Journal Pre-proof

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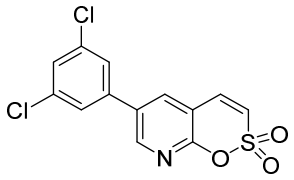
Graphical Abstract

Oxathiino[6,5-b]pyridine 2,2-dioxides and compound **17** in particular noticeably decreases viability of human cancer cells (MCF-7, HeLa, A549), having IC_{50} values in the range from 4.8 to 20 μM. Oxathiino[6,5-b]pyridine 2,2-dioxides very potent and selective inhibitors of cancer associated carbonic anhydrases isoforms – CA IX and CA XII. At the same time these compounds have poor or no inhibition of ubiquitous CA I and CA II.



17

CA **I** K_I = >100 μM
CA II K_I = >100 μM
CA IX K_I = 56.0 nM
CA XII K_I = 3.8 nM

Development of oxathiino[6,5-b]pyridine 2,2-dioxide derivatives as selective inhibitors of tumor-related carbonic anhydrases IX and XII

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Abstract

Oxathiino[6,5-b]pyridine 2,2-dioxides are identified as a new class of isoform-selective nanomolar inhibitors of tumor associated human carbonic anhydrases (hCA) IX and XII. At the same time they do not inhibit or poorly inhibit cytosolic isoforms hCA I and II. Oxathiino[6,5-b]pyridine 2,2-dioxides exhibited good antiproliferative properties on tumor cell lines MCF-7 (Human breast adenocarcinoma), A549 (human lung (alveolar) adenocarcinoma) and HeLa (epithelioid cervix carcinoma).

Keywords: Carbonic anhydrase; oxathiino[6,5-b]pyridine 2,2-dioxide; inhibitor; tumor.

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1. Introduction

Carbonic anhydrases (CAs, EC 4.2.1.1) are ubiquitous metalloenzymes, which are present in organisms all over the phylogenetic tree and being encoded by at least eight different genetic families[1-10]. CAs catalyze a crucial physiologic reaction – reversible hydratation of CO_2 to produce a weak base (bicarbonate) and a strong acid (hydronium ions). CAs are involved in many physiologic processes, starting with pH regulation and ending with metabolism[1-3, 7-10, 11-22].

These enzymes are also involved in different pathological processes and therefore are drug targets for decades, and pharmacological applications of CA inhibitors (CAI) have found place in many fields[1-3, 7-19]. The primary sulfonamides were discovered as CA inhibitors (CAIs) as early as in the 40s. In the next decades numerous CAIs based on this class of compounds were approved for clinical use as diuretics, antiepileptics, or antiglaucoma drugs[1-3, 7-19]. Although highly potent as CAIs[1-3], the sulfonamides are generally non-selective inhibitors of most of α-CA isoforms

present in humans and mammals[1-3] and CAs from the other genetic families (β-, γ-, δ-, ζ-, η-, θ- and ι-CAs)[4-19], therefore novel, isoform selective CAI classes were searched. A multitude of novel chemotypes and new CA inhibition mechanisms were reported in the last decade[1-3, 11-14, 23-30].

Highly enriched understanding of these enzymes allowed obtaining of isoform-selective CAIs that target physiologically relevant isoforms[11-14, 23-27]. Among the new chemotypes, which also exhibited the highest levels of isoform selectivity, were saccharin derivatives[31-33], the coumarins[27], the sulfocoumarins[23-26, 34] and homosulfocoumarins (3H-1,2-benzoxathiepine 2,2-dioxides)[35-37].

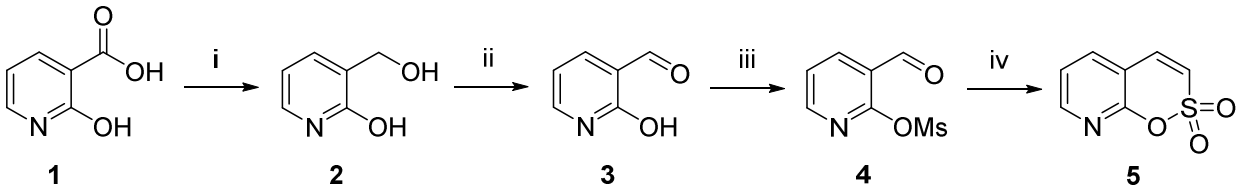
Considering high interest in design of non-sulfonamide CAIs for various potential applications, we report here a facile synthesis of a new class of compounds, namely oxathiino[6,5-b]pyridine 2,2-dioxides, their inhibitory profiles against the major human (h) CA isoforms, hCA I, II, IX, and XII, involved in many pathologies, including cancer, and *in vitro* cytotoxicity against cancer cell lines.

2. Results and discussion

2.1. Chemistry

The synthesis of oxathiino[6,5-b]pyridine 2,2-dioxides is presented in Schemes 1 and 2.

The synthesis of desired oxathiino[6,5-b]pyridine 2,2-dioxide (**5**) was started with the reduction of commercially available 2-hydroxynicotinic acid (**1**) by $LiAlH_4$ where intermediate **2** was isolated in good yield (Scheme 1). Alcohol **2** was oxidized by MnO_2 to corresponding aldehyde **3**. Mesylation of compound **3** provided compound **4**, which in turn was submitted to intramolecular aldol condensation affording **5** in acceptable yield.

