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Original article

Carbonic anhydrase inhibitors. Regioselective synthesis of novel series 1-substituted 1,4-dihydro-4-oxo-3-pyridinesulfonamides and their inhibition of the human cytosolic isozymes I and II and transmembrane cancer-associated isozymes IX and XII

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ABSTRACT

A series of novel 1-substituted 1,4-dihydro-4-oxo-3-pyridinesulfonamides **4**–**6**, **9**–**17** and **21**–**31** have been synthesized and investigated as inhibitors of four isoforms of zinc enzyme carbonic anhydrase (CA.EC 4.2.1.1), that is the cytosolic CA I and II, and cancer-associated isozymes CA IX and XII. Against the human isozymes hCA I the new compounds showed K_Is in the range of 224–4830 nM, whereas toward hCA II, K_Is = 318–873 nM. Isozyme hCA IX was inhibited with K_Is = 11.8–93.4 nM, and hCA XII with 23.5 –82.3 nM. Compounds **12**–**14**, **27** and **29**–**31** have an activity against hCA I (K_Is = 224–889 nM) which is comparable to the clinically used sulfonamide **DCP** (K_Is = 1200 nM). Several of new compounds, including **9**, **10**, **21**, **24**, **26**–**28** and **30** have an activity against hCA IX (K_Is = 11.8–38.6 nM) which is comparable or more effective than the clinically used sulfonamides **AAZ**, **MZA**, **EZA**, **DCP** and **IND** (K_Is = 24–50 nM). Compounds **9**, **10**, **13**, **21**–**23**, **26** and **27** were also very effective hCA XII inhibitors, with inhibition constants ranged from 23.5 to 47.2 nM comparable or more effective than sulfonamides **EZA** (K_Is = 22 nM) or **DCP** (K_Is = 50 nM), respectively.

1. Introduction

The connections between carbonic anhydrase and cancer is known for approximately fifteen years, since two tumor-associated transmembrane carbonic anhydrase isozymes (CA IX and CA XII) have been identified, cloned and sequenced [1–3]. Many reports have been published on the role of CA IX and CA XII in tumor physiology [4–14]. In addition its role in the controlling of the tumor pH, there are evidenced that CA IX can also influence other processes in the cell microenvironment that promote cell proliferation, invasion, and metastasis [7]. Upregulated expression of CA IX was reported in carcinoma cells derived from various organ including breast, cervix, bladder, esophagus, lung, kidney, colon and rectum, head and neck. This protein constitutes an endogenous marker of cellular hypoxia, a natural phenotype of solid tumors [15], and its predicative and prognostic potential has been demonstrated in various clinical studies [16]. Considering the abnormally high expression of CA IX in many hypoxic tumors and its demonstrated role in the tumor acidification processes and oncogenesis, CA IX constitutes a candidate target for anticancer therapy. Thus, agents that can selectively inhibit CA IX activity may have therapeutic value and offer opportunities for the treatment or prevention of a variety of cancers. The potential use of CA inhibitors as antitumor agents opens thus a new important research direction [4,17,18]. For example, it has been recently demonstrated that sulfonamide or coumaric potent CA IX inhibitors have a potent effect in inhibiting the growth of both primary tumors and metastases in an animal model of breast cancer [12,19].

In order to remind CA inhibition mechanism by sulfonamide inhibitors (Fig. 1) should be noted that the active domain of CAIs contains an active Zn(II) ion site; a strong Lewis acid that binds to, and activates a substrate H_2O molecule to catalyze the reversible hydration reaction of carbon dioxide. The metal ion is situated at the bottom of active site, being coordinated by three histidine residues (His 94, His 96 and His 119) and water molecule/hydroxide ion (Fig. 1) [6].

Sulfonamides are the most important CAIs, bind in a tetrahedral geometry of the Zn(II) ion, in deprotonated state (Fig. 1). On the

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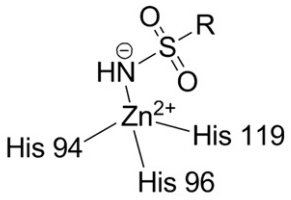


$$E\text{-}Zn^{2+}\text{-}OH_2 + I \longleftrightarrow En\text{-}Zn^{2+}\text{-}I + H_2O$$

Tetrahedral sulfonamide adduct

Fig. 1. CA inhibition mechanism by sulfonamide inhibitors [6].

basis of X-ray crystallographic structures for many adducts of sulfonamide inhibitors with ubiquitous isozymes CA I, II or IV [20–23] it clearly shows that the deprotonated sulfonamide is coordinated to the Zn(II) ion of the enzyme (Fig. 1), and its NH moiety participates in hydrogen bond with the Oγ of Thr 199, which in turn is engaged in another hydrogen bond to the carboxylate group of Glu 106 [22,23], whereas one of the oxygen atoms of the sulfonamide moiety also participated in hydrogen bond with backbone NH moiety of Thr 199 [22,23]. These structures provide close insight into why the sulfonamide group appears to have unique properties for CA inhibition. Recently, the report of the X-ray crystal structure of CA IX [24] showed that this isoform is a dimeric protein with a quaternary structure not evidenced earlier for this family of the enzymes, allows in near future for structure-based drug design investigations of the inhibitors against this novel antitumor target. Interestingly, as observed in the structure of other α-CA isozymes, the isoform IX active site is located in a large conical cavity, which spans from the surface to the center of the protein. The Zn(II) ion is located at the bottom of this cavity, and two distinct regions made of hydrophobic or hydrophilic amino acids delimit the active site [24].

Recently, we have reported on the strong inhibition of human cytosolic isozymes CA I and II and tumor-associated isozymes CA IX and XII with some 4-chloro-5-methyl-2-(R-thio)benzenesulfonamides of type **I** [25–27] (Fig. 2). We also described the syntheses of a number of 3-pyridinesulfonamides of types: **II** [28,29] and **III** [30] (Fig. 2) and inhibitory activities against human isozymes hCA I, II, IX, XII and XIV [19,25,26]. Numerous of these compounds exhibit excellent hCA IX inhibitory efficacy with inhibition constants 4.6–12.0 nM, being thus much more effective as compared to the clinically used sulfonamides **AAZ**, **MZA**, **EZA**, **DCP** and **IND** (K_Is = 24–50 nM). Generally, different aryl/heteroaryl sulfonamides do not show specificity for the inhibition of the cancer-associated isoforms versus the remaining CA isozymes CA I–VII and XII–XV found in mammals. So far, a lot of effort has been made in solving this low selectivity problem. This fact among others prompted us to synthesize and investigate the related 1-substituted 1,4-dihydro-4-oxo-3-pyridinesulfonamides of type **IV** (Fig. 2), to recognize the structural features contributing to the carbonic anhydrase inhibitors.

