound 9 was prepared according to the general procedure from phenylacetylene (13 mg, 0.13 mmol), azide 8 (30 mg, 0.13 mmol), CuSO₄·5H₂O (65 mg, 0.26 mmol), sodium ascorbate (103 mg, 0.52 mmol), AcOH (0.14 ml, 2.45 mmol) as white solid (41 mg, 95%). Mp 203–204 °C. IR (KBr, cm⁻¹) \( \nu_{\text{max}} = 1368 \) (S=O), 1171 (S=O); \(^1\)H NMR (400 MHz, DMSO-d₆) \( \delta = 4.61 \) (dd, 2H, \( J = 5.9, 12.0 \) Hz), 6.09–6.16 (m, 1H), 6.30–6.33 (m, 1H), 7.30–7.43 (m, 1H), 7.38–7.45 (m, 2H), 8.00 (dd, 1H, \( J = 8.8, 2.6 \) Hz), 8.13 (d, 1H, \( J = 2.6 \) Hz), 9.39 (s, 1H); \(^{13}\)C NMR (100 MHz, DMSO-d₆) \( \delta = 51.7, 119.9, 121.6, 122.1, 122.7, 124.0, 125.3, 128.4, 129.1, 129.6, 130.0, 130.1, 135.0, 146.3, 147.5; HRMS (ESI) m/z [M + H]^+ calcd for C₁₇H₁₄N₃O₃S: 340.0756, found 340.0755.

**4-(4-Chlorophenyl)-1-(2,2-dioxido-3H-1,2-benzoxathiepin-7-yl)-1H-1,2,3-triazole (10)**

Compound 10 was prepared according to the general procedure from 1-chloro-4-ethylnylbenzene (17 mg, 0.12 mmol), azide 8 (29 mg, 0.12 mmol), CuSO₄·5H₂O (61 mg, 0.24 mmol), sodium ascorbate (97 mg, 0.49 mmol), AcOH (0.13 ml, 2.27 mmol) as yellowish solid (34 mg, 74%). Mp 191–192 °C. IR (KBr, cm⁻¹) \( \nu_{\text{max}} = 1369 \) (S=O), 1356 (S=O), 1168 (S=O); \(^1\)H NMR (400 MHz, DMSO-d₆) \( \delta = 4.61 \) (dd, 2H, \( J = 5.9, 12.0 \) Hz), 6.09–6.16 (m, 1H), 7.01 (d, 1H, \( J = 11.5 \) Hz), 7.55–7.61 (m, 2H), 7.63 (d, 1H, \( J = 8.9 \) Hz), 7.71 (d, 1H, \( J = 2.7 \) Hz), 9.38 (s, 1H); \(^{13}\)C NMR (100 MHz, DMSO-d₆) \( \delta = 51.7, 120.3, 121.6, 122.1, 122.7, 124.1, 127.0, 129.0, 129.1, 129.6, 130.1, 132.8, 135.0, 146.3, 146.4; HRMS (ESI) m/z [M + H]^+ calcd for C₁₇H₁₃ClN₃O₃S: 374.0366, found 374.0366.