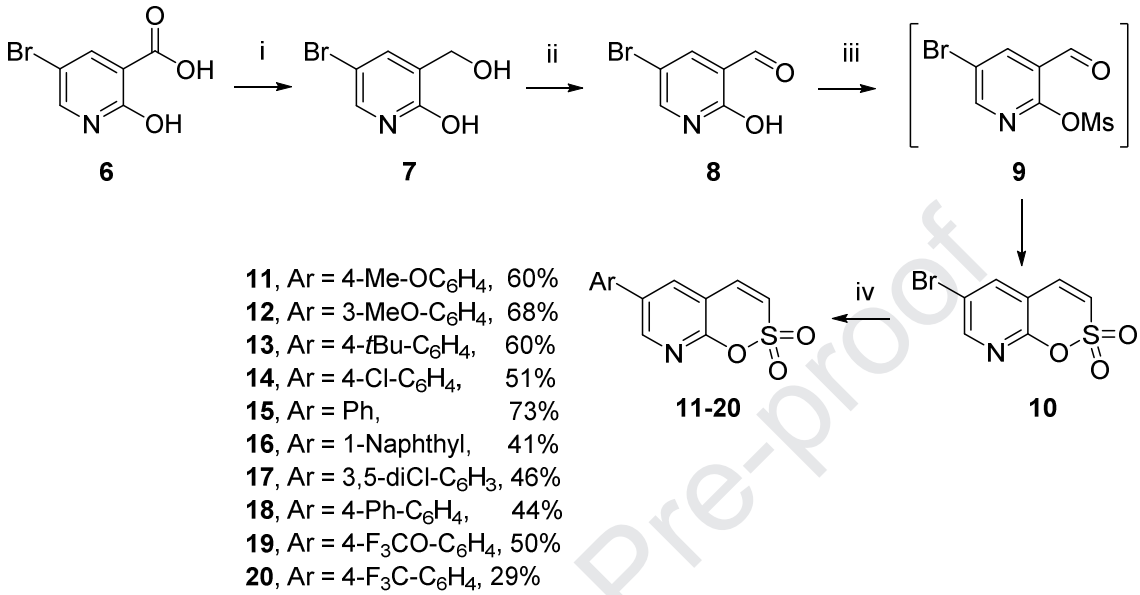


1 **2** **3** **4** **5**

Scheme 1. Reagents and conditions: (i) $LiAlH_4$, THF, relux, 2h, 80%. (ii) MnO_2, EtOAc, 50 °C, 70%. (iii) MsCl, NaH, THF, rt, 16 h, 30%. (iv) a) K_2CO_3, DMF rt, 1 h; b) MsCl, NEt_3, DCM, rt, 3 h, 40%.

Similar strategy was used for the synthesis of **11 – 20**, where synthesis was started with reduction of commercially available carboxylic acid **6** thus obtaining alcohol **7** in good yield (Scheme 2). Oxidation of **7** by MnO_2 provided aldehyde **8** also in good yield. Compound **8** was mesylated and

intermediate **9** formed was *in situ* submitted to intramolecular aldol condensation affording the key intermediate **10**. Bromide **10** was reacted with a series of organo-tin derivatives (prepared separately) under Stille reaction conditions providing a series of the inhibitors **11**-**20** in 29% to 73% yield.



11, Ar = 4-Me-OC_6H_4, 60%
12, Ar = 3-MeO-C_6H_4, 68%
13, Ar = 4-*t*Bu-C_6H_4, 60%
14, Ar = 4-Cl-C_6H_4, 51%
15, Ar = Ph, 73%
16, Ar = 1-Naphthyl, 41%
17, Ar = 3,5-diCl-C_6H_3, 46%
18, Ar = 4-Ph-C_6H_4, 44%
19, Ar = 4-F_3CO-C_6H_4, 50%
20, Ar = 4-F_3C-C_6H_4, 29%

Scheme 2. Reagents and conditions: (i) BH_3, THF, rt, 18 h, 53%. (ii) MnO_2, EtOAc, 60 °C, 18 h, 53%. (iii) a) MsCl, NaH, DMF, rt, 25 min; b) K_2CO_3, DMF, rt, 75 min, 58%. (iv) Ar-$SnBu_3$ or Ar-$SnMe_3$, $Pd(OAc)_2$, tri(2-furyl)phosphine, dioxane, 100 °C, 18 h.

2.2. Determination of inhibition of carbonic anhydrases *in vitro*

All synthesized oxathiino[6,5-b]pyridine 2,2-dioxides were studied for the inhibition of physiologically relevant human CA isoforms - the cytosolic, widespread hCA I and hCA II (off-targets in this case) and transmembrane tumor-associated hCA IX and hCA XII which have been defined as anti-cancer drug targets[19, 30, 38-43].

From the collected and presented data in Table 1, following SAR can be drawn:

All compounds studies except **5**, showed no inhibition of ubiquitous (off-target) CA I and CA II with K_I values greater than 100 μM (Table 1). Compound **5** exhibited moderate inhibition of CA I and CA II with K_I values of 64.11 and 14.05 μM, respectively.

All compounds showed very good inhibition of cancer associated CA IX and CA XII.