2. Results and discussion

2.1. Chemistry

The regioselective synthesis of the target 1,4-dihydro-4-oxo-3-pyridinesulfonamides **4**–**6**, **9**–**17** and **21**–**31** was achieved in satisfactory yields (64–85%) by a facile one-step procedure starting from previously described 4-methoxy-3-pyridinesulfonamide **1** [28] and the appropriate R-methyl halides in dry acetonitrile. The proposed mechanism leading to the formation of the products **4**–**6**, **9**–**17** and **21**–**31** is outlined in Schemes 1, 2 and 4. The initial step is believed to be the formation of intermediate1-pyridinium salts of type **A** (Scheme 1) **D**, **E** and **F** (Scheme 2) or **G** (Scheme 4) which undergoes S_N2 substitution on the methyl by halide to form the 4-pyridones **4**–**6**, **9**–**17** and **21**–**31**. However, we found that analog

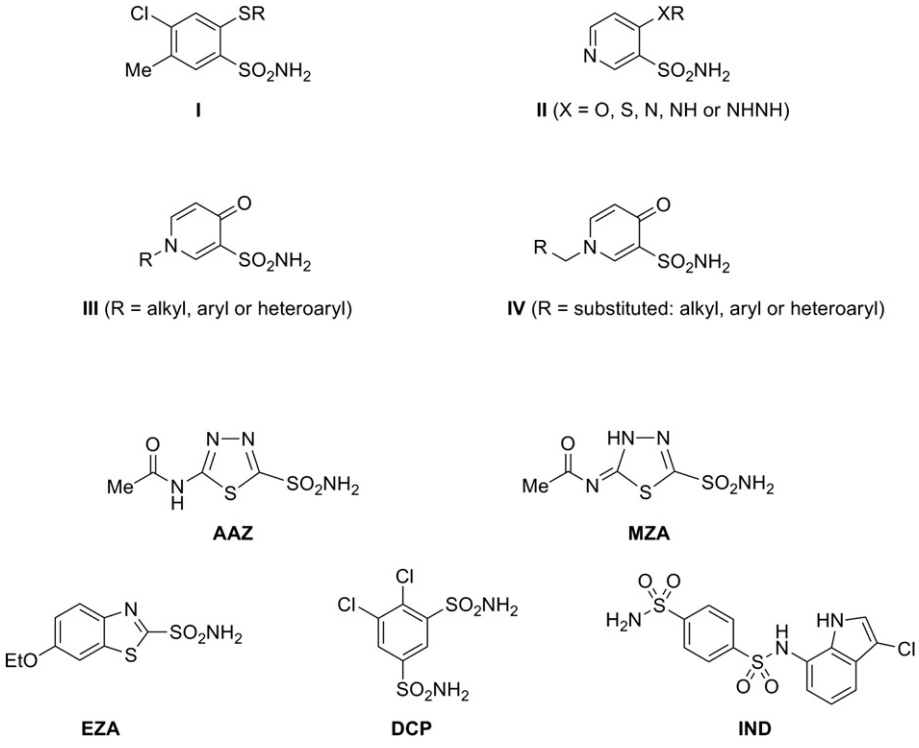
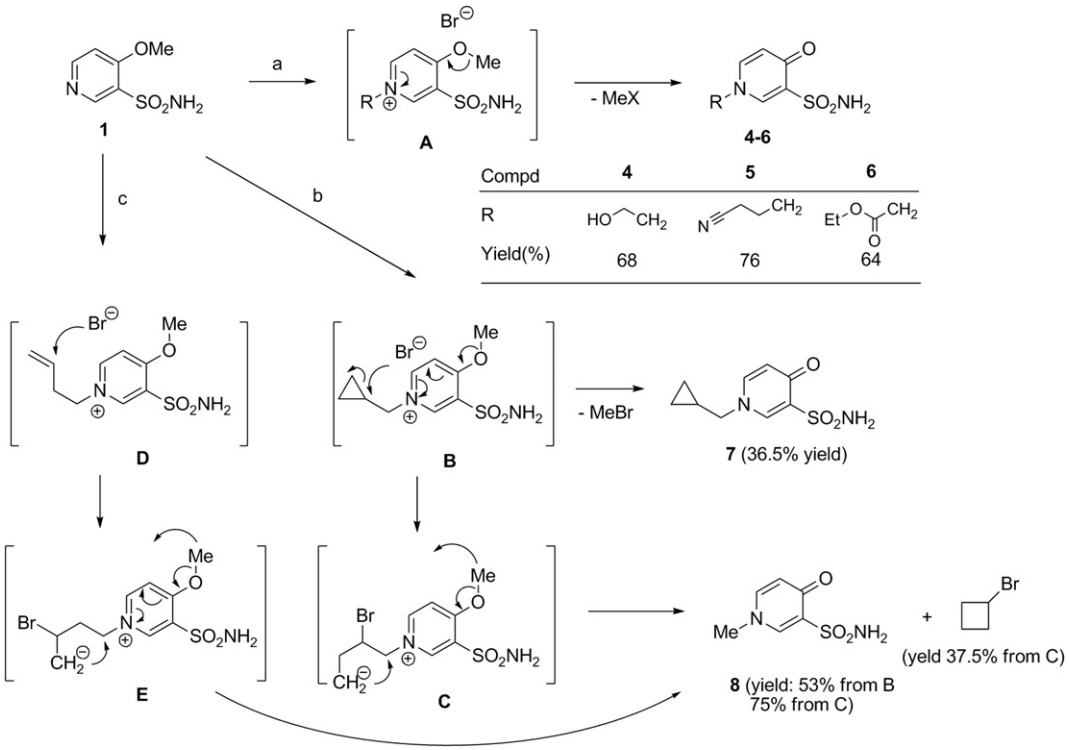
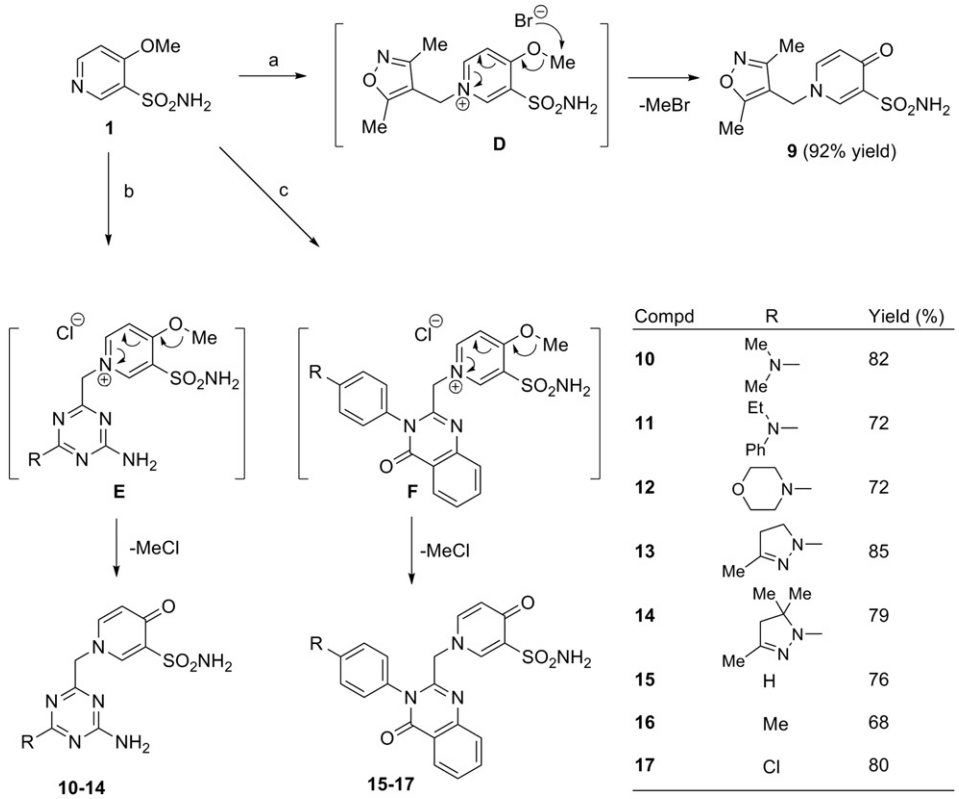


