

RESEARCH PAPER



Aryl derivatives of 3H-1,2-benzoxathiepine 2,2-dioxide as carbonic anhydrase inhibitors

Aleksandrs Pustenko^{a,b}, Alessio Nocentini^c, Anastasija Balašova^a, Ahmed Alafeefy^d, Mikhail Krasavin^e, Raivis Žalubovskis^{a,b} and Claudiu T. Supuran^c 

^aLatvian Institute of Organic Synthesis, Riga, Latvia; ^bInstitute of Technology of Organic Chemistry, Faculty of Materials Science and Applied Chemistry, Riga Technical University, Riga, Latvia; ^cDipartimento Neurofarba, Sezione di Scienze Farmaceutiche e Nutraceutiche, Università degli Studi di Firenze, Florence, Italy; ^dFaculty of Pharmacy, University Technology MARA, UiTM, Bandar, Malaysia; ^eChemistry Department, Saint Petersburg State University, Saint Petersburg, Russian Federation

ABSTRACT

A new series of homosulfocoumarins (3H-1,2-benzoxathiepine 2,2-dioxides) possessing various substitution patterns and moieties in the 7, 8 or 9 position of the heterocyclic ring were prepared by original procedures and investigated for the inhibition of four physiologically relevant carbonic anhydrase (CA, EC 4.2.1.1) isoforms, the human (h) hCA I, II, IX and XII. The 8-substituted homosulfocoumarins were the most effective hCA IX/XII inhibitors followed by the 7-substituted derivatives, whereas the substitution pattern in position 9 led to less effective binders for the transmembrane, tumour-associated isoforms IX/XII. The cytosolic isoforms hCA I and II were not inhibited by these compounds, similar to the sulfocoumarins/coumarins investigated earlier. As hCA IX and XII are validated anti-tumour targets, with one sulphonamide (SLC-0111) in Phase Ib/II clinical trials, finding derivatives with better selectivity for inhibiting the tumour-associated isoforms over the cytosolic ones, as the homosulfocoumarins reported here, is of crucial importance.

ARTICLE HISTORY

Received 7 October 2019
Revised 13 November 2019
Accepted 14 November 2019

KEYWORDS

Carbonic anhydrase; transmembrane isoforms; sulfocoumarin; homosulfocoumarin; isoform-selective inhibitor

1. Introduction



Carbonic anhydrases (CAs, EC 4.2.1.1) are metalloenzymes widespread in nature, being encoded by at least eight different genetic families, which have been identified in organisms all over the phylogenetic tree^{1–3}. By catalysing a crucial physiologic reaction, by which CO₂ is hydrated with the formation of a weak base (bicarbonate) and a strong acid (hydronium ions), these enzymes are involved in a multitude of physiologic processes, starting with pH regulation and ending with metabolism^{1,3–6}. As thus, CAs are drug targets for decades, with their inhibitors having pharmacological applications in a multitude of fields^{1,3–5}. The primary sulphonamides were discovered as CA inhibitors (CAIs) in the 40s, and most of the drugs that were launched in the next decades as diuretics, antiepileptics, or antiglaucoma agents targeting CAs belonged to this class of compounds^{1,3–5}. Although highly effective as CAIs¹, the sulphonamides generally indiscriminately inhibit most α -CA isoforms present in mammals (at least 15 in humans, and 16 in other vertebrates¹) as well as CAs belonging to the other genetic families (β -, γ -, δ -, ζ -, η -, θ - and ι -CAs)^{2–5} and for this reason alternative CAI classes were searched for. In fact, in the last 10 years, a multitude of new chemotypes as well as novel CA inhibition mechanisms were reported^{1,4,7–9}, which highly enriched our understanding of these enzymes and also allowed for obtaining isoform-selective CAIs targeting all the mammalian isoforms^{4,7–9}. Among the new such chemotypes, which also showed the highest levels of isoform selectivity, were the coumarins⁹, the sulfocoumarins^{7,8} and their congeners, homosulfocoumarins

(3H-1,2-benzoxathiepine 2,2-dioxides)¹⁰. Considering the fact that this last chemotype was only recently reported and rather poorly investigated¹⁰, we report here a series of new aryl-3H-1,2-benzoxathiepine 2,2-dioxides substituted in various positions of the heterocyclic ring, which have been designed in order to explore the chemical space around this new CA inhibitory chemotype and to see whether the presence of various moieties in position 7, 8 or 9 of the heterocyclic system maintains the desired enzyme inhibitory activity and selectivity for the target isoforms.

2. Materials and methods

2.1. 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 visualised with UV light (254 and 365 nm). Melting points were determined on an OptiMelt automated melting point system. IR spectra were recorded on Shimadzu FTIR IR Prestige-21 spectrometer. NMR spectra were recorded on Bruker Advance Neo (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. High-resolution mass spectra (HRMS) were recorded on a mass spectrometer with a Q-TOF micro mass analyser using the ESI technique. Elemental analyses were measured using Carlo Erba (EA1108) apparatus (Milan, Italy).

CONTACT Claudiu T. Supuran  claudiu.supuran@unifi.it  Neurofarba Department, University of Florence, Firenze 50121, Italy

© 2019 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.

2-Hydroxy-5-iodobenzaldehyde (2)

To a solution of salicylaldehyde (**1**) (8.73 mL, 81.9 mmol) in AcOH (40 mL) iodine monochloride (4.92 mL, 98.3 mmol) was added¹¹. Reaction mixture was stirred 24 h at 40 °C, then cooled to r.t. EtOH (60 mL) was added and all volatiles were removed in vacuum. CH₂Cl₂ (60 mL) and water (100 mL) were added, the phases were separated and the aqueous phase was extracted with CH₂Cl₂ (3 × 50 mL). The combined organic phases were washed with 10% Na₂S₂O₃ (1 × 60 mL), brine (1 × 60 mL), dried over Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography on silica gel (PE/EtOAc 3:1), the crude product was re-crystallised from EtOH to afford product **2** (17.1 g, 84%) as yellowish solid. ¹H NMR (400 MHz, DMSO-d₆) δ = 6.85 (d, 1H, *J* = 8.6 Hz), 7.77 (dd, 1H, *J* = 8.6, 2.4 Hz), 7.87 (d, 1H, *J* = 2.4 Hz), 10.16 (s, 1H), 10.92 (s, 1H) ppm. ¹³C NMR (100 MHz, DMSO-d₆) δ = 81.4, 120.1, 124.6, 136.7, 144.1, 160.3, 189.8 ppm.

Prop-2-ene-1-sulphonyl chloride (4)

Compound was synthesised using previously described procedure by our group¹⁰. To a solution of Na₂SO₃ (30.2 g; 0.24 mol) in water (140 mL) allyl bromide (17.4 mL; 0.20 mol) was added and the reaction mixture was refluxed overnight. After cooling to room temperature, reaction mixture was washed with Et₂O (3 × 50 mL). Aqueous phase was concentrated. Crude white solid was dried under high vacuum at 100 °C for 6 h. To the white solid at 0 °C POCl₃ (120 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-sulfonyl chloride (**4**) as colourless oil (18.6 g, 66%), which was used in further reactions without additional purification.

General procedure for the synthesis of ethenylphenols (3, 14, 18, 27)

To a stirred solution of methyltriphenylphosphonium bromide (2.60 eq) in dry THF (5 mL/1 mmol of methyltriphenylphosphonium bromide), was added tBuOK (3.2 eq) in several portions over 20 min. Reaction mixture was stirred for 1 h at r.t. Corresponding benzaldehyde (1 eq) was added and stirring continued at room temperature for 24 h. Reaction mixture was diluted with CH₂Cl₂ (4 mL/1 mmol of methyltriphenylphosphonium bromide). Organic layer was washed with water (2 × 20 mL) and brine (2 × 20 mL), and dried over Na₂SO₄, filtered and concentrated. The crude product was purified by column chromatography on silica gel (PE/EtOAc 4:1).

4-Iodo-2-ethenylphenol (3)

Compound **3** was prepared according to the general procedure from methyltriphenylphosphonium bromide (14.98 g, 37.0 mmol), *t*-BuOK (5.79 g, 51.6 mmol) and 2-hydroxy-5-iodobenzaldehyde (**2**) (4.00 g, 16.1 mmol) as yellowish solid (3.29 g, 83%)¹². ¹H NMR

(400 MHz, DMSO-d₆) δ = 5.23 (dd, 1H, *J* = 11.3, 1.4 Hz), 5.80 (dd, 1H, *J* = 17.8, 1.4 Hz), 6.67 (d, 1H, *J* = 8.6 Hz), 6.77–6.87 (m, 1H), 7.38 (dd, 1H, *J* = 8.5, 2.3 Hz), 7.70 (d, 1H, *J* = 2.3 Hz), 9.94 (s, 1H) ppm ¹³C NMR (100 MHz, DMSO-d₆) δ = 81.4, 115.1, 118.4, 126.9, 130.4, 134.4, 137.0, 154.6 ppm.