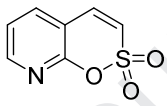
CA IX was inhibited with K_I values in range from 31.4 to 220.7 nM. Even though specific SAR cannot be depicted, the inhibitors could be split into two groups. Most active ones having K_Is from 31.4 to 86.8 nM are compounds **11**, **13**, **14**, **17** and **20**. This compounds, except **17**, have small substituents at 4-posion of phenyl ring. The most active compound **13** from this group having

K_I=31.4 nM is nearly as active as nonselective CA inhibitor acetazolamide (**AAZ**). The rest of the compounds having bulkier substituents on phenyl ring, except compounds **15** and **19**, have K_I values in range from 134.3 to 220.7 nM.

CA XII inhibited stronger than CA IX where K_I values are in the range from 3.8 to 63.8 nM. Also in case of CA XII distinct SAR cannot be concluded, but all compounds can be split into two group. In the first group compounds **11**, **13**, **14**, **17**, **19** and **20** with low nanomolar K_Is values ranging from 3.8 to 9.5 nM can be included. Compounds in this group, except **17**, have small substituents at 4-position of phenyl ring. Notably, compounds **17** and **20** have stronger inhibition of CA XII compare to nonselective **AAZ**. The other compounds having bulkier substituents on phenyl ring or no phenyl ring exhibited slightly lower inhibition of CA with K_Is values ranging from 18.4 to 63.8 nM.

It is interesting to note, that compare to previous results obtained on structurally similar compounds - sulfocoumarins all oxathiino[6,5-b]pyridine 2,2-dioxides except compound **5** also exhibited no inhibition of ubiquitous CA I and CA II. At the same time CA IX inhibition is similar to one observed for sulfocoumarins. However the oxathiino[6,5-b]pyridine 2,2-dioxide **17** exhibited better inhibition of CA IX compare to sulfocoumarins where best inhibitor had K_I=4.3 nM.[23, 44-46]

Table 1: Inhibition data of human CA isoforms hCA I, II, IX and XII by compounds **5**, **11**-**20** and the standard sulfonamide inhibitor acetazolamide (**AAZ**) by a stopped flow CO_2 hydrase assay[47].



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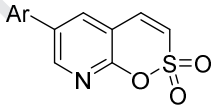
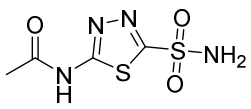


11-20



AAZ

Comp.	CA inhibition, K_I (nM)*			
	hCA I	**hCA II**	**hCA IX**	**hCA XII**
5	64110	14050	169.3	63.8
11	>100000	>100000	86.8	9.5
12	>100000	>100000	137.3	18.4
13	>100000	>100000	31.4	7.2
14	>100000	>100000	86.3	8.7
15	>100000	>100000	134.3	22.7
16	>100000	>100000	220.7	39.8
17	>100000	>100000	56.9	3.8
18	>100000	>100000	210.0	29.4
19	>100000	>100000	200.1	8.5
20	>100000	>100000	70.1	5.3
AAZ	250	12	25	6

*Mean from 3 different assays, by a stopped flow technique (errors were in the range of ± 5-10 % of the reported values). Data were collected after 6 h of incubation period of the enzyme and inhibitor solution.

It was observed, that after 15 min incubation of inhibitor and enzyme solution (data not shown) no inhibition of any of four isoforms studied was observed, therefore we assume that similar to

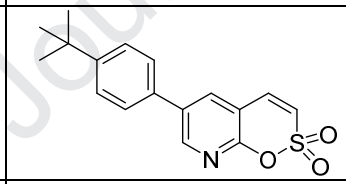
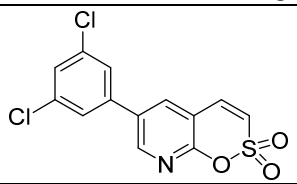
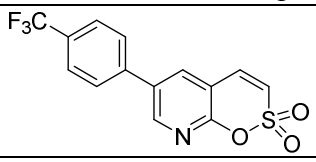
sulfocoumarins oxatiine ring opening takes place in oxathiino[6,5-b]pyridine 2,2-dioxide, and formed sulfonate binds in the active center of CA[23, 48].

2.3. Cell viability

Compounds **13**, **17** and **20** having best CA inhibition profile were used for determination of their antiproliferative properties *in vitro* on monolayer tumor cell lines MCF-7 (human breast adenocarcinoma), A549 (human lung (alveolar) adenocarcinoma) and HeLa (epithelioid cervix carcinoma), the results are summarized in Table 2.

Compounds **17** and **20** showed very good cytotoxic effect on MCF-7 cell line with IC_{50} values 20 and 75 μM, respectively. All three compounds showed moderate to very good cytotoxic effect on HeLa cell line with IC_{50} values from 171 to 4.8 μM. Also moderate to very good cytotoxic effect on A549 cell line was observed for **13**, **17** and **20** with IC_{50} values ranging from 149 to 14 μM. It is interesting to note, that compound **17** exhibited very good cytotoxic effect on all three cancer cell lines.

Table 2. *In vitro* cytotoxicity of compounds **5**, **11**-**20** on monolayer tumor cell lines MCF-7 (human breast adenocarcinoma), HeLa (epithelioid cervix carcinoma) and A549 (human lung (alveolar) adenocarcinoma).