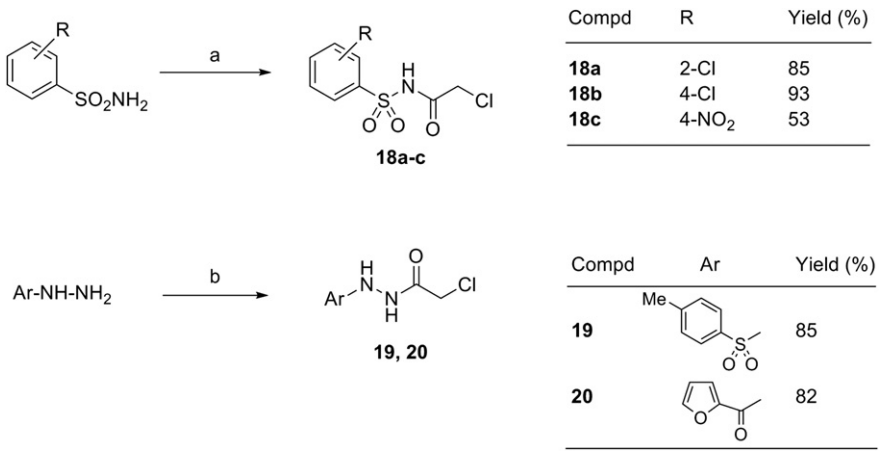
Fig. 2. General structures of known **I**–**III** and novel **IV** arylsulfonamides, and clinically used sulfonamides **AAZ**, **MZA**, **EZA**, **DCP** and **IND** (standard CA inhibitors).



Scheme 1. Synthesis and proposed mechanisms of the formation of 1-substituted 3-pyridinesulfonamides **4**–**8**. Reagents and conditions: (a) appropriate 2-bromoethanol, 4-bromobutyronitrile or ethyl chloroacetate (1.083 M equiv), dry acetonitrile, r.t. 24 h, reflux 60 h; (b) (bromomethyl)cyclopropane (1.083 M equiv) dry acetonitrile, r.t. 24 h, reflux 60 h; (c) 4-bromo-1-butene (1.083 M equiv.), dry acetonitrile, r.t. 24 h, reflux 60 h.



Scheme 2. Synthesis of 1-(heteroaryl)methyl-1,4-dihydro-4-oxo-3-pyridinesulfonamides **9**–**17**. Reagents and conditions: (a) 4-chloromethyl-3,5-dimethylisoxazole (1.05 M equiv), dry acetonitrile, r.t. 36 h, reflux 96 h; (b) appropriate 2-amino-6-chloromethyl-4-R-1,3,5-triazines **2a**–**e** (1.05 M equiv), dry acetonitrile, r.t. 24 h, reflux 50 h; (c) appropriate 2-chloromethyl-3-(4-R-phenyl)quinazolin-4(3*H*)-ones **3a**–**c** (1.0 M equiv), dry acetonitrile, r.t. 24 h, reflux 50 h.



Compd	R	Yield (%)
18a	2-Cl	85
18b	4-Cl	93
18c	4-NO_2	53

Compd	Ar	Yield (%)
19		85
20		82

Scheme 3. Synthesis of *N*-(chloroacetyl)benzenesulfonamides **18a**–**c** and new *N*-(chloroacetyl)hydrazines **19**, **20** as substrates for the synthesis of the target 3-pyridinesulfonamides **27**–**31**. Reagents and conditions: (a) chloroacetyl chloride (large excess), reflux, 16 h; (b) chloroacetyl chloride (1.13 M equiv), dry 1,4-dioxane, 0–10 °C, 1 h, reflux 2 h.

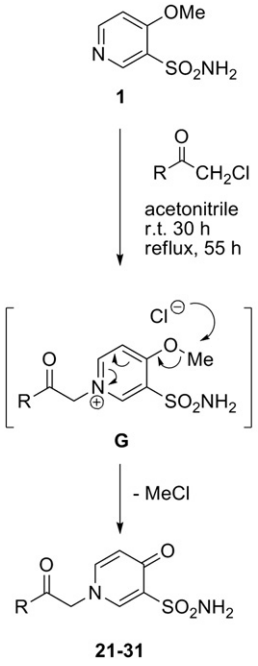
reaction of 4-methoxy-3-pyridinesulfonamide **1** with (bromomethyl)cyclopropane led to the formation of a mixture of target 1-(cyclopropylmethyl)-1,4-dihydro-4-oxo-3-pyridinesulfonamide **7** and 1-methyl-1,4-dihydro-3-pyridinesulfonamide **8** as main reaction products, which were separated in 36.5 and 53% yields, respectively. We propose a reaction sequence for these transformations as shown in Scheme 1. The initial step in this reactions would be formation of transitional salts of type **B** and **C**, which were stabilized by elimination of methyl bromide or cyclobutyl bromide to give the corresponding 3-pyridinesulfonamides **7** and **8** as final products.