3-Bromo-2-ethenylphenol (14)

Compound **14** was prepared according to the general procedure from methyltriphenylphosphonium bromide (18.48 g; 51.7 mmol), *t*-BuOK (7.15 g; 63.7 mmol) and 2-bromo-5-hydroxybenzaldehyde (**13**) (4.00 g, 19.9 mmol) as yellowish solid (3.25 g; 82%). ¹H NMR (400 MHz, DMSO-d₆) δ = 5.51 (dd, 1H, *J* = 12.0, 2.4 Hz), 6.06 (dd, 1H, *J* = 17.7, 2.4 Hz), 6.76 (dd, 1H, *J* = 17.7, 11.9 Hz), 6.86–6.91 (m, 1H), 6.98 (t, 1H, *J* = 8.0 Hz), 7.07 (dd, 1H, *J* = 8.0, 1.2 Hz), 10.18 (s, 1H) ppm ¹³C NMR (100 MHz, DMSO-d₆) δ = 115.4, 120.9, 123.2, 123.4, 124.2, 129.1, 132.2, 157.1 ppm.

5-Bromo-2-ethenylphenol (18)

Compound **18** was prepared according to the general procedure from methyltriphenylphosphonium bromide (18.48 g; 51.7 mmol), *t*-BuOK (7.15 g; 63.7 mmol) and 4-bromo-2-hydroxybenzaldehyde (**17**) (4.00 g, 19.9 mmol) as yellowish solid (3.01 g; 76%)¹³. ¹H NMR (400 MHz, DMSO-d₆) δ = 5.24 (dd, 1H, *J* = 11.3, 1.6 Hz), 5.79 (dd, 1H, *J* = 17.8, 1.6 Hz), 6.86 (dd, 1H, *J* = 17.8, 11.3 Hz), 6.93–6.97 (m, 1H), 7.00 (d, 1H, *J* = 2.0 Hz), 7.37 (d, 1H, *J* = 8.3 Hz), 10.13 (s, 1H) ppm ¹³C NMR (100 MHz, DMSO-d₆) δ = 114.6, 118.3, 120.8, 122.0, 123.5, 128.0, 130.8, 155.7 ppm.

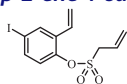
2-Bromo-6-ethenylphenol (27)

Compound **27** was prepared according to the general procedure from methyltriphenylphosphonium bromide (18.48 g; 51.7 mmol), *t*-BuOK (7.15 g; 63.7 mmol) and 3-bromo-2-hydroxybenzaldehyde (**26**) (4.00 g, 19.9 mmol) as yellowish solid (3.17 g; 80%)¹⁴.

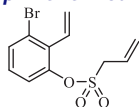
¹H NMR (400 MHz, DMSO-d₆) δ = 5.29 (dd, 1H, *J* = 11.2, 1.3 Hz), 5.78 (dd, 1H, *J* = 17.6, 1.4 Hz), 6.80 (t, 1H, *J* = 7.8 Hz), 7.02 (dd, 1H, *J* = 17.6, 11.2 Hz), 7.41–7.49 (m, 2H), 9.32 (s, 1H) ppm ¹³C NMR (100 MHz, DMSO-d₆) δ = 112.2, 115.5, 121.3, 125.4, 127.5, 131.4, 132.1, 150.7 ppm.

General procedure for diolefine (5, 15, 19, 28) synthesis

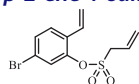
To a stirred solution of corresponding ethenylphenol (**3, 14, 18, 27**) (1 eq) in CH₂Cl₂ (10 mL/1 mmol corresponding ethenylphenol) at 0 °C was added prop-2-ene-1-sulphonyl chloride (**4**) (1.39 eq) and Et₃N (1.4 eq). Reaction mixture was stirred overnight (20 h) at room temperature. Water (30 mL) was added, reaction mixture was extracted with EtOAc (3 × 40 mL), combined organic extracts were washed with brine (2 × 40 mL), and dried over Na₂SO₄, filtered and concentrated. The crude product was purified by column chromatography on silica gel (EtOAc/PE 1:4).

4-Iodo-2-ethenylphenyl prop-2-ene-1-sulfonate (5)

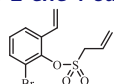
Compound **5** was prepared according to the general procedure from 4-iodo-2-ethenylphenol (**3**) (2.00 g; 8.13 mmol), prop-2-ene-1-sulphonyl chloride (**4**) (1.11 mL; 10.57 mmol) and NEt_3 (1.58 mL; 11.38 mmol) as yellowish oil (2.36 g; 83%). IR (film, cm^{-1}) $\nu_{\text{max}} = 1373$ (S=O), 1160 (S=O). ^1H NMR (400 MHz, DMSO-d_6) $\delta = 4.46\text{--}4.50$ (m, 2H), 5.44–5.55 (m, 2H), 5.56–5.63 (m, 1H), 5.85–5.97 (m, 1H), 6.02 (d, 1H, $J = 17.6$ Hz), 6.84 (dd, 1H, $J = 17.8$, 11.2 Hz), 7.15 (d, 1H, $J = 8.6$ Hz), 7.72 (dd, 1H, $J = 8.6$, 2.2 Hz), 8.10 (d, 1H, $J = 2.2$ Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 54.9$, 93.0, 119.0, 124.6, 125.0, 125.3, 128.4, 133.1, 134.9, 137.9, 145.7 ppm. HRMS (ESI) $[\text{M} + \text{H}]^+$: m/z calcd for $\text{C}_{11}\text{H}_{12}\text{O}_3\text{SI}$: 350.9552. Found 350.9542.

3-Bromo-2-vinylphenyl prop-2-ene-1-sulfonate (15)

Compound **15** was prepared according to the general procedure from 3-bromo-2-ethenylphenol (**14**) (2.00 g; 10.05 mmol), prop-2-ene-1-sulphonyl chloride (**4**) (1.37 mL; 13.06 mmol) and NEt_3 (1.96 mL; 14.07 mmol) as yellowish oil (2.01 g; 66%). IR (film, cm^{-1}) $\nu_{\text{max}} = 1368$ (S=O), 1174 (S=O), 1160 (S=O). ^1H NMR (400 MHz, DMSO-d_6) $\delta = 4.41$ (dt, 2H, $J = 7.2$, 1.0 Hz), 5.49–5.53 (m, 1H), 5.55–5.61 (m, 1H), 5.69–5.76 (m, 2H), 5.83–5.94 (m, 1H), 6.63 (dd, 1H, $J = 17.9$, 11.7 Hz), 7.30–7.35 (m, 1H), 7.43–7.46 (m, 1H), 7.67 (dd, 1H, $J = 8.0$, 1.1 Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 55.4$, 122.4, 123.5, 123.7, 124.5, 125.2, 129.8, 130.6, 131.6, 131.9, 146.9 ppm. HRMS (ESI) $[\text{M} + \text{H}]^+$: m/z calcd for $\text{C}_{11}\text{H}_{12}\text{O}_3\text{SBr}$: 302.9691. Found 302.9681.

5-Bromo-2-vinylphenyl prop-2-ene-1-sulfonate (19)

Compound **19** was prepared according to the general procedure from 5-bromo-2-ethenylphenol (**18**) (2.00 g; 10.05 mmol), prop-2-ene-1-sulphonyl chloride (**4**) (1.37 mL; 13.06 mmol) and NEt_3 (1.96 mL; 14.07 mmol) as yellowish oil (1.65 g; 54%). IR (film, cm^{-1}) $\nu_{\text{max}} = 1377$ (S=O), 1161 (S=O). ^1H NMR (400 MHz, DMSO-d_6) $\delta = 4.54$ (dt, 2H, $J = 7.2$, 1.0 Hz), 5.48 (dd, 1H, $J = 11.2$, 0.8 Hz), 5.52–5.56 (m, 1H), 5.58–5.64 (m, 1H), 5.86–5.98 (m, 1H), 5.99 (dd, 1H, $J = 17.6$, 0.9 Hz), 6.89 (dd, 1H, $J = 17.8$, 11.2 Hz), 7.55–7.59 (m, 2H), 7.73–7.77 (m, 1H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 55.1$, 118.4, 120.8, 124.5, 125.4, 125.6, 128.1, 128.7, 130.3, 130.5, 146.1 ppm. HRMS (ESI) $[\text{M} + \text{H}]^+$: m/z calcd for $\text{C}_{11}\text{H}_{12}\text{O}_3\text{SBr}$: 302.9691. Found 302.9684.

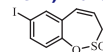
2-Bromo-6-vinylphenyl prop-2-ene-1-sulfonate (28)

Compound **28** was prepared according to the general procedure from 2-bromo-6-ethenylphenol (**27**) (2.00 g; 10.05 mmol), prop-2-ene-1-sulphonyl chloride (**4**) (1.37 mL; 13.06 mmol) and NEt_3 (1.96 mL; 14.07 mmol) as yellowish oil (2.62 g; 86%). IR (film, cm^{-1}) $\nu_{\text{max}} = 1367$ (S=O), 1179 (S=O), 1165 (S=O). ^1H NMR (400 MHz, CDCl_3) $\delta = 4.31$ (dt, 2H, $J = 7.2$, 1.0 Hz), 5.45 (dd, 1H, $J = 11.0$,

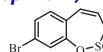
0.80 Hz), 5.57–5.65 (m, 2H), 5.81 (dd, 1H, $J = 17.5$, 0.8 Hz), 6.03–6.15 (m, 1H), 7.07–7.17 (m, 2H), 7.52–7.59 (m, 2H) ppm. ^{13}C NMR (100 MHz, CDCl_3) $\delta = 57.9$, 117.7, 118.4, 123.9, 125.7, 126.0, 128.3, 130.9, 133.1, 135.1, 144.4 ppm. HRMS (ESI) $[\text{M} + \text{H}]^+$: m/z calcd for $\text{C}_{11}\text{H}_{12}\text{O}_3\text{SBr}$: 302.9691. Found 302.9681.