Comp.	Formulas	Cytotoxicity, IC_{50} (μM)		
		MCF-7	**HeLa**	**A549**
13		>500	171±11	149±27
17		20±1	4.8±0.03	14±2
20		75±7	42±9	77±6

3. Conclusions

In conclusion, we report here the facile synthesis of new class of compounds – oxathiino[6,5-b]pyridine 2,2-dioxides. We have demonstrated that oxathiino[6,5-b]pyridine 2,2-dioxides are a new class of tumor associated carbonic anhydrases IX and XII isoform-selective inhibitors. The title

compounds were investigated for the inhibition of four hCA isoforms with medicinal chemistry applications, the cytosolic hCA I and II, and the transmembrane, tumor-associated hCA IX and XII. Oxathiino[6,5-b]pyridine 2,2-dioxides generally do not inhibit the ubiquitous, off-target cytosolic isoforms hCA I and II. However, these compounds showed selective and strong inhibition of the two transmembrane hCAs, with K_Is ranging from 31.4 to 220.7 nM against hCA IX, and from 3.8 to 63.8 nM against hCA XII. The compounds **13**, **17** and **20** having best CA inhibition profile were studied on antiproliferative properties on tumor cell lines MCF-7 (human breast adenocarcinoma), A549 (human lung (alveolar) adenocarcinoma) and HeLa (epithelioid cervix carcinoma), where very good IC_{50} values were observed on all three cell line. Compound **17** showed excellent activities on all three cell lines with IC_{50} ranging from 4.8 to 20 μM. Thus we have demonstrated that oxathiino[6,5-b]pyridine 2,2-dioxides is suitable class of compounds for further studies to identify suitable drug candidates for preclinical and clinical studies.

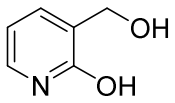
4. Experimental

4.1. Chemistry

All reagents were purchased from commercial sources and used as received without further purification. Thin layer chromatography was performed on *Merck* TLC-plates with fluorescence indication (silica type 60, F_{254}), spots were visualized using UV-light or $KMnO_4$ stains. Flash chromatography was performed using silica with a grain size of 40–63 μm from *Macherey-Nagel*. Deuterated chloroform was purchased from *Deutero*. NMR spectra were recorded on *Bruker 300 Fourier*, *Bruker AV 300* and *Bruker AV 400* spectrometers. The chemical shifts (δ) for ^1H in $CDCl_3$ are given in parts per million (ppm) and referenced to 7.26, respectively. Coupling constants are expressed in Hertz (Hz). The following abbreviations are used: s= singlet, d= doublet, t= triplet, q= quadruplet, m= multiplet.

4.2. Synthesis of inhibitors

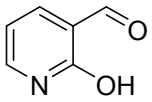
3-(Hydroxymethyl)pyridin-2-ol (**2**)



2-Hydroxynicotinic acid (**1**) (1.00 g, 7.19 mmol) was suspended in dry THF (100 mL). The suspension was cooled to 0 °C and $LiAlH_4$ (0.655 g, 17.3 mmol) was added. The mixture was refluxed for 2 h, then cooled to room temperature and poured onto ice. To the mixture 10% HCl was added (pH=1), then $NaHCO_3$ was added (pH=8). The volatiles were evaporated. The crude

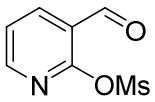
solid was washed with EtOAc (5×100 mL). The combined organic layer was evaporated to give **2** (0.720 g, 80%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 4.28 (s, 2H), 4.99 (br s, 1H), 6.19 (t, *J* = 6.6 Hz, 1H), 7.22–7.27 (m, 1H), 7.38–7.43 (m, 1H), 11.4 (br s, 1H).

2-Hydroxynicotinaldehyde (**3**)



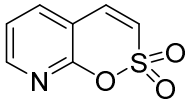
3-(Hydroxymethyl)pyridin-2-ol (**2**) (1.50 g, 12.0 mmol) was suspended in EtOAc (150 mL). To the suspension MnO_2 (10.4 g, 120 mmol) was added. The resulting suspension was stirred at 50 °C for 18 h. The mixture was filtered through celite pad and celite was washed with EtOAc. The solvent was evaporated to yield **3** (1.03 g, 70%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 6.37 (t, *J* = 6.7 Hz, 1H), 7.82 (dd, *J* = 6.3, 2.4 Hz, 1H), 7.98 (dd, *J* = 7.1, 2.3 Hz, 1H), 10.09 (d, *J* = 0.7 Hz, 1H), 12.3 (br s, 1H).

3-Formylpyridin-2-yl methanesulfonate (**4**)



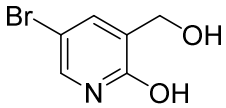
2-Hydroxynicotinaldehyde (**3**) (206 mg, 1.67 mmol) was suspended in dry THF (20 mL). The suspension was cooled to 0 °C and 57-63% NaH on mineral oil (240 mg, 6.0 mmol) was added. The mixture was stirred at 0 °C under argon for 15 min. To the mixture mesyl chloride (0.65 mL, 8.40 mmol) was added. The resulting mixture was stirred at room temperature under argon for 16 h. To the mixture sat. NH_4Cl (15 mL) was added. The mixture was extracted with EtOAc (3×25 mL). The organic layer was dried over Na_2SO_4 and the volatiles were evaporated. The crude was purified by reversed phase column chromatography (sorbent C-18 modified silica gel, eluent MeCN/H_2O gradient) to yield **4** (100 mg, 30%) as a yellow oil. IR (KBr, cm^{-1}) υ_{max}: 1707 (C=O), 1328 (S=O), 1153 (S=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 3.86 (s, 3H), 6.61 (t, *J* = 7.0 Hz, 1H), 8.14 (dd, *J* = 7.0, 2.0 Hz, 1H), 8.26 (dd, *J* = 7.0, 2.0 Hz, 1H), 10.08 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 41.5, 106.4, 126.5, 138.7, 145.1, 160.1, 188.1. HRMS-ESI (m/z) calcd for $C_7H_7NO_4S$ $[M]^+$: 201.1960. Found: 201.1957.