**1-(2,2-Dioxido-3H-1,2-benzothiazepine-7-yl)-4-(3-methoxyphenyl)-1H-1,2,3-triazole (11)**

Compound 11 was prepared according to the general procedure from 3-ethynylanisole (17 mg, 0.13 mmol), azide 8 (30 mg, 0.13 mmol), CuSO₄·5H₂O (63 mg, 0.25 mmol), sodium ascorbate (100 mg, 0.50 mmol), AcOH (0.14 ml, 2.45 mmol) as yellowish solid (24 mg, 51%). Mp 210–211 °C (KBr, cm⁻¹) \( \nu_{\text{max}} = 1372 \) (S=O), 1162 (S=O); \(^1\)H NMR (400 MHz, DMSO-d₆) \( \delta = 3.84 \) (s, 3H), 4.61 (dd, 2H, \( J = 5.8, 1.2 \) Hz), 6.09–6.16 (m, 1H), 6.94–6.99 (m, 1H), 7.02 (d, 1H, \( J = 11.5 \) Hz), 7.39–7.45 (m, 1H), 7.49–7.55 (m, 2H), 7.63 (d, 1H, \( J = 8.9 \) Hz), 8.03 (dd, 1H, \( J = 8.9, 2.7 \) Hz), 8.12 (d, 1H, \( J = 2.7 \) Hz), 9.36 (s, 1H); \(^{13}\)C NMR (100 MHz, DMSO-d₆) \( \delta = 51.7, 55.2, 110.6, 114.1, 117.6, 120.1, 121.6, 122.1, 122.6, 124.0, 129.6, 130.1, 132.0, 134.5, 146.3, 147.4, 159.8; HRMS (ESI) m/z [M + H]^+ calcd for C₁₈H₁₆N₃O₅S: 370.0862, found 370.0876.
149.5, 149.6; HRMS (ESI) m/z [M + H]^+ calcd for C_{18}H_{13}F_{3}N_{2}O_{5}S: 424.0579, found 424.0553.

1-(2,2-Dioxido-3H-1,2-benzoxathiepin-7-yl)-4-(3-fluorophenyl)-1H-1,2,3-triazole (14)

Compound 14 was prepared according to the general procedure from 1-ethynyl-3-fluorobenzene (25 mg, 0.21 mmol), azide 8 (50 mg, 0.21 mmol), CuSO_4·5H_2O (105 mg, 0.42 mmol), sodium ascorbate (166 mg, 0.84 mmol), AcOH (0.25 ml, 4.37 mmol) as brownish solid (56 mg, 74%). Mp 188–189°C. IR (KBr, cm⁻¹) v_max = 1354 (S = O), 1175 (S = O); ¹H NMR (400 MHz, DMSO-d₆) δ = 4.62 (dd, 2H, J = 6.0, 13.3 Hz), 6.09–6.16 (m, 1H), 7.01 (d, 1H, J = 11.6 Hz), 7.20–7.26 (m, 1H), 7.52–7.60 (m, 1H), 7.64 (d, 1H, J = 8.8 Hz), 7.70–7.75 (m, 1H), 7.77–7.81 (m, 1H), 8.02 (dd, 1H, J = 8.9, 2.7 Hz), 8.10 (d, 1H, J = 2.7 Hz), 9.42 (s, 1H); ¹³C NMR (100 MHz, DMSO-d₆) δ = 51.7, 111.9 (d, J = 23.0 Hz), 115.1 (d, J = 20.8 Hz), 120.7, 121.3 (d, J = 2.5 Hz), 121.6, 122.1, 122.7, 124.1, 129.6, 130.0, 131.2 (d, J = 8.7 Hz), 132.4 (d, J = 8.4 Hz), 134.9, 146.3, 146.4, 162.6 (d, J = 243.5 Hz); HRMS (ESI) m/z [M + H]^+ calcd for C_{17}H_{13}FN_{3}O_{3}S: 358.0662, found 358.0667.

2-[1-(2,2-Dioxido-3H-1,2-benzoxathiepin-7-yl)-1H-1,2,3-triazol-4-yl]aniline (15)

Compound 15 was prepared according to the general procedure from 2-ethynylaniline (25 mg, 0.21 mmol), azide 8 (50 mg, 0.21 mmol), CuSO_4·5H_2O (105 mg, 0.42 mmol), sodium ascorbate (166 mg, 0.84 mmol), AcOH (0.25 ml, 4.37 mmol) as yellowish solid (73 mg, 85%). Mp 192–193°C. IR (KBr, cm⁻¹) v_max = 3364 (N-H), 1365 (S = O), 1167 (S = O), 1163 (S = O); ¹H NMR (400 MHz, DMSO-d₆) δ = 4.61 (dd, 2H, J = 6.0, 1.2 Hz), 6.09–6.16 (m, 1H), 6.49–6.85 (m, 2H), 7.01 (d, 1H, J = 11.3 Hz), 7.10–7.18 (m, 1H), 7.59–7.66 (m, 2H), 8.08 (dd, 1H, J = 8.9, 2.4 Hz), 8.16 (d, 1H, J = 2.4 Hz), 9.26 (s, 1H); ¹³C NMR (100 MHz, DMSO-d₆) δ = 51.7, 112.1, 115.9, 116.1, 119.8, 121.8, 122.1, 122.8, 124.0, 127.9, 129.0, 129.6, 130.1, 135.0, 145.8, 146.3, 148.1; HRMS (ESI) m/z [M + H]^+ calcd for C_{17}H_{15}N_{4}O_{3}S: 408.0630, found 408.0626.