General method for 3H-1,2-benzoxathiepine 2,2-dioxide halogen derivative (7, 20, 29) synthesis

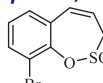
To a solution of corresponding diolefine (**5**, **15**, **19**, **28**) (1.0 eq) in dry, degassed toluene (15 mL/1 mmol corresponding diolefine) ruthenium catalyst **6** (5 mol %) was added. Reaction mixture was bubbled with argon for 5 min and sealed, stirred at 70 °C for 4 h. After cooling to r.t. it was concentrated, and the crude product was purified by column chromatography on silica gel (EtOAc/PE 1:4). Products were re-crystallised from EtOH.

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

Compound **7** was prepared according to the general procedure from diolefine (**5**) (1.00 g; 2.86 mmol) and ruthenium catalyst **6** (0.14 g; 0.14 mmol) as yellowish solid (0.82 g; 89%). Mp 127–128 °C. IR (film, cm^{-1}) $\nu_{\text{max}} = 1370$ (S=O), 1164 (S=O), 1155 (S=O). ^1H NMR (400 MHz, DMSO-d_6) $\delta = 4.52$ (dd, 2H, $J = 5.8$, 1.3 Hz), 5.97–6.04 (m, 1H), 6.82–6.87 (m, 1H), 7.14 (d, 1H, $J = 8.5$ Hz), 7.79 (dd, 1H, $J = 8.5$, 2.2 Hz), 7.88 (d, 1H, $J = 2.2$ Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 51.6$, 92.3, 121.5, 124.5, 129.8, 130.4, 138.7, 139.6, 146.7 ppm. Anal. Calcd for $\text{C}_9\text{H}_7\text{IO}_3\text{S}$: C, 33.56; H, 2.19. Found: C, 33.55; H, 2.21.

8-Bromo-3H-1,2-benzoxathiepine 2,2-dioxide (20)

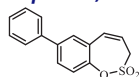
Compound **20** was prepared according to the general procedure from diolefine (**19**) (1.23 g; 4.06 mmol) and ruthenium catalyst **6** (0.19 g; 0.20 mmol) as white solid (1.0 g; 90%). Mp 144–145 °C. IR (film, cm^{-1}) $\nu_{\text{max}} = 1359$ (S=O), 1182 (S=O), 1165 (S=O). ^1H NMR (400 MHz, DMSO-d_6) $\delta = 4.54$ (dd, 2H, $J = 5.8$, 1.0 Hz), 5.95–6.05 (m, 1H), 6.87 (d, 1H, $J = 11.4$ Hz), 7.42–7.47 (m, 1H), 7.58–7.66 (m, 2H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 51.9$, 120.9, 122.0, 125.2, 127.5, 130.1, 130.3, 133.0, 147.1 ppm. Anal. Calcd for $\text{C}_9\text{H}_7\text{BrO}_3\text{S}$: C, 39.29; H, 2.56. Found: C, 39.28; H, 2.59.

9-Bromo-3H-1,2-benzoxathiepine 2,2-dioxide (29)

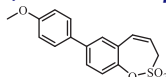
Compound **29** was prepared according to the general procedure from diolefine (**28**) (2.20 g; 7.26 mmol) and ruthenium catalyst **6** (0.34 g; 0.36 mmol) as yellowish solid (1.55 g; 78%). Mp 113–114 °C. IR (film, cm^{-1}) $\nu_{\text{max}} = 1364$ (S=O), 1177 (S=O). ^1H NMR (400 MHz, CDCl_3) $\delta = 4.10$ (dd, 2H, $J = 6.0$, 1.2 Hz), 5.95–6.03 (m, 1H), 6.82–6.87 (m, 1H), 7.18 (t, 1H, $J = 7.8$ Hz), 7.24–7.28 (m, 1H), 7.66 (dd, 1H, $J = 7.9$, 1.6 Hz) ppm. ^{13}C NMR (100 MHz, CDCl_3) $\delta = 51.8$, 117.7, 120.1, 128.0, 130.0, 130.1, 132.2, 134.2, 144.9 ppm. Anal. Calcd for $\text{C}_9\text{H}_7\text{BrO}_3\text{S}$: C, 39.29; H, 2.56. Found: C, 39.28; H, 2.58.

General method for 3H-1,2-benzoxathiepine 2,2-dioxide aryl derivative (8–12, 21–25 and 30–34) synthesis

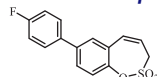
In a pressure tube corresponding 3H-1,2-benzoxathiepine 2,2-dioxide halogen derivative (**7**, **20**, **29**) (1.0 eq) was dissolved in dry toluene (6 mL/1 mmol corresponding 3H-1,2-benzoxathiepine 2,2-dioxide halogen derivative), degassed water was added (5% from toluene volume), corresponding boronic acid (1.5 eq), K_3PO_4 (2.0 eq) and $Pd(PPh_3)_4$ (0.1 eq). Reaction mixture was bubbled with argon 5 min, tube was sealed and heated for 16 h at 100 °C temperature. Reaction mixture was cooled to r.t., filtered through celite was washed with EtOAc (40 mL). Mixture was evaporated and crude product was purified by column chromatography on silica gel (EtOAc/PE 1:3). Products were re-crystallised from EtOH.

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

Compound **8** was prepared according to the general procedure from 7-iodo-3H-1,2-benzoxathiepine 2,2-dioxide (**7**) (0.20 g; 0.62 mmol) phenylboronic acid (0.11 g; 0.93 mmol), K_3PO_4 (0.26 g; 1.24 mmol) and $Pd(PPh_3)_4$ (72 mg; 0.062 mmol) as white solid (95 mg; 56%). Mp 144–145 °C. IR (film, cm^{-1}) ν_{max} =1366 (S=O), 1363 (S=O), 1172 (S=O), 1164 (S=O). 1H NMR (400 MHz, $CDCl_3$) δ =4.06 (dd, 2H, J =6.2, 0.8 Hz), 5.99–6.07 (m, 1H), 6.95 (d, 1H, J =11.0 Hz), 7.38–7.43 (m, 2H), 7.44–7.52 (m, 3H), 7.54–7.58 (m, 2H), 7.62 (dd, 1H, J =8.4, 2.2 Hz) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ =51.4, 119.8, 123.3, 127.3, 128.1, 128.5, 129.1, 129.3, 129.4, 132.9, 139.4, 140.6, 147.1 ppm. Anal. Calcd for $C_{15}H_{12}O_3S$: C, 66.16; H, 4.44. Found: C, 66.06; H, 4.45.

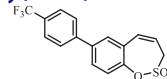
7-(4-Methoxyphenyl)-3H-1,2-benzoxathiepine 2,2-dioxide (9)

Compound **9** was prepared according to the general procedure from 7-iodo-3H-1,2-benzoxathiepine 2,2-dioxide (**7**) (0.20 g; 0.62 mmol) 4-methoxyphenylboronic acid (0.14 g; 0.93 mmol), K_3PO_4 (0.26 g; 1.24 mmol) and $Pd(PPh_3)_4$ (72 mg; 0.062 mmol) as yellowish solid (115 mg; 61%). Mp 162–163 °C. IR (film, cm^{-1}) ν_{max} =1395 (S=O), 1375 (S=O), 1179 (S=O), 1156 (S=O). 1H NMR (400 MHz, $DMSO-d_6$) δ =3.80 (s, 3H), 4.50 (dd, 2H, J =5.8, 1.0 Hz), 5.98–6.06 (m, 1H), 6.97 (d, 1H, J =11.2 Hz), 7.02–7.07 (m, 2H), 7.38 (d, 1H, J =8.4 Hz), 7.62–7.67 (m, 2H), 7.69 (dd, 1H, J =8.4, 2.4 Hz), 7.73 (d, 1H, J =2.4 Hz) ppm. ^{13}C NMR (100 MHz, $DMSO-d_6$) δ =51.6, 55.2, 114.5, 120.5, 122.7, 127.9, 128.0, 128.4, 129.0, 130.8, 131.2, 138.7, 145.8, 159.3 ppm. Anal. Calcd for $C_{16}H_{14}O_4S$: C, 63.56; H, 4.67. Found: C, 63.38; H, 4.68.

7-(4-Fluorophenyl)-3H-1,2-benzoxathiepine 2,2-dioxide (10)

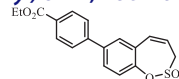
Compound **10** was prepared according to the general procedure from 7-iodo-3H-1,2-benzoxathiepine 2,2-dioxide (**7**) (0.20 g; 0.62 mmol) (4-fluorophenyl)boronic acid (0.13 g; 0.93 mmol), K_3PO_4 (0.26 g; 1.24 mmol) and $Pd(PPh_3)_4$ (72 mg; 0.062 mmol) as white solid (79 mg; 44%). Mp 117–118 °C. IR (film, cm^{-1}) ν_{max} =1373 (S=O), 1181 (S=O), 1168 (S=O). 1H NMR (400 MHz, $CDCl_3$) δ =4.06 (dd, 2H, J =6.2, 1.2 Hz), 5.99–6.06 (m, 1H), 6.93 (d, 1H, J =11.0 Hz), 7.12–7.18 (m, 2H), 7.39 (d, 1H, J =8.4 Hz), 7.45 (d, 1H, J =2.3 Hz), 7.49–7.55 (m, 2H), 7.57 (dd, 1H, J =8.4, 2.3 Hz) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ =51.4, 116.1 (d, J =21.6 Hz), 119.9, 123.4, 128.6, 128.9, 129.0, 129.2, 129.3, 132.8, 135.6 (d, J =3.4 Hz), 139.6, 147.1, 163.0 (d, J =247.0 Hz) ppm. Anal. Calcd for $C_{15}H_{11}FO_3S$: C, 62.06; H, 3.82. Found: C, 62.34; H, 3.83.