[1,2]Oxathiino[6,5-b]pyridine 2,2-dioxide (**5**)



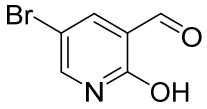
3-Formylpyridin-2-yl methanesulfonate (**4**) (147 mg, 0.731 mmol) and K_2CO_3 (131 mg, 0.950 mmol) were suspended in dry DMF (5 mL). The suspension was stirred at room temperature under argon for 1 h. To the mixture 10% HCl was added (pH=1). The resulting mixture was extracted with EtOAc (3×20 mL). The organic layer was washed with H_2O (3×20 mL) and brine (1×20 mL), dried over Na_2SO_4. The volatiles were evaporated. The crude was dissolved in dry CH_2Cl_2 (5 mL). The solution was cooled to 0 °C. To the solution mesyl chloride (0.08 mL, 1.03 mmol) and NEt_3 (0.14 mL, 1.01 mmol) were added. The resulting mixture was stirred at room temperature under argon for 3 h. The volatiles were evaporated. The crude was purified by reversed phase column chromatography (sorbent C-18 modified silica gel, eluent MeCN/H_2O gradient) to yield **5** (54 mg, 40%) as a light pink solid. Mp 103–105 °C. IR (KBr, cm^{-1}) υ_{max}: 1378 (S=O), 1169 (S=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.57 (dd, *J* = 7.6, 4.9 Hz, 1H), 7.68 (d, *J* = 10.3 Hz, 1H), 7.81 (d, *J* = 10.3 Hz, 1H), 8.26 (dd, *J* = 7.6, 1.9 Hz, 1H), 8.47 (dd, *J* = 4.9, 1.9 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 114.3, 123.3, 123.5, 134.9, 140.2, 150.1, 155.5. Anal. Calcd for $C_7H_5NO_3S$: C, 45.90; H, 2.75; N, 7.65. Found: C, 45.99; H, 2.84; N, 7.55.

5-Bromo-3-(hydroxymethyl)pyridin-2-ol (**7**)



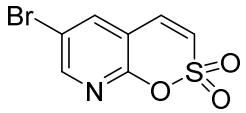
5-Bromo-2-hydroxynicotinic acid (**6**) (2.00 g, 9.17 mmol) was suspended in dry THF (100 mL). The suspension was cooled to 0 °C and 1M BH_3/THF (36.7 mL, 36.7 mmol) was added. The mixture was stirred at room temperature under argon for 18 h. To the mixture 10% HCl was added (pH=1) and the resulting mixture was refluxed for 1 h. The volatiles were evaporated. The crude was purified by reversed phase column chromatography (sorbent C-18 modified silica gel, eluent MeCN/H_2O gradient) to yield **7** (0.993 g, 53%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6, δ): 4.26-4.29 (m, 2H), 7.41–7.44 (m, 1H), 7.52–7.55 (m, 1H), 11.82 (br s, 1H).

5-Bromo-2-hydroxynicotinaldehyde (**8**)



5-Bromo-3-(hydroxymethyl)pyridin-2-ol (**7**) (0.820 g, 4.02 mmol) was suspended in EtOAc (100 mL). To the suspension MnO_2 (3.49 g, 40.2 mmol) was added. The suspension was stirred at 60 °C for 18 h. The resulting mixture was filtered through celite pad and celite was washed with EtOAc. The solvent was evaporated to yield **8** (0.432 g, 53%) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6, δ): 7.97 (d, *J* = 3.0 Hz, 1H), 8.12 (d, *J* = 3.0 Hz, 1H), 10.01 (s, 1H), 12.7 (br s, 1H).

6-Bromo-[1,2]oxathiino[6,5-b]pyridine 2,2-dioxide (**10**)