CA inhibition assay

An SX.18MV-R Applied Photophysics (Oxford, UK) stopped-flow instrument has been used to assay the catalytic/inhibition of various CA isozymes. Phenol Red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 10 mM Hepes (pH 7.4) as buffer, 0.1 M Na₂SO₄ or NaClO₄ (for maintaining constant the ionic strength; these anions are not inhibitory in the used concentration, and the values reported throughout the paper are the means of seven different inhibitor concentrations have been used for the CA inhibition assay, in order to allow for the formation of the E-I complex. Three different determinations. All CA isozymes used here were purchased from Calbiochem, and the values reported throughout the paper are the means of seven different inhibitor concentrations have been used for the CA inhibition assay, in order to allow for the formation of the E-I complex. Triple experiments were done for each inhibitor concentration. The values reported throughout the paper are the means of such results. The inhibition constants were obtained by non-linear least-squares methods using the Cheng–Prusoff equation, as reported earlier, and represent the mean from at least three different determinations. All CA isozymes used here were recombinant proteins obtained as reported earlier by our group.
X-Ray structure determination

X-Ray diffraction data for compound 6c were collected using a NoniusKappaCCD diffractometer (MoKα radiation, λ = 0.71073 Å), equipped with low temperature Oxford Cryosystems Cryostream Plus device (Delft, the Netherlands). Data were collected using KappaCCD Server Software, cell refined by SCALEPACK19, data reduction performed by DENZO20 and SCALEPACK19, structures solved by direct method using SIR2004 and refined by SHELXL9721 as implemented in the program package WinGX.22 Software used to prepare CIF file was SHELXL9721 and graphics–ORTEP322.

Crystal data for 6c: C9H7NO5S (M = 241.22), monoclinic, P21/a, a = 7.3194(3), b = 14.9000(7) and c = 18.3387(8) Å, β = 101.325(10)*, V = 1961.06(15) Å3, T = 173(2) K, Z = 2, μ(MoKα) = 0.34 mm−1, 9545 reflections measured, 2150 independent reflections (Rint = 0.083), R1( obs) = 0.058, wR1(obs) = 0.1500, R1(all) = 0.1893, wR1(all) = 0.1096, S = 0.94.

CCDC 1526002 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk.

Results and discussion

Chemistry

The synthesis of homo-sulfocoumarins began with a Wittig reaction in which salicylic aldehydes 1 were converted to the corresponding mono-olefins 2a–c in good yields (Scheme 2). Treatment of compounds 2a–c with allyl sulfonyl chloride (3) provided bis-olefins 4a–c as the key intermediates, again in good yields (see Experimental for details). In the next step, olefin metathesis with the commercially available Ru-catalyst 5 was used, in which bis-olefins 4a–c were converted to 3H-1,2-benzoxathiepine 2,2-dioxides 6a–b in 84–96% yields. To obtain a series of 7-substituted homo-sulfocoumarins, the synthesis of 1,4-triazolyl derivatives 9–17 was thereafter performed. For this purpose, 7-nitro derivative 6c was reduced by elemental iron to the corresponding amine 7 in nearly quantitative yield. Further diazotation of amine 7 followed by in situ treatment with sodium azide afforded the azide 8. Treatment of azide 8 with alkynes under click chemistry condition provides a series of 1,4-triazolyl homo-sulfocoumarins 9–17 in good to excellent yields (see Experimental for details).

The structures of all synthesized 3H-1,2-benzoxathiepine 2,2-dioxides 6–17 were fully supported by 1H, 13C NMR and IR spectroscopy, MS or elemental analysis. Additionally, the final unequivocal identification of the scaffold of 3H-1,2-benzoxathiepine 2,2-dioxide was established by a single-crystal X-ray structure for compound 6c, shown in Figure 2.