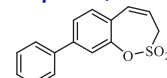
7-(4-(Trifluoromethyl)phenyl)-3H-1,2-benzoxathiepine 2,2-dioxide (11)

Compound **11** was prepared according to the general procedure from 7-iodo-3H-1,2-benzoxathiepine 2,2-dioxide (**7**) (0.20 g; 0.62 mmol) (4-(trifluoromethyl)phenyl)boronic acid (0.18 g; 0.93 mmol), K_3PO_4 (0.26 g; 1.24 mmol) and $Pd(PPh_3)_4$ (72 mg; 0.062 mmol) as white solid (140 mg; 66%). Mp 166–168 °C.

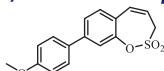
IR (film, cm^{-1}) ν_{max} =1357 (S=O), 1332 (S=O), 1166 (S=O). 1H NMR (400 MHz, $DMSO-d_6$) δ =4.56 (dd, 2H, J =5.8, 1.0 Hz), 6.00–6.08 (m, 1H), 6.99 (d, 1H, J =11.4 Hz), 7.47 (d, 1H, J =8.4 Hz), 7.81–7.86 (m, 3H), 7.88 (d, 1H, J =2.2 Hz), 7.94 (d, 2H, J =8.2 Hz) ppm. ^{13}C NMR (100 MHz, $DMSO-d_6$) δ =51.8, 120.8, 123.0, 124.3 (q, J =273.0 Hz), 125.9 (q, J =3.7 Hz), 127.7, 128.3 (q, J =32.0 Hz), 128.6, 128.9, 130.3, 130.8, 137.4, 142.5, 146.9 ppm. Anal. Calcd for $C_{16}H_{11}F_3O_3S$: C, 56.47; H, 3.26. Found: C, 56.46; H, 3.28.

7-(4-(Ethoxycarbonyl)phenyl)-3H-1,2-benzoxathiepine 2,2-dioxide (12)

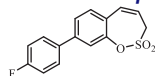
Compound **12** was prepared according to the general procedure from 7-iodo-3H-1,2-benzoxathiepine 2,2-dioxide (**7**) (0.20 g; 0.62 mmol) (4-(ethoxycarbonyl)phenyl)boronic acid (0.18 g; 0.93 mmol), K_3PO_4 (0.26 g; 1.24 mmol) and $Pd(PPh_3)_4$ (72 mg; 0.062 mmol) as yellowish solid (96 mg; 44%). Mp 141–142 °C. IR (film, cm^{-1}) ν_{max} =1701 (C=O), 1380 (S=O), 1184 (S=O), 1170 (S=O). 1H NMR (400 MHz, $DMSO-d_6$) δ =1.34 (t, 3H, J =7.1 Hz), 4.34 (q, 2H, J =7.1 Hz), 4.55 (dd, 2H, J =5.8, 1.2 Hz), 6.00–6.08 (m, 1H), 6.99 (d, 1H, J =11.5 Hz), 7.46 (d, 1H, J =8.5 Hz), 7.83 (dd, 1H, J =8.5, 2.3 Hz), 7.85–7.90 (m, 3H), 8.03–8.08 (m, 2H) ppm. ^{13}C NMR (100 MHz, $DMSO-d_6$) δ =14.2, 51.7, 60.8, 120.8, 123.0, 127.1, 128.6, 128.8, 129.2, 129.8, 130.1, 130.8, 137.7, 142.9, 146.9, 165.4 ppm. Anal. Calcd for $C_{18}H_{16}O_5S$: C, 62.78; H, 4.68. Found: C, 62.76; H, 4.71.

8-Phenyl-3H-1,2-benzoxathiepine 2,2-dioxide (21)

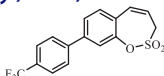
Compound **21** was prepared according to the general procedure from 8-bromo-3H-1,2-benzoxathiepine 2,2-dioxide (**20**) (0.25 g; 0.91 mmol) phenylboronic acid (0.17 g; 1.36 mmol), K_3PO_4 (0.39 g; 1.82 mmol) and $Pd(PPh_3)_4$ (105 mg; 0.091 mmol) as yellowish solid (109 mg; 44%). Mp 103–104 °C. IR (film, cm^{-1}) ν_{max} =1376 (S=O), 1177 (S=O). 1H NMR (400 MHz, $CDCl_3$) δ =4.08 (dd, 2H, J =6.1, 1.2 Hz), 5.94–6.01 (m, 1H), 6.88–6.93 (m, 1H), 7.36–7.43 (m, 2H), 7.44–7.50 (m, 2H), 7.55–7.63 (m, 4H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ =51.6, 119.2, 121.3, 125.7, 126.8, 127.2, 128.5, 129.2, 131.3, 132.5, 138.9, 144.0, 148.1 ppm. Anal. Calcd for $C_{15}H_{12}O_3S$: C, 66.16; H, 4.44. Found: C, 66.15; H, 4.46.

8-(4-Methoxyphenyl)-3H-1,2-benzoxathiepine 2,2-dioxide (22)

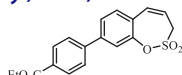
Compound **22** was prepared according to the general procedure from 8-bromo-3H-1,2-benzoxathiepine 2,2-dioxide (**20**) (0.25 g; 0.91 mmol) 4-methoxyphenylboronic acid (0.21 g; 1.36 mmol), K_3PO_4 (0.39 g; 1.82 mmol) and $Pd(PPh_3)_4$ (105 mg; 0.091 mmol) as yellowish solid (121 mg; 44%). Mp 142–143 °C. IR (film, cm^{-1}) ν_{max} = 1369 (S=O), 1177 (S=O), 1164 (S=O). 1H NMR (400 MHz, $CDCl_3$) δ = 3.86 (s, 3H), 4.07 (dd, 2H, J = 6.1, 1.1 Hz), 5.92–5.99 (m, 1H), 6.88 (d, 1H, J = 11.1 Hz), 6.97–7.02 (m, 2H), 7.32–7.36 (m, 1H), 7.50–7.58 (m, 4H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 51.6, 55.5, 114.6, 118.8, 120.6, 125.2, 126.1, 128.3, 131.3, 132.6, 143.6, 148.2, 160.1 ppm. Anal. Calcd for $C_{16}H_{14}O_4S$: C, 63.56; H, 4.67. Found: C, 63.20; H, 4.69.

8-(4-Fluorophenyl)-3H-1,2-benzoxathiepine 2,2-dioxide (23)

Compound **23** was prepared according to the general procedure from 8-bromo-3H-1,2-benzoxathiepine 2,2-dioxide (**20**) (0.25 g; 0.91 mmol) (4-fluorophenyl)boronic acid (0.19 g; 1.36 mmol), K_3PO_4 (0.39 g; 1.82 mmol) and $Pd(PPh_3)_4$ (105 mg; 0.091 mmol) as white solid (108 mg; 41%). Mp 111–112 °C. IR (film, cm^{-1}) ν_{max} = 1371 (S=O), 1168 (S=O). 1H NMR (400 MHz, $CDCl_3$) δ = 4.08 (dd, 2H, J = 6.1, 1.2 Hz), 5.94–6.01 (m, 1H), 6.90 (d, 1H, J = 11.0 Hz), 7.12–7.19 (m, 2H), 7.35–7.40 (m, 1H), 7.50–7.53 (m, 2H), 7.54–7.60 (m, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 51.7, 116.2 (d, J = 21.6 Hz), 119.3, 121.2, 125.6, 126.9, 128.9, 129.0, 131.5, 132.4, 135.0 (d, J = 3.3 Hz), 142.9, 148.1, 163.2 (d, J = 248.0 Hz) ppm. Anal. Calcd for $C_{15}H_{11}FO_3S$: C, 62.06; H, 3.82. Found: C, 62.04; H, 3.86.

8-(4-(Trifluoromethyl)phenyl)-3H-1,2-benzoxathiepine 2,2-dioxide (24)

Compound **24** was prepared according to the general procedure from 8-bromo-3H-1,2-benzoxathiepine 2,2-dioxide (**20**) (0.25 g; 0.91 mmol) (4-(trifluoromethyl)phenyl)boronic acid (0.26 g; 1.36 mmol), K_3PO_4 (0.39 g; 1.82 mmol) and $Pd(PPh_3)_4$ (105 mg; 0.091 mmol) as white solid (142 mg; 46%). Mp 121–122 °C. IR (film, cm^{-1}) ν_{max} = 1366 (S=O), 1324 (S=O), 1172 (S=O). 1H NMR (400 MHz, $CDCl_3$) δ = 4.11 (dd, 2H, J = 6.1, 1.2 Hz), 5.97–6.04 (m, 1H), 6.90 (d, 1H, J = 11.2 Hz), 7.40–7.44 (m, 1H), 7.55–7.60 (m, 2H), 7.70–7.75 (m, 4H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 51.8, 119.7, 121.6, 124.2 (q, J = 273.0 Hz), 125.9, 126.2 (q, J = 3.8 Hz), 127.6, 127.8, 130.5 (q, J = 32.9 Hz), 131.7, 132.2, 142.3, 142.4, 148.1 ppm. Anal. Calcd for $C_{16}H_{11}F_3O_3S$: C, 56.47; H, 3.26. Found: C, 56.23; H, 3.23.