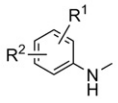
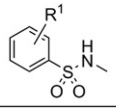
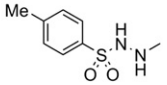
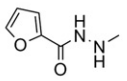
The attempted transformation of **1** into target 1-(3-butenyl)-1,4-dihydro-4-oxo-3-pyridinesulfonamide by reaction with 4-bromo-1-butenal failed. As shown in Scheme 1 the initial step of the reaction is believed to be formation of 1-(3-butenyl)-4-methoxy-3-sulfamoyl-1-pyridinium bromide intermediate **D**, and then intermediate **E**, which was stabilized by elimination of cyclobutyl bromide, to give 1-methyl-1,4-dihydro-4-oxo-3-pyridinesulfonamide **8** as main product in 75% separated yield.

In turn, as shown in Scheme 3, the *N*-(chloroacetyl)benzenesulfonamides (**18a**–**c**) and *N*-(chloroacetyl)hydrazines (**19**–**20**) as substrates for the synthesis of the target 3-pyridinesulfonamides **27**–**31** were achieved in good (53–93%) yields by reaction of the appropriate benzenesulfonamides with an excess of boiling chloroacetyl chloride or arylsulfonylhydrazines with chloroacetyl chloride in boiling 1,4-dioxane.

It should be noted however, that the compounds **18a**–**c** which were obtained by the previously described method [31] in the reaction of chloroacetyl chloride with benzenesulfonamides in alkaline (NaOH) aqueous solution, proved to be insufficiently pure for our purposes.

All finale products were characterized by IR and NMR spectroscopy as shown in experimental protocols. Elemental analyses (C, H, N) were in accordance with the proposed structures.



Compd	R	R^1	R^2	Yield (%)
21		2-Me	6-Me	74.5
22		4-Cl	H	76.8
23		4-Cl	3-NO_2	84.0
24		H	3-NO_2	71.0
25		2-MeOCO	H	82.1
26		4-SO_2NH_2	H	77.6
27		2-Cl		80.0
28		4-Cl		77.0
29		4-NO_2		78.0
30				73.1
31				69.7

Scheme 4. Synthesis of 1-substituted 1,4-dihydro-4-oxo-3-pyridinesulfonamides **21**–**31**.

2.2. CA inhibition studies

The compounds **4**–**7**, **9**–**17** and **21**–**31** as well as standard, clinical used CAIs, such as acetazolamide **AAZ**, methazolamide **MZA**, ethoxzolamide **EZA**, dichlorophenamide **DCP**, and indisulam **IND** (Fig. 1) have been tested for the inhibition of two transmembrane cancer-associated isoforms hCA IX and XII (Table 1). Data for the inhibition of the dominant human isoforms hCA I and II with these compounds are also included in Table 1, for comparison reasons. The following structure–activity relationships can be observed from data of Table 1:

(a) The compounds **9**, **10**, **21**, **24**, **26**–**28** and **30** showed high hCA IX inhibitory activity, with K_is in the range of 11.8–38.6 nM, being comparable or more effective, than those clinically used sulfonamides **AAZ**, **MZA**, **EZA**, **DCP** and **IND** (Table 1), and show good selectivity ratios CA IX/CA II in the range of 8.8–46.5, while this selectivity ratios for the clinical used standard drugs presented above are in the range of 0.33–0.76. These compounds are generally 1-(R^1-methy)-1,4-dihydro-4-oxo-3-pyridinesulfonamide derivatives incorporating substituents (R^1) such as heteroaryl (**9**, **10**), phenylcarbamoyl (**21**, **24** and **26**), phenylsulfonamidocarbonyl (**27**, **28**) or phenylsulfonylhydrazinocarbonyl (**30**) moieties. It should be observed that most of these compounds act as ineffective hCA I inhibitors (micromolar range) and rather weak effective hCA II ones (nanomolar range Table 1). It is pertinent to note, that even slight structural modification in heteroaryl substituent R^1 causes significant decrease in inhibitory potency in the series of similar scaffolds, for example, replacement of $R^2 = Me_2N-$ in active **10** by Ph(Et)N– (**11**) or 1-morfolinyl (**12**) results in weak active compound against hCA IX with K_is = 50.1 or 72.5 nM in **11** and **12**, respectively, versus K_is = 16.5 nM of **10**. The same situation is observed in a series of compounds **21**–**26**, where replacement of two Me groups in positions 2 and 6 on benzene ring in substituent R^1 in active **21**, by chlorine atom in position 4 (**22**) or methoxycarbonyl mioety in position 2 (**25**), lead to significantly lowest inhibitory activity against isoform IX K_is = 17.3 nM (**21**), versus K_is = 93.4 or 86.4 nM in **22** and **25**, respectively). In the series of structurally related **27**–**29**, the presence of chlorine atom in positions 2 or 4 in benzene ring (**27**, **28**) is favorable for activity against hCA IX, whereas their exchange on nitro group (NO_2) in position 4, two-fold decrease the potency of **29** (K_is = 29.7 or 38.6 nM for **27**, **28**), versus K_is = 65.2 nM in **29**, respectively). The other adequate example of substituent influences on inhibitory activity in a series of structurally related 1,4-dihydro-4-oxo-3-pyridinesulfonamide derivatives, may be the comparison of compounds **30** and **31** in which presence of *p*-tosyl group attached to the hydrazide moiety in R^1 is favorable for activity of **30** (K_is = 11.8 nM), while exchange of this group by 2-furoyl moiety results in 4-fold lowering of activity for hCA IX in **31** (K_is = 56.1 nM) (Table 1).