Carbonic anhydrase inhibition

All the synthesized derivatives 6c–17 were evaluated for their efficacy in inhibiting four relevant CA isoforms, i.e. hCA I, II, IX and XII, by using the stopped flow carbon dioxide hydrase assay, in comparison to the sulfonamide acetazolamide (AAZ, 5-acetamido-1,3,4-thiadiazole-2-sulfonamide) as a standard CAI.

Data of Table 1 show that the cytosolic isoforms hCA I and II (widely distributed enzymes, with important physiological roles in many tissues) were generally not inhibited by the investigated homo-sulfocoumarins, up to 50 μM concentration of inhibitors in

| Compound | IC50 (μM) |
|----------|-----------|
| 6c       | 10        |
| 7b       | 20        |
| 8c       | 50        |
| 9b       | 100       |
| 10c      | 200       |
| 11b      | 500       |

Scheme 2. Reagents and conditions: (i) MePPh3Br, tBuOK, THF, RT, 24 h; (ii) NEt3, CH2Cl2, RT, 20 h; (iii) Fe, AcOH, EtOH, H2O, 70 °C, 1 h, 98%; (iv) NaNO2, H2O, TFA, 2) NaN3, H2O, 69%; (v) alkyne, tBuOH/H2O (1:1), CuSO4, sodium ascorbate, acetic acid, 30 min.
the assay system. Only one derivative, 13, showed a moderate inhibitory profile against hCA II, with an inhibition constant of 5.77 μM.

The tumour-associated isoform hCA IX, a validated drug target for antitumor/antimetastatic agents13,24, was on the other hand effectively inhibited by the investigated homo-sulfocoumarins, with K_S ranging between 27 nM and 3.59 μM (Table 1). The structure activity relationship (SAR) was very interesting, as the best inhibitor (6c) incorporated a compact, powerful electron attracting moiety (NO_2) whereas the remaining derivatives, incorporating substituted 1,2,3-triazole moieties in position 7 of the homo-sulfocoumarin ring were less effective hCA IX inhibitors. Four submicromolar hCA IX inhibitors were however detected apart 6c, derivatives 13, 15, 16 and 17, which incorporate either the compact hydroxymethyl group at the triazole fragment of the molecule, or substituted phenyls with 4-trifluoromethoxy-, 2-amino-, or 4-trifluoromethyl substituents on the aryl fragment. These derivatives showed K_S ranging between 0.34 and 0.87 μM. The remaining homo-sulfocoumarins were low micromolar hCA IX inhibitors.

The SAR for inhibition of the second tumour-associated isoform, hCA XII, was more complex compared to what discussed above for hCA IX (Table 1). Thus, 8 out of 11 derivatives were inactive (K_S > 50 μM) whereas the remaining ones, 6c, 13 and 15, inhibited hCA XII with K_S in the range of 0.64–2.32 μM.

This inhibition profile is rather similar to the one of sulfocoumarins1–6 and coumarins7–9, which are generally selective inhibitors for the tumour-associated over the cytosolic isoforms. However, some homo-sulfocoumarins showed a very specific, and unique up until now inhibition profile among all classes of CAIs known to date9,10, as they are highly selective for hCA IX over hCA I, II and XII (e.g. 7–12, 14, 16 and 17).

In conclusion, we report here a new chemotype with effective and isoform-selective CAIs, the homo-sulfocoumarins, which show a unique inhibition profile for the tumour-associated CA isoforms hCA IX (and XII) over the cytosolic ones. Although the CA inhibition mechanism with these new compounds is unknown for the moment, we hypothesize that it may be similar to that of the sulfocoumarins, i.e. hydrolysis to the corresponding sulfonic acids which thereafter anchor to the zinc-coordinated water molecule within the enzyme active site.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**References**

1. a) Grandane A, Belyakov S, Trapencieris P, et al. Facile synthesis of coumarin bioisosteres – 1,2-benzoxathiine 2,2-dioxides. Tetrahedron 2012;68:5541–6. b) Tars K, Vullo D, Kazaks A, et al. Sulfocoumarins (1,2-benzoxathiine-2,2-dioxides): a class of potent and isoform-selective inhibitors of tumor-associated carbonic anhydrases. J Med Chem 2013; 56:293–300.