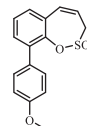
8-(4-(Ethoxycarbonyl)phenyl)-3H-1,2-benzoxathiepine 2,2-dioxide (25)

Compound **25** was prepared according to the general procedure from 8-bromo-3H-1,2-benzoxathiepine 2,2-dioxide (**20**) (0.25 g; 0.91 mmol) (4-(ethoxycarbonyl)phenyl)boronic acid (0.26 g; 1.36 mmol), K_3PO_4 (0.39 g; 1.82 mmol) and $Pd(PPh_3)_4$ (105 mg; 0.091 mmol) as white solid (119 mg; 38%). Mp 151–152 °C. IR (film, cm^{-1}) ν_{max} = 1703 (C=O), 1366 (S=O), 1175 (S=O). 1H NMR (400 MHz, $CDCl_3$) δ = 1.42 (t, 3H, J = 7.1 Hz), 4.10 (dd, 2H, J = 6.1,

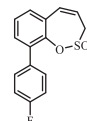
1.2 Hz), 4.41 (q, 2H, J = 7.1 Hz), 5.96–6.03 (m, 1H), 6.90 (d, 1H, J = 11.2 Hz), 7.39–7.43 (m, 1H), 7.57–7.62 (m, 2H), 7.65–7.70 (m, 2H), 8.11–8.16 (m, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 14.5, 51.7, 61.3, 119.6, 121.5, 125.9, 127.1, 127.7, 130.4, 131.6, 132.3, 142.7, 143.0, 148.1, 166.3 ppm. Anal. Calcd for $C_{18}H_{16}O_5S$: C, 62.78; H, 4.68. Found: C, 62.50; H, 4.70.

9-Phenyl-3H-1,2-benzoxathiepine 2,2-dioxide (30)

Compound **30** was prepared according to the general procedure from 9-bromo-3H-1,2-benzoxathiepine 2,2-dioxide (**29**) (0.25 g; 0.91 mmol) phenylboronic acid (0.17 g; 1.36 mmol), K_3PO_4 (0.39 g; 1.82 mmol) and $Pd(PPh_3)_4$ (105 mg; 0.091 mmol) as white solid (104 mg; 42%). Mp 135–136 °C. IR (film, cm^{-1}) ν_{max} = 1370 (S=O), 1162 (S=O). 1H NMR (400 MHz, $CDCl_3$) δ = 4.08 (dd, 2H, J = 5.8, 1.3 Hz), 5.87–5.94 (m, 1H), 6.85–6.90 (m, 1H), 7.29 (dd, 1H, J = 7.6, 1.8 Hz), 7.35–7.42 (m, 2H), 7.43–7.49 (m, 3H), 7.51–7.55 (m, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 52.1, 118.9, 127.1, 128.1, 128.5, 128.6, 129.6, 130.5, 132.1, 132.5, 136.3, 136.5, 144.7 ppm. Anal. Calcd for $C_{15}H_{12}O_3S$: C, 66.16; H, 4.44. Found: C, 66.15; H, 4.46.

9-(4-Methoxyphenyl)-3H-1,2-benzoxathiepine 2,2-dioxide (31)

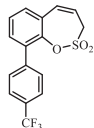
Compound **31** was prepared according to the general procedure from 9-bromo-3H-1,2-benzoxathiepine 2,2-dioxide (**29**) (0.25 g; 0.91 mmol) 4-methoxyphenylboronic acid (0.21 g; 1.36 mmol), K_3PO_4 (0.39 g; 1.82 mmol) and $Pd(PPh_3)_4$ (105 mg; 0.091 mmol) as white solid (110 mg; 40%). Mp 113–114 °C. IR (film, cm^{-1}) ν_{max} = 1369 (S=O), 1181 (S=O), 1154 (S=O). 1H NMR (400 MHz, $CDCl_3$) δ = 3.85 (s, 3H), 4.08 (dd, 2H, J = 5.8, 1.3 Hz), 5.86–5.94 (m, 1H), 6.84–6.89 (m, 1H), 6.97–7.02 (m, 2H), 7.23–7.27 (m, 1H), 7.34 (t, 1H, J = 7.6 Hz), 7.42 (dd, 1H, J = 7.6, 1.8 Hz), 7.45–7.50 (m, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 52.0, 55.4, 114.0, 118.9, 127.1, 128.6, 128.7, 130.1, 130.8, 132.0, 132.6, 136.2, 144.7, 159.5 ppm. Anal. Calcd for $C_{16}H_{14}O_4S$: C, 63.56; H, 4.67. Found: C, 63.58; H, 4.70.

9-(4-Fluorophenyl)-3H-1,2-benzoxathiepine 2,2-dioxide (32)

Compound **32** was prepared according to the general procedure from 9-bromo-3H-1,2-benzoxathiepine 2,2-dioxide (**29**) (0.25 g; 0.91 mmol) (4-fluorophenyl)boronic acid (0.19 g; 1.36 mmol), K_3PO_4 (0.39 g; 1.82 mmol) and $Pd(PPh_3)_4$ (105 mg; 0.091 mmol) as white solid (103 mg; 39%). Mp 130–131 °C. IR (film, cm^{-1}) ν_{max} = 1370 (S=O), 1154 (S=O). 1H NMR (400 MHz, $CDCl_3$) δ = 4.08 (dd, 2H, J = 5.8, 1.3 Hz), 5.88–5.95 (m, 1H), 6.85–6.90 (m, 1H), 7.10–7.18 (m, 2H), 7.30 (dd, 1H, J = 7.5, 2.0 Hz), 7.37 (t, 1H, J = 7.5 Hz), 7.41 (dd, 1H, J = 7.5, 2.0 Hz), 7.47–7.53 (m, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 52.1, 115.5 (d, J = 21.6 Hz), 119.1, 127.2, 128.7, 130.6, 131.3, 131.4, 132.0, 132.3 (d, J = 3.3 Hz), 132.5, 135.6, 144.7, 162.8

(d, $J = 247.0$ Hz) ppm. Anal. Calcd for $C_{15}H_{11}FO_3S$: C, 62.06; H, 3.82. Found: C, 62.05; H, 3.84.

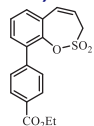
9-(4-(Trifluoromethyl)phenyl)-3H-1,2-benzoxathiepine 2,2-dioxide (33)



Compound **33** was prepared according to the general procedure from 9-bromo-3H-1,2-benzoxathiepine 2,2-dioxide (**29**) (0.25 g; 0.91 mmol) 4-(trifluoromethyl)phenylboronic acid (0.26 g; 1.36 mmol), K_3PO_4 (0.39 g; 1.82 mmol) and $Pd(PPh_3)_4$ (105 mg; 0.091 mmol) as white solid (136 mg; 44%). Mp 115–116 °C.

IR (film, cm^{-1}) $\nu_{max} = 1333$ (S=O), 1166 (S=O). 1H NMR (400 MHz, $CDCl_3$) $\delta = 4.10$ (dd, 2H, $J = 5.8, 1.3$ Hz), 5.90–5.97 (m, 1H), 6.86–6.91 (m, 1H), 7.35 (dd, 1H, $J = 7.0, 2.6$ Hz), 7.38–7.45 (m, 2H), 7.62–7.67 (m, 2H), 7.70–7.74 (m, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 52.2, 119.2, 124.5$ (q, $J = 273.0$ Hz), 125.5 (q, $J = 3.8$ Hz), 127.3, 128.9, 130.0, 130.2 (q, $J = 32.0$ Hz), 131.3, 131.9, 132.3, 135.2, 140.0 (q, $J = 1.5$ Hz), 144.6 ppm. Anal. Calcd for $C_{16}H_{11}F_3O_3S$: C, 56.47; H, 3.26. Found: C, 56.21; H, 3.29.

9-(4-(Ethoxycarbonyl)phenyl)-3H-1,2-benzoxathiepine 2,2-dioxide (34)



Compound **34** was prepared according to the general procedure from 9-bromo-3H-1,2-benzoxathiepine 2,2-dioxide (**29**) (0.25 g; 0.91 mmol) 4-(ethoxycarbonyl)phenylboronic acid (0.26 g; 1.36 mmol), K_3PO_4 (0.39 g; 1.82 mmol) and $Pd(PPh_3)_4$ (105 mg; 0.091 mmol) as white solid (113 mg; 36%). Mp 105–106 °C.

IR (film, cm^{-1}) $\nu_{max} = 1714$ (C=O), 1375 (S=O), 1157 (S=O). 1H NMR (400 MHz, $CDCl_3$) $\delta = 1.41$ (t, 3H, $J = 7.1$ Hz), 4.08 (dd, 2H, $J = 5.8, 1.1$ Hz), 4.40 (q, 2H, $J = 7.1$ Hz), 5.89–5.97 (m, 1H), 6.88 (d, 1H, $J = 11.4$ Hz), 7.31–7.46 (m, 3H), 7.58–7.63 (m, 2H), 8.11–8.16 (m, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 14.5, 52.1, 61.1, 119.2, 127.2, 128.8, 129.6, 129.7, 130.1, 131.1, 131.8, 132.4, 135.6, 140.9$.