(b) A rather large numbers of the investigated 1-substituted 1,4-dihydro-4-oxo-pyridine-3-sulfonamide derivatives such as: **4**–**7**, **11**–**17**, **22**, **23**, **25**, **29** and **31** showed moderate to low hCA IX inhibitory activity, with K_Is in the range of 50.1–93.4 nM (Table 1). These compounds except of **12**–**14**, **29** and **31** act as ineffective hCA I inhibitors (K_is in the range of 1571–4830 nM) and rather very weak effective hCA II inhibitors (K_is of 358–873 nM) (Table 1).

(c) In the most cases, among 1-substituted 1,4-dihydro-4-oxo-pyridine-3-sulfonamide derivatives which inhibited effectively transmembrane isoform hCA IX, was also found activity against the second transmembrane cancer-associated isoform hCA XII. For example compounds **9**, **10**, **21**, **26** and **27** acting as strong inhibitors of isoform XII with K_is in the range of 23.5–43.3 nM, with the exception of compounds **25**, **28** and **30** (K_is of 61.3–79.8 nM) (Table 1). On the other hand the relatively high potency of compounds **13** and **22** (K_is of 32.4–45.2 nM) against hCA XII, did not find confirmation in their activity against hCA IX (K_is of 77.8 and 93.4 nM), respectively (Table 1).

(d) Generally, it should be pointed out, that the investigated pyridine-3-sulfonamide derivatives **4**–**7**, **11**, **12**, **14**–**17**, **24**, **25** and **28**–**31** showed moderate inhibition of isoform XII with K_Is in the range of 58.0–82.3 nM, whereas remaining derivatives **9**, **10**, **13**, **21**–**23**, **26** and **27** were very effective hCA XII inhibitors with K_Is ranging from 23.5 to 47.2 nM, which are comparable or more effective than sulfonamides **EZA** and **DCP**, respectively (Table 1).

In conclusion, structure–activity relationships is thus very difficult to interpret for the inhibition of transmembrane cancer-associated hCA IX and XII with these compounds, as no X-ray crystal structures of the enzymes in adducts with inhibitors, are available. However, presented data prove that many 1-substituted 1,4-dihydro-4-oxo-pyridine-3-sulfonamide derivatives incorporating heteroaryl, phenylcarbamoyl, phenylsulfonamidocarbonyl or phenylsulfonylhydrazinocarbonyl moieties can be used for designing efficient transmembrane cancer-associated hCA IX and XII inhibitors, with good selectivity ratios CA IX/CA II in the range of 16.5–46.5 for the most active compounds **9**, **10**, **21**, **24**, **26** and **30**.

3. Conclusions

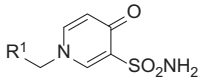
We have developed methods for the regioselective synthesis of 1-substituted 1,4-dihydro-4-oxo-3-pyridinesulfonamides. The 24 new compounds have been assayed for the inhibition of four physiological CA isozymes, such as CA I and II and the cancer-associated isozymes CA IX and XII. Against the human isozyme hCA I the new sulfonamides showed inhibition constants in the range of 224–4830 nM, against hCA II in the range of 318–873 nM, against hCA IX K_Is = 11.8–93.4 nM, while toward hCA XII K_Is = 23.5–82.3 nM. Compounds **12**–**14**, **27** and **29**–**31** have an activity against hCA I (K_Is = 224–889 nM), which is comparable to the clinically used sulfonamides **DCP** (K_Is = 1200 nM). Several of new compounds including **9**, **10**, **21**, **24**, **26**–**28** and **30** have an activity against hCA IX (K_Is = 11.8–38.6 nM), thus showed efficacy comparable to the clinically used sulfonamides **AAZ**, **MZA**, **EZA**, **DCP** and **IND** (K_Is = 24–50 nM). Compounds **9**, **10**, **13**, **21**–**23**, **26** and **27** were also very effective hCA XII inhibitors, with inhibition constants 23.5–47.2 nM, comparable or more effective than sulfonamides **EZA** (K_Is = 22 nM) or **DCP** (K_Is = 50 nM), respectively. Some of these compounds **9**, **10**, **21**, **24**, **26** and **30** are selective CA IX inhibitors over CA II inhibitors with selectivity ratios in the range of 16.5–46.5, making them interesting candidates for targeting hypoxic tumors overexpressing CA IX and/or CA XII.

4. Experimental protocols

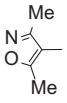
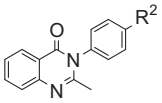
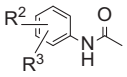
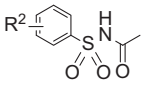
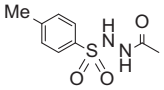
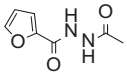
4.1. Synthesis

The following instruments and parameters were used: melting points Buchi 535 apparatus, IR spectra: KBr pellets, 400–4000 cm^{-1} Perkin Elmer FT IR spectrophotometer; ^1H, ^{13}C NMR: Varian Gemini 200 apparatus at 200 and 50 MHz, respectively; chemical shifts are expressed in parts per million (ppm) relative to TMS as internal standard. The results of elemental analyses for C, H and N were within ±0.4% of theoretical values. The commercially unavailable substrates were obtained according to

Table 1
Carbonic anhydrase inhibition data for compounds **4**–**7**, **9**–**17** and **21**–**31** and standard inhibitors against human isozymes hCA I, II, IX and XII by a stopped-flow CO_2 hydrase assay [37].



4-7, 9-17 and **21-31**

Compd.	R^1	R^2	R^3	K_i[a] (nM)			
				hCA I	hCA II	hCA IX	hCA XII
AAZ				301	12	25.0	5.7
MZA				780	14	27.0	3.4
EZA				25	8	34.0	22.0
DCP				1200	38	50.0	50.0
IND				31	15	24.0	3.4
4	HO–CH_2–			4065	873	54.7	58.0
5	N≡C–CH_2CH_2–			4830	688	67.2	66.3
6	EtO(C=O)–			1925	674	60.1	70.6
7				2496	453	85.1	81.8
9				3613	389	23.1	23.5
10		Me_2N–		3217	387	16.5	36.9
11		Ph(Et)N–		3742	573	50.1	73.8
12				899	461	72.5	72.4
13				774	669	77.8	45.2
14				776	568	66.3	73.1
15		H		4364	490	76.2	82.3
16		Me		4790	700	70.3	70.5
17		Cl		4563	567	71.2	68.4
21		2-Me	6-Me	3226	804	17.3	26.5
22		4-Cl	H	1571	457	93.4	32.4
23		4-Cl	3-NO_2	1883	726	60.6	47.2
24		H	3-NO_2	4065	675	33.4	61.3
25		2-MeO(C=O)–	H	2437	620	86.4	62.9
26		4-H_2NO_2S–	H	1573	513	31.0	40.5
27		2-Cl		235	404	29.7	43.3
28		4-Cl		3106	339	38.6	73.7
29		4-NO_2		583	358	65.2	81.6
30				224	318	11.8	79.8
31				580	379	56.1	75.4

[a] Mean from 3 different determinations (errors were in the range of ±5–10% of the reported values, data not shown).

the following methods described previously: 4-methoxy-3-pyridinesulfonamide **1** [28], 2,4-diamino-6-chloromethyl-1,3,5-triazines **2a–e** [32] and 2-choromethyl-3-phenylquinazolin-4(3*H*)-ones **3a–c** [33].