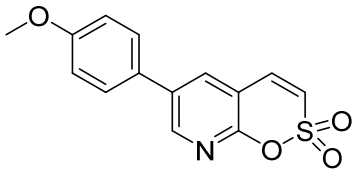


5-Bromo-2-hydroxynicotinaldehyde (**8**) (1.39 g, 6.88 mmol) was dissolved in dry DMF (54 mL). The solution was cooled to 0 ºC and 57–63% NaH on mineral oil (0.339 g, 8.26 mmol) was added. The mixture was stirred under argon at 0 ºC for 10 min. To the mixture mesyl chloride (0.64 mL, 8.26 mmol) was added. The resulting mixture was stirred at room temperature for 25 min. To the mixture K_2CO_3 (1.23 g, 8.90 mmol) was added. The resulting suspension was stirred at room temperature under argon for 75 min. The mixture was poured into 10% HCl (pH=1) and extracted with EtOAc (3×50 mL). The organic layer was washed with H_2O (2×40 mL) and sat. NaCl (1×40 mL) and dried over Na_2SO_4. The volatiles were evaporated. The crude was dissolved in dry DCM (15 mL). The solution was cooled to 0 ºC and NEt_3 (1.44 mL, 10.3 mmol) and mesyl chloride (0.80 mL, 10.3 mmol) were added. The resulting mixture was stirred at room temperature under argon for 3 h. To the mixture H_2O (10 mL) was added and the resulting mixture was extracted with EtOAc (3×20 mL). The organic layer was dried over Na_2SO_4 and the volatiles were evaporated. The crude was purified by reversed phase column chromatography (sorbent C-18 modified silica gel, eluent MeCN/H_2O gradient) to yield **10** (1.04 g, 58%) as a yellow solid. Mp 164–166 °C. IR (neat, cm^{-1}) υ_{max}: 1366 (S=O), 1177 (S=O), 1114 (S=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.74 (d, *J* = 10.3 Hz, 1H), 7.78 (d, *J* = 10.3 Hz, 1H), 8.56 (d, *J* = 2.5 Hz, 1H), 8.62 (d, *J* = 2.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 115.8, 117.3, 124.4, 133.9, 142.0, 150.4, 154.4. Anal. Calcd for $C_7H_4BrNO_3S$: C, 32.08; H, 1.54; N, 5.34. Found: C, 32.58; H, 1.80; N, 5.41.

General procedure for the synthesis of 6-aryl-[1,2]oxathiino[6,5-b]pyridine 2,2-dioxides

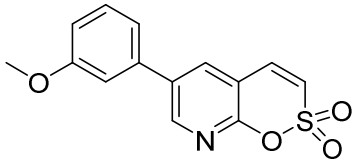
10 (200 mg, 0.763 mmol), appropriate organotin derivative (1.3 equiv.), tri(2-furyl)phosphine (71 mg, 0.305 mmol) and $Pd(OAc)_2$ (34 mg, 0.151 mmol) were suspended in dry 1,4-dioxane (0.7 mL). The suspension was stirred under argon at 100 ºC for 18 h. To the mixture EtOAc (5 mL) was added and the resulting mixture was filtrated through celite pad and the volatiles were evaporated. The crude was purified by reversed phase column chromatography (sorbent C-18 modified silica gel, eluent MeCN/H_2O gradient).

6-(4-Methoxyphenyl)-[1,2]oxathiino[6,5-b]pyridine 2,2-dioxide (**11**)



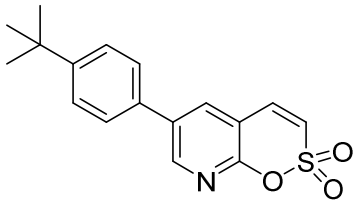
Obtained according to the general procedure from tributyl(4-methoxyphenyl)stannane (394 mg, 0.992 mmol). The crude was recrystallized from EtOH to yield **11** (133 mg, 60%) as a yellow solid. Mp 165–166 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 3.82 (s, 3H), 7.07–7.12 (m, 2H), 7.69–7.74 (m, 3H), 7.81 (d, *J* = 10.3 Hz, 1H), 8.52 (d, *J* = 2.5 Hz, 1H), 8.72 (d, *J* = 2.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 55.3, 114.1, 114.7, 123.8, 127.4, 128.3, 134.9, 135.0, 137.3, 147.2, 154.3, 159.9. Anal. Calcd for $C_{14}H_{11}NO_4S$: C, 58.12; H, 3.83; N, 4.84. Found: C, 58.15; H, 3.81; N, 4.70.

6-(3-Methoxyphenyl)-[1,2]oxathiino[6,5-b]pyridine 2,2-dioxide (**12**)



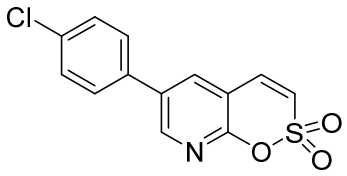
Obtained according to the general procedure from tributyl(3-methoxyphenyl)stannane (394 mg, 0.992 mmol). The crude was recrystallized from EtOH to yield **12** (150 mg, 68%) as a yellow solid. Mp 151–152 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 3.84 (s, 3H), 7.02–7.06 (m, 1H), 7.31–7.35 (m, 2H), 7.42–7.48 (m, 1H), 7.73 (d, *J* = 10.3 Hz, 1H), 7.82 (d, *J* = 10.3 Hz, 1H), 8.59 (d, *J* = 2.5 Hz, 1H), 8.79 (d, *J* = 2.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 55.3, 112.5, 114.0, 114.4, 119.2, 123.9, 130.4, 134.9, 135.0, 136.5, 138.1, 147.9, 154.8, 159.9. Anal. Calcd for $C_{14}H_{11}NO_4S$: C, 58.12; H, 3.83; N, 4.84. Found: C, 58.12; H, 3.79; N, 4.72.

6-(4-(*tert*-Butyl)phenyl)-[1,2]oxathiino[6,5-b]pyridine 2,2-dioxide (**13**)



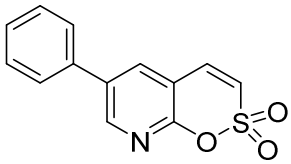
Obtained according to the general procedure from tributyl(4-(tert-butyl)phenyl)stannane (420 mg, 0.992 mmol) as yellow needles (144 mg, 60%). Mp 212–214 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 1.32 (s, 9H), 7.53–7.58 (m, 2H), 7.67–7.72 (m, 2H), 7.73 (d, *J* = 10.3 Hz, 1H), 7.84 (d, *J* = 10.3 Hz, 1H), 8.56 (d, *J* = 2.5 Hz, 1H), 8.76 (d, *J* = 2.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 31.0, 34.4, 114.1, 123.9, 126.1, 126.7, 132.3, 135.0, 135.1, 137.7, 147.6, 151.3, 154.6. Anal. Calcd for $C_{17}H_{17}NO_3S$: C, 64.74; H, 5.43; N, 4.44. Found: C, 64.54; H, 5.73; N, 4.43.