144.6, 166.5 ppm. Anal. Calcd for $C_{18}H_{16}O_5S$: C, 62.78; H, 4.68. Found: C, 62.28; H, 4.69.

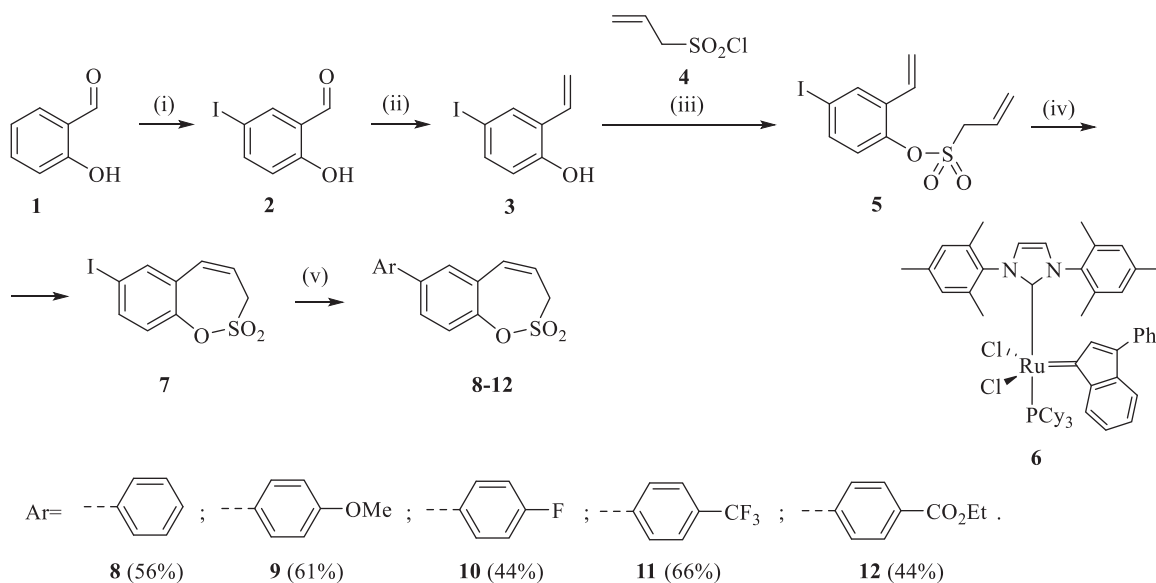
2.2. CA inhibitory assay

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalysed CO_2 hydration activity¹⁵. Phenol red (at a concentration of 0.2 mM) was used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5) as buffer and 20 mM Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalysed 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 uncatalysed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled – deionised water, and dilutions up to 0.01 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 6 h 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^{16–19}, and represent the mean from at least three different determinations. All CA isoforms were recombinant ones obtained in-house as reported earlier^{19,20}.

3. Results and discussion

3.1. Chemistry

The synthesis of desired compounds is partly based on the strategy previously developed by our groups¹⁰. The synthesis of 7-aryl 3H-1,2-benzoxathiepine 2,2-dioxides starts with the iodination of salicylaldehyde (**1**) by iodine monochloride and corresponding iodo derivative **2** was isolated in good yield (Scheme 1)¹¹. Under Wittig reaction conditions aldehyde **2** was converted to olefin **3**, which was treated by sulphonyl chloride **4** thus providing bis-olefin **5** in 83% yield. To obtain the key intermediate **7**, the ring closure in compound **5** was performed in olefin metathesis



Scheme 1. Reagents and conditions for the preparation of derivatives **8–12**: (i) ICl , $AcOH$, 40 °C, 24 h, 84%; (ii) $KOtBu$, $CH_3P(C_6H_5)_3Br$, THF, RT, 18 h, 83%; (iii) NEt_3 , CH_2Cl_2 , 0 °C to RT, 4 h, 83%; (iv) toluene, 70 °C, 4 h, 89%; (v) $Ar-B(OH)_2$, $Pd(PPh_3)_4$, K_3PO_4 , toluene/ H_2O , 100 °C, 16 h.

conditions, using Ru-catalyst **6**. The key intermediate **7** was reacted with a series of aryl boronic acids under Suzuki reaction conditions and the desired 7-aryl 3H-1,2-benzoxathiepine 2,2-dioxides **8–12** were isolated in acceptable yields (44–66%) (Scheme 1).

In an attempt to prepare 6-aryl 3H-1,2-benzoxathiepine 2,2-dioxides, the commercially available bromo salicylaldehyde **13** was first converted to olefin **14** under Wittig reaction conditions, followed by treatment with sulphonyl chloride **4**, thus providing bis-olefin **15** for olefin metathesis ring closure reaction (Scheme 2). Utilisation of the Ru-catalyst **6** as described above did not provide the formation of the desired key intermediate 6-bromo 3H-1,2-benzoxathiepine 2,2-dioxide (**16**) even at prolonged reaction times. By doubling catalyst **6** amount (10 mol%) only traces of compound **16** were observed after 40 h. No product formation was observed also when using Schrock and Schrock–Hoveyda Mo-catalysts. Probably olefin metathesis ring closure reaction did not take place due to sterical constraints due to the bulky Br atom at 3-position of bis-olefin **15**.

The synthesis of 8-bromo intermediate **20** was started from commercially available aldehyde **17**, when under Wittig reaction conditions olefin **18** was obtained, which was thereafter treated with sulphonyl chloride **4** and provided the bis-olefin **19** in good yield (Scheme 3). Ru-catalysed olefin metathesis afforded the key intermediate **20** which in turn, by reaction with a series of aryl boronic acids under Suzuki reaction condition, provided the desired compounds **21–25**.

The same strategy was successfully utilised for the synthesis of a series of 9-aryl 3H-1,2-benzoxathiepine 2,2-dioxides starting by

the treatment of aldehyde **26** with methyltriphenylphosphonium bromide under Wittig reaction conditions (Scheme 4). The obtained phenol **27** was reacted with sulphonyl chloride **4** and ring closure of isolated **28** was successfully performed in Ru-catalysed olefin metathesis conditions, providing bromide **29**. Further reaction of compound **9** with aryl boronic acids provided the desired derivatives **30–34** in moderate yields.

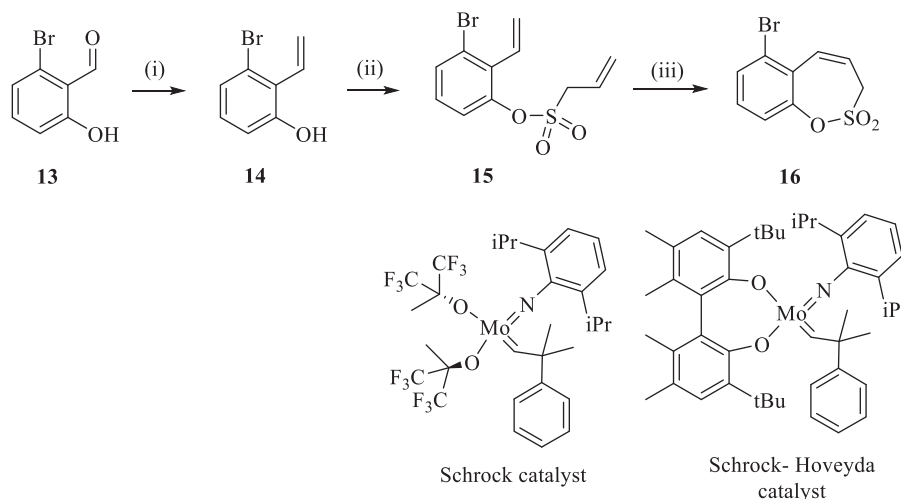
3.2. Carbonic anhydrase inhibition

The obtained homosulfocoumarins **7–34** were investigated for their CA inhibitory properties by using a stopped-flow CO₂ hydrase assay¹⁵ and four human CA isoforms (hCA I, II, IX and XII) known to be drug targets¹ (Table 1).

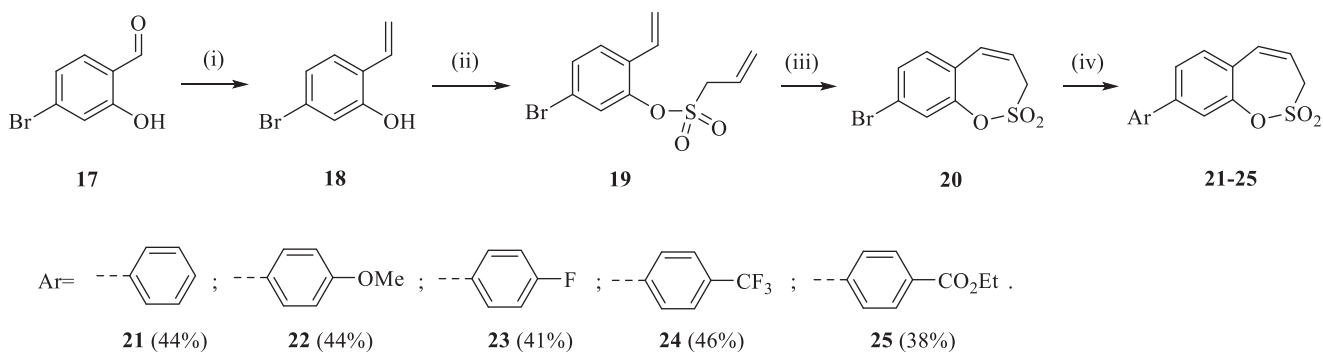
The following structure-activity relationship (SAR) can be observed from the inhibition data of Table 1.