*4.1.1. Procedures for the preparation of 1,4-dihydro-4-oxo-3-pyridinesulfonamides (**4–8**)*

To a solution of the corresponding bromide derivatives RBr (6.5 mmol) in dry acetonitrile (15 ml) 4-methoxy-3-pyridinesulfonamide **1** (1.13 g, 6 mmol) was added. The reaction mixture was stirred at room temperature for 24 h, followed at reflux for 60 h. After cooling to room temperature and standing overnight the precipitate of adequate 1,4-dihydro-3-pyridinesulfonamide was collected by filtration, washed with acetonitrile (4 × 1.5 ml) and dried. In this manner the following sulfonamides were obtained.

*4.1.1.1. 1-(2-Hydroxyethyl)-1,4-dihydro-4-oxo-3-pyridinesulfonamide (**4**).* Starting from 2-bromoethanol (0.81 g), the title compound **4** was obtained (0.9 g, 68%): m.p. 173–174 °C IR (KBr) 3510 (OH), 3320, 3230 (SO_2NH_2), 1650 (C=O), 1330, 1310, 1150, 1140 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.64 (d, J = 4.4 Hz, 2H, CH_2N), 4.02 (d, J = 4.4 Hz, 2H, OCH_2), 5.05 (t, J = 4.4 Hz, 1H, OH), 6.34 (d, J = 7.3 Hz, 1H, H-5), 6.78 (s, 2H, SO_2NH_2), 7.78 (dd, J_{ortho} = 7.3 Hz, J_{meta} = 1.9 Hz, H-6), 8.27 (d, J_{meta} = 1.9 Hz, 1H, H-2) ppm. Anal. ($C_7H_{10}N_2O_4S$) C, H, N.

*4.1.1.2. 1-(3-Cyanopropyl)-1,4-dihydro-4-oxo-3-pyridinesulfonamide (**5**).* Starting from 4-bromobutyronitrile (0.96 g), the title compound **5** was obtained (1.1 g, 76%): m.p. 241–243 °C dec; IR (KBr) 3350, 3245 (SO_2NH_2), 2245 (C≡N), 1650 (C=O), 1345, 1330, 1155 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.04 (pent, J = 6.8 Hz, 2H, N≡C–CH_2–CH_2–CH_2N), 2.53 (t, J = 6.8 Hz, 2H, N≡C–CH_2–CH_2–CH_2N), 4.05 (t, J = 6.8 Hz, 2H, N≡C–CH_2–CH_2–CH_2N), 6.38 (d, J = 7.2 Hz, 1H, H-6), 6.75 (s, 2H, SO_2NH_2), 7.86 (d, J = 7.2 Hz, 1H, H-5), 8.3 (s, 1H, H-2) ppm; ^{13}C NMR (DMSO-d_6) δ 13.71, 26.27, 55.02, 118.97, 120.36, 130.31, 141.50, 142.35, 172.56 ppm. Anal. ($C_9H_{11}N_3O_3S$) C, H, N.

*4.1.1.3. Ethyl 1,4-dihydro-4-oxo-3-sulfamoyl-1-pyridineacetate (**6**).* Starting from ethyl bromoacetate (1.81 g), the title compound was obtained (1.0 g, 64%): m.p. 185–186 °C; IR (KBr) 3335, 3150 (SO_2NH_2), 1750 (O=COEt), 1650 (C=O), 1325, 1155 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.22 (t, J = 7.1 Hz, 3H, CH_3CH_2O), 4.17 (q, J = 7.1 Hz, 2H, CH_3CH_2O), 5.17 (s, 2H, O=C–CH_2N), 6.37 (d, J = 7.8 Hz, 1H, H-5), 6.83 (s, 2H, SO_2NH_2), 7.76 (dd, J_{ortho} = 7.8 Hz, J_{meta} = 1.9 Hz, H-6), 8.36 (d, J_{meta} = 1.9 Hz, 1H, H-2) ppm. Anal. ($C_9H_{12}N_2O_5S$) C, H, N.

*4.1.1.4. 1-(Cyclopropylmethyl)-1,4-dihydro-4-oxo-3-pyridinesulfonamide (**7**) and 1-methyl-1,4-dihydro-4-oxo-3-pyridinesulfonamide (**8**) as a main product.* Starting from bromomethyl cyclopropane (0.88 g), the title compound **8** was obtained (0.6 g, 53%): m.p. 246–247 °C; IR and ^1H NMR data were in accordance with those reported below to the authentic sample of **8** [25].

The acetonitrile filtrate mixture was evaporated under reduced pressure and the resulting residue was triturated with water (10 ml), and then precipitate of title compound **7** was filtered off, washed with water (5 × 2 ml), and dried at temperatures gradually increasing to 100 °C. Yield 0.5 g (36.5%): m.p. 164–165 °C; IR (KBr) 3350, 3315, 3235 (SO_2NH_2), 1650 (C=O), 1330, 1150 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.39 (d, J_{trans} = 3.9 Hz, 2H, cyclopropyl), 0.53 (d, J_{cis} = 6.8 Hz, 2H, cyclopropyl), 1.19 (sext, 1H, cyclopropyl), 3.88 (d, J = 7.3 Hz, 2H, CH_2N), 6.37 (d, J = 7.3 Hz, 1H, H-5, pyrid.), 6.79 (s, 2H, SO_2NH_2), 7.91 (dd, J_{ortho} = 7.3 Hz, J_{meta} = 1.9 Hz, 1H, H-6, pyrid.), 8.38 (d, J_{meta} = 1.9 Hz, 1H, H-2, pyrid.) ppm. Anal. ($C_9H_{12}N_2O_3S$) C, H, N.