2. Tanc M, Barzi F, Bozdag M, et al. 7-Substituted-sulfocoumarins are isoform-selective, potent carbonic anhydrase II inhibitors. Bioorg Med Chem 2013;21:4502–10.

3. a) Grandane A, Tanc M, Zalubovskis R, et al. Synthesis of 6-tetrazolyl-substituted sulfocoumarins acting as highly potent and selective inhibitors of the tumor-associated carbonic anhydrase isozymes IX and XII. Bioorg Med Chem 2014;22:1522–8. b) Grandane A, Tanc M, Zalubovskis R, et al. 6-Triazolyl-substituted sulfocoumarins are potent, selective inhibitors of the tumor-associated carbonic anhydrases IX and XII. Bioorg Med Chem Lett 2014;24:1256–60.

4. Grandane A, Tanc M, Di Cesare Mannelli L, et al. 6-Substituted sulfocoumarins are selective carbonic anhydrase IX and XII Inhibitors with significant cytotoxicity against colorectal cancer cells. J Med Chem 2015;58: 9375–83.

5. Grandane A, Tanc M, Žalubovskis R, et al. Synthesis of 6-aryl-substituted sulfocoumarins and investigation of their carbonic anhydrase inhibitory action. Bioorg Med Chem 2015;23:1430–6.

6. Nocentini A, Ceruso M, Carta F, et al. 7-Aryl-triazolyl-substituted sulfocoumarins are potent, selective inhibitors of the tumor-associated carbonic anhydrase IX and XII. J Enzyme Inhib Med Chem 2016;31:1226–33.

7. (a) Maresca A, Temperini C, Vu H, et al. Non-zinc mediated inhibition of carbonic anhydrases: coumarins are a new class of suicide inhibitors. J Am Chem Soc 2009;131:3057–62. b) Maresca A, Temperini C, Pochet L, et al. Deciphering the mechanism of carbonic anhydrase inhibition with coumarins and thiocoumarins. J Med Chem 2010;53:335–44. c) Maresca A, Supuran CT. Coumarins incorporating hydroxy- and chloro-moieties selectively inhibit the transmembrane, tumor-associated carbonic anhydrase isozymes IX and XII over the cytosolic ones I and II. Bioorg Med Chem Lett 2010;20:4511–14.

8. a) Maresca A, Scozzafava A, Supuran CT. 7,8-Disubstituted— but not 6,7-disubstituted coumarins selectively inhibit the
transmembrane, tumor-associated carbonic anhydrase isoforms IX and XII over the cystolic ones I and II in the low nanomolar/subnanomolar range. Bioorg Med Chem Lett 2010;20:7255–58. b) Touissi N, Maresa A, McDonald PC, et al. Glycosyl coumarin carbonic anhydrase IX and XII inhibitors strongly attenuate the growth of primary breast tumors. J Med Chem 2011;54:8271–7. c) Carta F, Maresa A, Scozzafava A, et al. Novel coumarins and 2-thioxo-coumarins as inhibitors of the tumor-associated carbonic anhydrases IX and XII. Bioorg Med Chem 2012;20:2266–73. d) Davis RA, Vullo D, Maresa A, et al. Natural product coumarins that inhibit human carbonic anhydrases. Bioorg Med Chem 2013;21:1539–43. e) Sharma A, Tiwari M, Supuran CT. Novel coumarins and benzocoumarins acting as isoform-selective inhibitors against the tumor-associated carbonic anhydrase IX. J Enzyme Inhib Med Chem 2014;29:292–6. f) Ferraroni M, Carta F, Scozzafava A, Supuran CT. Thioxocoumarins show an alternative carbonic anhydrase inhibition mechanism compared to coumarins. J Med Chem 2016;59:462–73.