6-(4-Chlorophenyl)-[1,2]oxathiino[6,5-b]pyridine 2,2-dioxide (**14**)



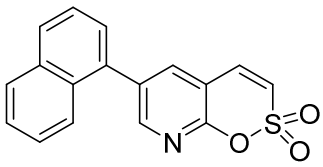
Obtained according to the general procedure from tributyl(4-chlorophenyl)stannane (398 mg, 0.992 mmol). The crude was recrystallized from EtOH to yield **14** (115 mg, 51%) as an orange solid. Mp 203–205 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.58–7.64 (m, 2H), 7.74 (d, *J* = 10.2 Hz, 1H), 7.79–7.84 (m, 3H), 8.60 (d, *J* = 2.5 Hz, 1H), 8.79 (d, *J* = 2.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 114.1, 124.0, 128.8, 129.2, 133.7, 133.9, 134.0, 134.9, 138.1, 147.8, 154.9. Anal. Calcd for $C_{13}H_8ClNO_3S$: C, 53.16; H, 2.75; N, 4.77. Found: C, 53.06; H, 2.80; N, 4.64.

6-Phenyl-[1,2]oxathiino[6,5-b]pyridine 2,2-dioxide (**15**)



Obtained according to the general procedure from tributyl(phenyl)stannane (364 mg, 0.992 mmol). The crude was recrystallized from EtOH to yield **15** (145 mg, 73%) as a yellow solid. Mp 187–188 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.44–7.50 (m, 1H), 7.51–7.57 (m, 2H), 7.74 (d, *J* = 10.3 Hz, 1H), 7.76–7.79 (m, 2H), 7.84 (d, *J* = 10.3 Hz, 1H), 8.59 (d, *J* = 2.5 Hz, 1H), 8.78 (d, *J* = 2.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 114.1, 123.9, 127.0, 128.7, 129.3, 134.9, 135.1, 135.2, 138.0, 147.8, 154.8. Anal. Calcd for $C_{13}H_9NO_3S$: C, 60.22; H, 3.50; N, 5.40. Found: C, 60.02; H, 3.43; N, 5.27.

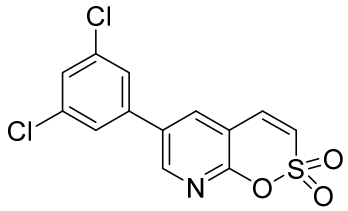
6-(Naphthalen-1-yl)-[1,2]oxathiino[6,5-b]pyridine 2,2-dioxide (**16**)



Obtained according to the general procedure from tributyl(naphthalen-1-yl)stannane (414 mg, 0.992 mmol). The crude was crystallized from Et_2O to yield **16** (96 mg, 41%) as a yellow solid. Mp 148–150 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.53–7.67 (m, 4H), 7.75–7.81 (m, 2H), 7.87 (d, *J* = 10.3 Hz, 1H), 8.04–8.09 (m, 2H), 8.45 (d, *J* = 2.4 Hz, 1H), 8.58 (d, *J* = 2.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 113.8, 123.8, 124.7, 125.6, 126.4, 127.1, 127.9, 128.5, 129.0, 130.7, 133.4,

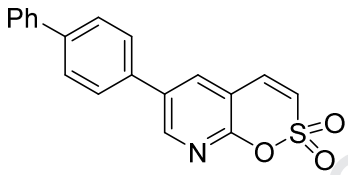
133.8, 134.9, 135.0, 141.0, 150.0, 154.8. Anal. Calcd for $C_{17}H_{11}NO_3S$: C, 66.01; H, 3.58; N, 4.53. Found: C, 65.79; H, 3.90; N, 4.51.

6-(3,5-Dichlorophenyl)-[1,2]oxathiino[6,5-b]pyridine 2,2-dioxide (**17**)



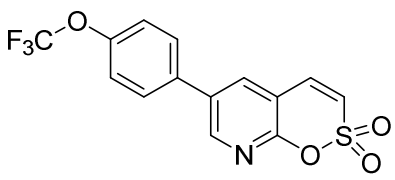
Obtained according to the general procedure from (3,5-dichlorophenyl)trimethylstannane (307 mg, 0.992 mmol). The crude was recrystallized from EtOH to yield **17** (116 mg, 46%) as a yellow solid. Mp 220–222 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.71 (t, *J* = 1.9 Hz, 1H), 7.75 (d, *J* = 10.3 Hz, 1H), 7.78 (d, *J* = 10.3 Hz, 1H), 7.90 (d, *J* = 1.9 Hz, 2H), 8.68 (d, *J* = 2.5 Hz, 1H), 8.86 (d, *J* = 2.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 114.0, 124.1, 125.8, 128.1, 132.3, 134.7, 134.9, 138.6, 138.7, 148.3, 155.3. Anal. Calcd for $C_{13}H_7Cl_2NO_3S$: C, 47.58; H, 2.15; N, 4.27. Found: C, 47.45; H, 2.02; N, 4.29.