*4.1.1.5. 1-Methyl-1,4-dihydro-4-oxo-3-pyridinesulfonamide (**8**).* Starting from 4-bromo-1-butene (0.88 g), the title compound **8** was obtained (0.85 g, 75%): m.p. 246–247 °C; IR and ^1H NMR data were in accordance with those reported above to the authentic sample of **8** [30]. The acetonitrile filtrate mixture was fractional distilled under normal pressure to give the fraction of acetonitrile (b.p. 76–78 °C), followed 0.30 g (37.5%) of cyclobutyl bromide: b.p. 106–108 °C [34]: IR [35] and ^1H NMR [36] data were in accordance with those reported in literature.

*4.1.2. Synthesis of 1-(3,5-dimethylisoxazol-4-yl)methyl-1,4-dihydro-4-oxo-3-pyridinesulfonamide (**9**)*

A mixture of 4-methoxy-3-pyridinesulfonamide **1** (1.5 g, 8 mmol) and 4-chlomethyl-3,5-dimethylisoxazole (1.23 g, 8.4 mmol) in dry acetonitrile (25 ml) was stirred at room temperature for 36 h, followed at reflux for 96 h. After cooling to room temperature and standing overnight, the precipitate was filtered off, washed with acetonitrile (3 × 1.5 ml) and dried. Yield 2.1 g (92.6%): m.p. 251–252 °C; IR (KBr) 3400, 3280, 3170 (SO_2NH_2), 1650 (C=O), 1590, 1560 (C=N and C=C), 1340, 1330, 1180, 1155 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.12 (s, 3H, CH_3-3), 2.45 (s, 2H, CH_3-5), 5.13 (s, 2H, CH_2), 6.37 (d, J = 7.5 Hz, 1H, H-5, pyrid.), 6.83 (s, 2H, SO_2NH_2), 7.76 (dd, J_{ortho} = 7.5 Hz, J_{meta} = 1.9 Hz, 1H, H-6, pyrid.), 8.36 (d, J_{meta} = 1.9 Hz, 1H, H-2, pyrid.) ppm; ^{13}C NMR (DMSO-d_6) δ 10.08, 10.93, 47.89, 109.64, 120.57, 130.27, 141.18, 141.93, 159.93, 168.77, 172.47 ppm. Anal. ($C_{11}H_{13}N_3O_4S$) C, H, N.

*4.1.3. Procedure for the preparation of 1-(heteroaryl)methyl-1,1-dihydro-4-oxo-3-pyridinesulfonamides (**10–17**)*

A mixture of 4-methoxy-3-pyridinesulfonamide **1** (0.85 g, 4.5 mmol) and the corresponding 2-amino-4-R-6-chloromethyl-1,3,5-triazine (4.7 mmol) or 2-chloromethyl-3-(4-R-phenyl)quinazolin-4(3*H*)-one (4.5 mmol) in dry acetonitrile (20 ml) was stirred at room temperature for 24 h, followed at reflux for 50 h. After cooling to room temperature and standing overnight, the precipitate was filtered off, washed with acetonitrile (3 × 1.2 ml) and dried. In this manner the following sulfonamides were obtained.

*4.1.3.1. 1-(2-Amino-4-dimetyhlamino-1,3,5-triazin-6-yl)methyl-1,4-dihydro-4-oxo-3-pyridinesulfonamide (**10**).* Starting from 2-amino-4-dimethylamino-6-chloromethyl-1,3,5-triazine (0.88 g), the title compound **10** was obtained (1.2 g, 82%): m.p. 269–270 °C dec.; IR (KBr) 3460, 3365, 3250, 3215 (NH_2 and SO_2NH_2), 1655 (C=O), 1595, 1570 (C=N and C=C), 1335, 1155 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.95 and 3.00 (2s, 2 × 3H, CH_3–N–CH_3), 5.03 (s, 2H, CH_2), 6.35 (d, J = 7.6 Hz, 1H, H-5, pyrid.), 6.80 (s, 2H, SO_2NH_2), 6.92 (s, 2H, NH_2), 7.79 (dd, J_{ortho} = 7.6 Hz, J_{meta} = 2.1 Hz, 1H, H-6, pyrid.), 8.34 (d, J_{meta} = 2.1 Hz, 1H, H-2, pyrid.) ppm. Anal. ($C_{11}H_{15}N_7O_3S$) C, H, N.

*4.1.3.2. 1-[2-Amino-4-(N-ethylanilino)-1,3,5-triazin-6-yl]methyl-1,4-dihydro-4-oxo-3-pyridinesulfonamide (**11**).* Starting from 2-amino-4-(*N*-ethylanilino)-6-chloromethyl-1,3,5-triazine (1.24 g), the title compound **11** was obtained (1.3 g, 72%): m.p. 245–246 °C; IR (KBr) 3445, 3325, 3200, 3175 (NH_2 and SO_2NH_2), 1645 (C=O), 1600, 1575, (C=N and C=C), 1320, 1155 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.02 (t, J = 6.8 Hz, 3H, CH_3CH_2N), 3.79 (q, J = 6.8 Hz, 2H, CH_2CH_2N), 5.03 (s, 2H, CH_2), 6.30 (d, J = 7.6 Hz, 1H, H-5, pyrid.), 6.78 (s, 2H, SO_2NH_2), 6.98 (s, 2H, NH_2), 7.17–7.26 (m, 3H, Ph), 7.34–7.41 (m, 2H, Ph), 7.75 (d, J = 7.6 Hz, 1H, H-6, pyrid.), 8.34 (s, 1H, H-2, pyrid.) ppm. Anal. ($C_{17}H_{19}N_7O_3S$) C, H, N.

*4.1.3.3. 1-(2-Amino-4-morpholino-1,3,5-triazin-6-yl)methyl-1,4-dihydro-4-oxo-3-pyridinesulfonamide (**12**).* Starting from 2-amino-4-morpholino-6-chloromethyl-1,3,5-triazine (1.08 g), the title compound **12** was obtained (1.2 g, 72.5%): m.p. 288–289 °C dec; IR (KBr) 3415, 3350, 3160, 3140 (NH_2 and SO_2NH_2), 1675 (C=O), 1650, 1590, 1560 (C=N and C=C), 1320, 1160 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.45–3.57 (m, 8H, morpholine), 5.04 (s, 2H, CH_2), 6.34 (d, J = 7.5 Hz, 1H, H-5, pyrid.), 6.79 (s, 2H, SO_2NH_2), 7.01 (s, 2H, NH_2), 7.78 (dd, J_{ortho} = 7.5 Hz, J_{meta} = 1.3 Hz, 1H, H-6, pyrid.), 8.34 (d, J_{meta} = 1.3 Hz, 1H, H-2, pyrid.) ppm. Anal. ($C_{13}H_{17}N_7O_4S$) C, H, N.