9. a) Supuran CT. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. Nat Rev Drug Discov 2008;7:168–81. b) Neri D, Supuran CT. Interfering with pH regulation in tumors as a therapeutic strategy. Nat Rev Drug Discov 2011;10:767–77. c) Durdagi S, Vullo D, Pan P, et al. Protein-protein interactions: Inhibition of mammalian carbonic anhydrases I-IX by the murine inhibitor of carbonic anhydrase and other members of the transferrin family. J Med Chem 2012;55:5529–35. d) Di Cesare Mannelli L, Micheli L, Carta F, et al. 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.

10. a) Supuran CT. Structure and function of carbonic anhydrases. Biochem J 2016;473:2023–32. b) Supuran CT. How many carbonic anhydrase inhibition mechanisms exist? J Enzyme Inhib Med Chem 2016;31:345–60. c) Supuran CT. Carbonic anhydrases. Bioorg Med Chem 2013;21:1377–8. d) Supuran CT. Advances in structure-based drug discovery of carbonic anhydrase inhibitors. Expert Opin Drug Discov 2017;12:61–88. e) 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. f) Supuran CT. Legionella pneumophila carbonic anhydrases: underexplored antibacterial drug targets. Pathogens 2016;5:E44.

11. a) De Simone G, Alterio V, Supuran CT. Exploiting the hydrophobic and hydrophilic binding sites for designing carbonic anhydrase inhibitors. Expert Opin Drug Discov 2013;8:793–810. b) Masini E, Carta F, Scozzafava A, et al. Antiglaucoma carbonic anhydrase inhibitors: a patent review. Expert Opin Ther Pat 2013;23:705–16. c) Supuran CT, Carbonic anhydrase inhibitors. Bioorg Med Chem Lett 2010;2:3467–74. d) Supuran CT. Carbonic anhydrases: from biomedical applications of the inhibitors and activators to biotechnological use for CO2 capture. J Enzyme Inhib Med Chem 2013;28:229–30. e) D’Ambrosio K, Carradori S, Monti SM, et al. Out of the active site binding pocket for carbonic anhydrase inhibitors. Chem Commun 2015;51:302–5.

12. a) Supuran CT. Structure-based drug discovery of carbonic anhydrase inhibitors. J Enzyme Inhib Med Chem 2012;27:759–72. b) Supuran CT. Carbonic anhydrase inhibitors: an editorial. Expert Opin Ther Pat 2013;23:677–9. c) Winum JY, Supuran CT. Recent advances in the discovery of zinc-binding motifs for the development of carbonic anhydrase inhibitors. J Enzyme Inhib Med Chem 2015;30:321–4. d) De Luca V, Del Prete S, Supuran CT, et al. Protonography, a new technique for the analysis of carbonic anhydrase activity. J Enzyme Inhib Med Chem 2015;30:277–82. e) Lomelino CL, Supuran CT, McKenna R. Non-classical inhibition of carbonic anhydrase. Int J Mol Sci 2016;11:E1150.
23. a) Gieling RG, Babur M, Mamnani L, et al. Antimetastatic effect of sulfamate carbonic anhydrase IX inhibitors in breast carcinoma xenografts. J Med Chem 2012;55:5591–600. b) Winum JY, Maresca A, Carta F, et al. Polypharmacology of sulfonamides: pazopanib, a multitargeted receptor tyrosine kinase inhibitor in clinical use, potently inhibits several mammalian carbonic anhydrases. Chem Commun 2012;48:8177–9. c) Lock EF, McDonald PC, Lou Y, et al. Targeting carbonic anhydrase IX depletes breast cancer stem cells within the hypoxic niche. Oncogene 2013;32:5210–19. d) Ward C, Langdon SP, Mullen P, et al. New strategies for targeting the hypoxic tumour microenvironment in breast cancer. Cancer Treat Rev 2013;39:171–9.

24. a) Pan J, Lau J, Mesak F, et al. Synthesis and evaluation of 18F-labeled carbonic anhydrase IX inhibitors for imaging with positron emission tomography. J Enzyme Inhib Med Chem 2014;29:249–55. b) Pettersen EO, Ebbesen P, Gieling RG, et al. Targeting tumour hypoxia to prevent cancer metastasis. From biology, biosensing and technology to drug development: The METOXIA consortium. J Enzyme Inhib Med Chem 2015;30:689–721.