6-([1,1'-Biphenyl]-4-yl)-[1,2]oxathiino[6,5-b]pyridine 2,2-dioxide (**18**)



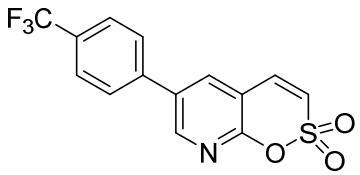
Obtained according to the general procedure from [1,1'-biphenyl]-4-yltrimethylstannane (314 mg, 0.992 mmol). The crude was recrystallized from EtOH to yield **18** (112 mg, 44%) as a brown solid. Mp 239–240 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.37–7.43 (m, 1H), 7.47–7.53 (m, 2H), 7.72–7.78 (m, 3H), 7.82–7.91 (m, 5H), 8.65 (d, *J* = 2.5 Hz, 1H), 8.84 (d, *J* = 2.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 114.1, 123.9, 126.7, 127.4, 127.5, 127.8, 129.0, 134.1, 134.6, 134.9, 137.9, 139.2, 140.4, 147.6, 154.8. Anal. Calcd for $C_{19}H_{13}NO_3S$: C, 68.05; H, 3.91; N, 4.18. Found: C, 67.84; H, 4.20; N, 4.16.

6-(4-(Trifluoromethoxy)phenyl)-[1,2]oxathiino[6,5-b]pyridine 2,2-dioxide (**19**)



Obtained according to the general procedure from tributyl(4-(trifluoromethoxy)phenyl)stannane (448 mg, 0.992 mmol). The crude was recrystallized from EtOH to yield **19** (131 mg, 50%) as a yellow solid. Mp 143–144 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.52–7.57 (m, 2H), 7.75 (d, *J* = 10.3 Hz, 1H), 7.83 (d, *J* = 10.3 Hz, 1H), 7.89–7.93 (m, 2H), 8.61 (d, *J* = 2.5 Hz, 1H), 8.80 (d, *J* = 2.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 114.1, 120.0 (q, *J* = 256.6 Hz), 121.7, 124.0, 129.1, 133.8, 134.5, 134.8, 138.3, 148.0, 148.7 (q, *J* = 1.9 Hz), 155.0. Anal. Calcd for $C_{14}H_8F_3NO_4S$: C, 48.99; H, 2.35; N, 4.08. Found: C, 48.78; H, 2.32; N, 3.92.

6-(4-(Trifluoromethyl)phenyl)-[1,2]oxathiino[6,5-b]pyridine 2,2-dioxide (**20**)



Obtained according to the general procedure from tributyl(4-(trifluoromethyl)phenyl)stannane (432 mg, 0.992 mmol). The crude was recrystallized from EtOH to yield **20** (72 mg, 29%) as a yellow solid. Mp 185–187 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.76 (d, *J* = 10.3 Hz, 1H), 7.85 (d, *J* = 10.3 Hz, 1H), 7.89–7.93 (m, 2H), 7.99–8.03 (m, 2H), 8.68 (d, *J* = 2.5 Hz, 1H), 8.86 (d, *J* = 2.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 114.2, 124.0, 124.2 (q, *J* = 272.0 Hz), 126.1 (q, *J* = 3.9 Hz), 127.9, 129.0 (q, *J* = 32.0 Hz), 133.6, 134.8, 138.6, 139.3 (q, *J* = 1.2 Hz), 148.3, 155.3. Anal. Calcd for $C_{14}H_8F_3NO_3S$: C, 51.38; H, 2.46; N, 4.28. Found: C, 51.07; H, 2.74; N, 4.25.

4.3. CA inhibition

An Applied Photophysics stoppedflow instrument has been used for assaying the CA catalysed CO_2 hydration activity. Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5) as buffer, and 20 mM 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 0.01 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. Data from Table 1 were obtained after 6 h incubation of enzyme and inhibitor, as for coumarins reported earlier[2, 3, 27, 28, 30, 49-52]. The inhibition constants were obtained by

non-linear leastsquares methods using PRISM 3, as reported earlier[53-56], and represent the mean from at least three different determinations. All CA isofoms were recombinant ones obtained in-house as reported earlier[2, 3, 27, 28, 30, 49-52, 57-59].

4.4. *In vitro* viability

Cells were seeded on a 96-well plate in DMEM medium supplemented with 10% fetal bovine serum and 2 mM glutamine and growth 24 h. Then the tested compound was added and cultivated in thermostat 48 hours. Cell viability was measured by colouring with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). After incubation with tested compound the medium was replaced with new one containing 0.2 mg/ml MTT. After incubated 3 hours in thermostat at 37 °C with 5% CO_2, removal of medium, the dye was extracted with 0.2 ml DMSO. Optical density (OD) determine using multichannel spectrophotometer (*Tecan multiplate reader Infinite100)* at 540 nm. Then calculate IC_{50} – the concentration of tested compound providing the death of 50% of cells by the usage of program Graph Pad Prism® 3.0.

Supplementary data

Can be found on...

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Highlights

- The facile synthesis of oxathiino[6,5-b]pyridine 2,2-dioxides (OPDs) was developed.
- OPDs are selectively and effectively inhibitors of CA IX and CA IX.
- Compound **17** have good antiproliferative properties on MCF-7, A549 and HeLa cells.

Declaration of interest statement

Dear Editor,

the authors have declared no conflicts of interest.

Prof. Raivis Žalubovskis