(i) as the previously reported homosulfocoumarins¹⁰ and similar to sulfocoumarins^{7–9}, also the derivatives reported here did not significantly inhibit the cytosolic isoforms hCA I and II, unlike the sulphonamide acetazolamide (used as standard CAI), which has a very good affinity (in the nanomolar range) for hCA II and a micromolar one for hCA I (Table 1).

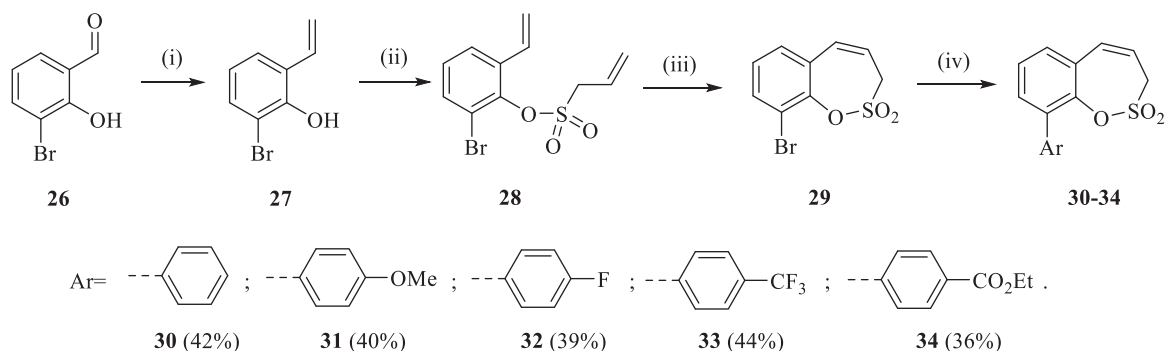
(ii) the transmembrane, tumour-associated isoforms hCA IX and XII were effectively inhibited by derivatives **7–29** reported here (in the low – medium nanomolar range) and were poorly inhibited, in the micromolar range by the 9-substituted-homosulfocoumarins **30–34** (K_s in the range of 16.4–60.9 μM against hCA IX and >100 μM



Scheme 2. Reagents and conditions: (i) KOtBu, CH₃P(C₆H₅)₃Br, THF, RT, 18 h, 82%; (ii) **4**, NEt₃, CH₂Cl₂, 0 °C to RT, 4 h, 66%; (iii) a) **6** (5 mol% and 10 mol%), toluene, 70 °C, 40 h, 0%; b) Schrock catalyst [Mo] (10 mol%), toluene, 70 °C, 16 h, 0%; c) Schrock–Hoveyda [Mo] (10 mol%), toluene, 70 °C, 16 h, 0%;



Scheme 3. Reagents and conditions: (i) KOtBu, CH₃P(C₆H₅)₃Br, THF, RT, 18 h, 76%; (ii) **4**, NEt₃, CH₂Cl₂, 0 °C to RT, 4 h, 54%; (iii) **6**, toluene, 70 °C, 4 h, 90%; (iv) Ar-B(OH)₂, Pd(PPh₃)₄, K₃PO₄, toluene/H₂O, 100 °C, 16 h.



Scheme 4. Reagents and conditions: (i) KOtBu, $\text{CH}_3\text{P}(\text{C}_6\text{H}_5)_3\text{Br}$, THF, RT, 18 h, 80%; (ii) **4**, NEt₃, CH_2Cl_2 , 0 °C to RT, 4 h, 86%; (iii) **6**, toluene, 70 °C, 4 h, 78%; (iv) Ar-B(OH)₂, Pd(PPh₃)₄, K₃PO₄, toluene/H₂O, 100 °C, 16 h.

Table 1. Inhibition data of human CA isoforms CA I, II, IX and XII with 3H-1,2-benzoxathiepine 2,2-dioxides **7–34** using AAZ as a standard drug.

Cmpd	7 / 8 / 9	R	<i>K_i</i> (nM)*			
			CA I	CA II	CA IX	CA XII
7	7	H	>100 μM	>100 μM	66.2	455.5
8	7	H	>100 μM	>100 μM	654.8	1376
9	7	OCH ₃	>100 μM	>100 μM	407.6	2934
10	7	F	>100 μM	>100 μM	330.8	890.5
11	7	CF ₃	>100 μM	>100 μM	221.4	4017
12	7	CO ₂ CH ₂ CH ₃	>100 μM	>100 μM	620.8	2398
20	8	Br	>100 μM	>100 μM	47.5	132.9
21	8	H	>100 μM	>100 μM	104.8	473.2
22	8	OCH ₃	>100 μM	>100 μM	63.1	168.6
23	8	F	>100 μM	>100 μM	95.2	77.9
24	8	CF ₃	>100 μM	>100 μM	44.0	247.8
25	8	CO ₂ CH ₂ CH ₃	>100 μM	>100 μM	79.8	289.3
29	9	Br	>100 μM	>100 μM	754.8	3824
30	9	H	>100 μM	>100 μM	21.1 μM	>100 μM
31	9	OCH ₃	>100 μM	>100 μM	60.9 μM	>100 μM
32	9	F	>100 μM	>100 μM	33.7 μM	>100 μM
33	9	CF ₃	>100 μM	>100 μM	47.1 μM	>100 μM
34	9	CO ₂ CH ₂ CH ₃	>100 μM	>100 μM	16.4 μM	>100 μM
AAZ	–	–	250	12	25	5.7

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

against hCA XII). Thus, although weak inhibitors, these sulfocoumarins are anyhow highly selective for the inhibition of hCA IX, whereas their activity against hCA I, II and XII is absent (Table 1). As already anticipated above, the most important factors associated with CA IX/XII inhibitory activity are the position and the nature of the moieties present on the six-membered ring of the homosulfocoumarin. Indeed, for 9-substituted derivatives, the presence of bulky, substituted aryls as in **30–34** leads to low activity, as mentioned above. Only the 9-bromo-derivative **29** had a medium potency inhibitory action against the two isoforms, with *K_s* in the range of 754.8 – 3824 nM. On the contrary, the 8-substituted derivatives **20–25** showed a much better inhibitory power against both isoforms, being generally more potent than the corresponding 7-substituted derivatives **7–12**. Indeed, for the 7-substituted homosulfocoumarins the *K_s* were in the range of 66.2 – 620.8 nM against hCA IX and of 455.5 –

2934 nM against hCA XII. On the contrary, for the 8-substituted homosulfocoumarins, the *K_s* were in the range of 44.0 – 104.8 nM against hCA IX and in the range of 77.9 – 473.2 nM for hCA XII (Table 1). The 8-(4-trifluoromethyl)phenyl-substituted homosulfocoumarin **24** was the most effective hCA IX inhibitor (potency in the same range as AAZ), whereas the corresponding 4-fluorophenyl derivative **23** was the best hCA XII inhibitor in the new series of compounds investigated here but it was an order of magnitude less effective compared to acetazolamide.

4. Conclusions

A new series of homosulfocoumarins (3H-1,2-benzoxathiepine 2,2-dioxides) possessing various moieties in the 7, 8 or 9 position of the heterocyclic ring were prepared by original procedures and investigated for the inhibition of four physiologically relevant CA isoforms, hCA I, II, IX and XII. The 8-substituted homosulfocoumarins were the most effective hCA IX/XII inhibitors followed by the 7-substituted derivatives, whereas the substitution pattern in position 9 led to less effective inhibitors for these transmembrane, tumour-associated isoforms. The cytosolic isoforms hCA I and II were not inhibited by these compounds, similar to the sulfocoumarins/coumarins investigated earlier. As hCA IX and XII are validated anti-tumour targets⁵, with one sulphonamide (SLC-0111) in Phase Ib/II clinical trials, finding derivatives with a better selectivity for inhibiting the tumour-associated isoforms over the cytosolic ones, as the homosulfocoumarins reported here, is of crucial importance.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by ERA.Net RUS plus joint programme project THIOREDIN (State Education Development Agency of Republic of Latvia) [grant number RUS_ST2017-309] and the Russian Foundation for Basic Research [grant number 18-515-76001].

ORCID

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

References

- (a) Supuran CT. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. *Nature Rev Drug Discov* 2008;7:168–81.(b) 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.(c) Supuran CT. Structure and function of carbonic anhydrases. *Biochem J* 2016;473:2023–32.
- (a) Xu Y, Feng L, Jeffrey PD, et al. Structure and metal exchange in the cadmium carbonic anhydrase of marine diatoms. *Nature* 2008;452:56–61.(b) Del Prete S, Vullo D, Fisher GM, et al. Discovery of a new family of carbonic anhydrases in the malaria pathogen *Plasmodium falciparum* – the η -carbonic anhydrases. *Bioorg Med Chem Lett* 2014;24:4389–96.(c) Jensen EL, Clement R, Kosta A, et al. A new widespread subclass of carbonic anhydrase in marine phytoplankton. *ISME J* 2019;13:2094–106.
- (a) Capasso C, Supuran CT. Anti-infective carbonic anhydrase inhibitors: a patent and literature review. *Expert Opin Ther Pat* 2013;23:693–704.(b) Capasso C, Supuran CT. An overview of the alpha-, beta- and gamma-carbonic anhydrases from bacteria: can bacterial carbonic anhydrases shed new light on evolution of bacteria? *J Enzyme Inhib Med Chem* 2015;30:325–32.(c) Capasso C, Supuran CT. Bacterial, fungal and protozoan carbonic anhydrases as drug targets. *Expert Opin Ther Targets* 2015;19:1689–704.(d) Supuran CT, Capasso C. Biomedical applications of prokaryotic carbonic anhydrases. *Expert Opin Ther Pat* 2018;28:745–54.
- (a) Supuran CT. How many carbonic anhydrase inhibition mechanisms exist? *J Enzyme Inhib Med Chem* 2016;31:345–60.(b) Nocentini A, Supuran CT. Advances in the structural annotation of human carbonic anhydrases and impact on future drug discovery. *Expert Opin Drug Discov* 2019;14:1175–97.(c) Supuran CT. Advances in structure-based drug discovery of carbonic anhydrase inhibitors. *Expert Opin Drug Discov* 2017;12:61–88.(d) De Simone G, Supuran CT. (In)organic anions as carbonic anhydrase inhibitors. *J Inorg Biochem* 2012;111:117–29.
- (a) Supuran CT. Carbonic anhydrase inhibitors as emerging agents for the treatment and imaging of hypoxic tumors. *Expert Opin Investig Drugs* 2018;27:963–70.(b) Supuran CT. Carbonic anhydrase inhibitors and their potential in a range of therapeutic areas. *Expert Opin Ther Pat* 2018;28:709–12.(c) Supuran CT. Applications of carbonic anhydrases inhibitors in renal and central nervous system diseases. *Expert Opin Ther Pat* 2018; 28:713–21.(d) Neri D, Supuran CT. Interfering with pH regulation in tumours as a therapeutic strategy. *Nat Rev Drug Discov* 2011;10:767–77.(e) Supuran CT, Alterio V, Di Fiore A, et al. Inhibition of carbonic anhydrase IX targets primary tumors, metastases, and cancer stem cells: three for the price of one. *Med Res Rev* 2018;38:1799–836.
- (a) Supuran CT. Carbonic anhydrases and metabolism. *Metabolites* 2018;8:25.(b) Supuran CT. Carbonic anhydrase inhibition and the management of hypoxic tumors. *Metabolites* 2017;7:E48.(c) Da'dara AA, Angeli A, Ferraroni M, et al. Crystal structure and chemical inhibition of essential schistosome host-interactive virulence factor carbonic anhydrase SmCA. *Commun Biol* 2019;2:333.
- 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.
- (a) Tanc M, Carta F, Bozdog M, et al. 7-Substituted-sulfocoumarins are isoform-selective, potent carbonic anhydrase II inhibitors. *Bioorg Med Chem* 2013;21:4502–10.(b) Nocentini A, Ceruso M, Carta F, Supuran CT. 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.(c) Grandane A, Tanc M, Mannelli LDC, et al. Substituted sulfocoumarins are selective carbonic anhydrase IX and XII inhibitors with significant cytotoxicity against colorectal cancer cells. *J. Med. Chem* 2015;58:3975–83.
- (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) Temperini C, Innocenti A, Scozzafava A, et al. The coumarin-binding site in carbonic anhydrase accommodates structurally diverse inhibitors: the antiepileptic lacosamide as an example. *J Med Chem* 2010;53:850–4.(d) Touisni N, Maresca A, McDonald PC, et al. Glycosylcoumarin carbonic anhydrase IX and XII inhibitors strongly attenuate the growth of primary breast tumors. *J Med Chem* 2011;54:8271–7.
- Pustenko A, Stepanovs D, Žalubovskis R, et al. 3H-1,2-benzoxathiepine 2,2-dioxides: a new class of isoform-selective carbonic anhydrase inhibitors. *J Enzyme Inhib Med Chem* 2017;32:767–75.
- Yin H, Zhang B, Yu H, et al. Two-photon fluorescent probes for biological Mg²⁺ detection based on 7-substituted coumarin. *J Org Chem* 2015;80:4306–12.
- Gillis EP, Burke MD. A simple and modular strategy for small molecule synthesis: iterative Suzuki–Miyaura coupling of B-protected haloboronic acid building blocks. *J Am Chem Soc* 2007;129:6716–7.
- Seoane A, Casanova N, Quinones N, et al. Straightforward assembly of benzoxepines by means of a rhodium(III)-catalyzed C–H functionalization of o-vinylphenols. *J Am Chem Soc* 2014;136:834–7.
- Hoveyda HR, Marsault E, Gagnon R, et al. Optimization of the potency and pharmacokinetic properties of a macrocyclic ghrelin receptor agonist (Part I): development of ulimorelin (TZP-101) from hit to clinic. *J Med Chem* 2011;54:8305–20.
- Khalifah RG. The carbon dioxide hydration activity of carbonic anhydrase. *J Biol Chem* 1971;246:2561–73.
- (a) Nocentini A, Bua S, Del Prete S, et al. *Malassezia globosa*: activity and modeling studies. *ChemMedChem* 2018;13:816–23.(b) D'Ascenzio M, Guglielmi P, Carradori S, et al. Open saccharin-based secondary sulfonamides as potent and selective inhibitors of cancer-related carbonic anhydrase IX and XII isoforms. *J Enzyme Inhib Med Chem* 2017;32:51–9.(c) Nocentini A, Ceruso M, Bua S, et al. Discovery of β -adrenergic receptors blocker-carbonic anhydrase inhibitor hybrids for multitargeted antiglaucoma therapy. *J Med Chem* 2018;61:5380–94.(d) Köhler K, Hillebrecht A, Schulze Wischeler J, et al. Saccharin inhibits carbonic anhydrases: possible explanation for its unpleasant metallic aftertaste. *Angew Chem Int Ed Engl* 2007;46:7697–9.
- (a) Vermelho AB, da Silva Cardoso V, Ricci Junior E, et al. Nanoemulsions of sulfonamide carbonic anhydrase inhibitors strongly inhibit the growth of *Trypanosoma cruzi*. *J*

- Enzyme Inhib Med Chem 2018;33:139–46.(b) Nocentini A, Carta F, Tanc M, et al. Deciphering the mechanism of human carbonic anhydrases inhibition with sulfocoumarins: computational and experimental studies. *Chemistry* 2018;24:7840–4.(c) Awadallah FM, Bua S, Mahmoud WR, et al. Inhibition studies on a panel of human carbonic anhydrases with N1-substituted secondary sulfonamides incorporating thiazolinone or imidazolone-indole tails. *J Enzyme Inhib Med Chem* 2018;33:629–38.
18. (a) Bua S, Bozdog M, Del Prete S, et al. Mono- and di-thio-carbamate inhibition studies of the δ -carbonic anhydrase TweCA δ from the marine diatom *Thalassiosira weissflogii*. *J Enzyme Inhib Med Chem* 2018;33:707–13.(b) Ferraroni M, Gaspari R, Scozzafava A, et al. Dioxygen, an unexpected carbonic anhydrase ligand. *J Enzyme Inhib Med Chem* 2018;33:999–1005.(c) El-Gazzar MG, Nafie NH, Nocentini A, et al. Carbonic anhydrase inhibition with a series of novel benzenesulfonamide-triazole conjugates. *J Enzyme Inhib Med Chem* 2018;33:1565–74.(d) Akocak S, Lolak N, Bua S, Supuran CT. Discovery of novel 1,3-diaryltriazene sulfonamides as carbonic anhydrase I, II, VII, and IX inhibitors. *J Enzyme Inhib Med Chem* 2018;33:1575–80.
19. (a) Nocentini A, Bonardi A, Gratteri P, et al. Steroids interfere with human carbonic anhydrase activity by using alternative binding mechanisms. *J Enzyme Inhib Med Chem* 2018;33:1453–9.(b) Nocentini A, Trallori E, Singh S, et al. 4-Hydroxy-3-nitro-5-ureido-benzenesulfonamides selectively target the tumor-associated carbonic anhydrase isoforms IX and XII showing hypoxia-enhanced antiproliferative profiles. *J Med Chem* 2018;61:10860–74.(c) Chohan ZH, Munawar A, Supuran CT. Transition metal ion complexes of Schiff bases. Synthesis, characterization and antibacterial properties. *Met Based Drugs* 2001;8:137–43.(d) Oztürk Sarikaya SB, Topal F, Sentürk M, et al. In vitro inhibition of α -carbonic anhydrase isozymes by some phenolic compounds. *Bioorg Med Chem Lett* 2011;21:4259–62.
20. (a) Supuran CT, Clare BW. Carbonic anhydrase inhibitors. Part 57. Quantum chemical QSAR of a group of 1,3,4-thiadiazole and 1,3,4-thiadiazoline disulfonamides with carbonic anhydrase inhibitory properties. *Eur J Med Chem* 1999;34:41–50.(b) Supuran CT, Ilies MA, Scozzafava A. Carbonic anhydrase inhibitors. Part 29. Interaction of isozymes I, II and IV with benzamide-like derivatives. *Eur J Med Chem* 1998;33:739–52.(c) Sentürk M, Gülçin I, Daştan A, et al. Carbonic anhydrase inhibitors. Inhibition of human erythrocyte isozymes I and II with a series of antioxidant phenols. *Bioorg Med Chem* 2009;17:3207–11.