*4.1.3.4. 1-[2-Amino-4-(3-methyl-2-pyrazolino)-1,3,5-triazine-6-yl] methyl-1,4-dihydro-4-oxo-3-pyridinesulfonamide (**13**).* Starting from 2-amino-4-(3-methyl-2-pyrazolidino)-6-chloromethyl-1,3,5-triazine (1.06 g), the title compound **13** was obtained (1.4 g, 85%): m.p. 298–299 °C dec; IR (KBr) 3435, 3330, 3240 (NH_2 and SO_2NH_2), 1650 (C=O), 1625, 1585 (C=N and C=C), 1330, 1160 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.93 (s, 3H, CH_3), 2.82 (t, J = 8.9 Hz, 2H, CH_2, pyrazoline), 3.74 (t, J = 8.9 Hz, 2H, CH_2, pyrazoline), 5.07 (s, 2H, CH_2), 6.35 (d, J = 7.6 Hz, 1H, H-5, pyrid.), 6.80 (s, 2H, SO_2NH_2), 7.10 (br s, 2H, NH_2), 7.79 (d, J = 7.6 Hz, 1H, H-6, pyrid.), 8.34 (s, 1H, H-2, pyrid.) ppm ^{13}C NMR (DMSO-d_6) δ 16.09, 35.49, 45.58, 59.55, 119.52, 129.69, 142.86, 143.92, 158.57, 161.73, 166.79, 172.21, 172.75 ppm. Anal. ($C_{13}H_{16}N_8O_3S$) C, H, N.

*4.1.3.5. 1-[2-Amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazin-6-yl]methyl-1,4-dihydro-4-oxo-3-pyridinesulfonamide (**14**).* Starting from 2-amino-4-(3,5,5-trimethyl-2-pyrazolino)-6-chloromethyl-1,3,5-triazine (1.2 g), the title compound **14** was obtained (1.4 g, 79%): m.p. 295–296 °C dec; IR (KBr) 3415, 3360, 3235 (NH_2 and SO_2NH_2), 1650 (C=O), 1580, 1540 (C=N and C=C), 1335, 1150 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.32 (s, 6H, 5,5-diCH_3, pyrazoline), 1.94 (s, 3H, 3-CH_3, pyrazoline), 2.76 (s, 2H, CH_2-4, pyrazoline), 5.07 (s, 2H, CH_2), 6.36 (d, J = 7.4 Hz, 1H, H-5, pyrid.), 6.76 (s, 2H, SO_2NH_2), 6.97 (s, 2H, NH_2), 7.82 (d, J = 7.4 Hz, 1H, H-6, pyrid.), 8.36 (s, 1H, H-2, pyrid.) ppm. Anal. ($C_{15}H_{20}N_8O_3S$) C, H, N.

*4.1.3.6. 1-[(4-Oxo-3-phenyl-3,4-dihydroquinazolin-2-yl)methyl]-1,4-dihydro-4-oxo-3-pyridinesulfonamide (**15**).* Starting from 2-chloromethyl-3-phenylquinazolin-4(3*H*)-one (1.22 g), the title compound **15** was obtained (1.4 g, 76%): m.p. 286–287 °C dec; IR (KBr) 3350, 3210 (SO_2NH_2), 1695 (C=O), 1650 (C=O), 1610, 1580 (C=N and C=C), 1325, 1160 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.92 (s, 2H, CH_2), 6.37 (d, J = 7.7 Hz, 1H, H-5, pyrid.), 6.82 (s, 2H, SO_2NH_2), 7.53–7.63 (m, 7H, arom.), 7.72–7.84 (m, 2H, arom.), 8.14 (dd, J_{ortho} = 7.7 Hz, J_{meta} = 2.1 Hz, 1H, H-6, pyrid.), 8.37 (d, J_{meta} = 2.1 Hz, 1H, H-2, pyrid.) ppm; ^{13}C NMR (DMSO-d_6) δ 57.24, 119.31, 120.93, 126.70, 127.61, 128.19, 129.74, 129.89, 129.99, 135.18, 135.51, 143.25, 144.41, 146.75, 152.25, 161.31, 172.93 ppm. Anal. ($C_{20}H_{16}N_4O_4S$) C, H, N.

*4.1.3.7. 1-[(4-Oxo-3-p-tolyl-3,4-dihydroquinazolin-2-yl)methyl]-1,4-dihydro-4-oxo-3-pyridinesulfonamide (**16**).* Starting from 2-chloromethyl-3-*p*-totylquinazolin-4(3*H*)-one (1.28 g), the title compound **16** was obtained (1.3 g, 68%): m.p. 294–295 °C dec; IR (KBr) 3420, 3320 (SO_2NH_2), 1695 (C=O), 1655 (C=O), 1615, 1570 (C=N and C=C), 1330, 1160 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.42 (s, 3H, CH_3), 4.93 (s, 2H, CH_2), 6.38 (d, J = 7.7 Hz, 1H, H-5, pyrid.), 6.83 (s, 2H, SO_2NH_2), 7.42–7.59 (m, 6H, arom.), 7.76 (t, J = 7.3 Hz, 1H, arom.), 7.85 (d, J = 7.3 Hz, 1H, arom.), 8.13 (d, J = 7.7 Hz, 1H, H-6, pyrid.), 8.36 (s, 1H, H-2, pyrid.) ppm. Anal. ($C_{21}H_{18}N_4O_4S$) C, H, N.

*4.1.3.8. 1-[4-Oxo-3-(4-chlorophenyl)-3,4-dihydroquinazolin-2-ylmethyl]-1,4-dihydro-4-oxo-3-pyridinesulfonamide (**17**).* Starting from 2-chloromethyl-3-(4-chlorophenyl)quinazolin-4(3*H*)-one (1.38 g), the title compound **17** was obtained (1.6 g, 80%): m.p. 304–305 °C dec; IR (KBr) 3370, 3165 (SO_2NH_2), 1695 (C=O), 1650 (C=O), 1610, 1585 (C=N and C=C), 1320, 1160 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.94 (s, 2H, CH_2), 6.39 (d, J = 7.7 Hz, 1H, H-5, pyrid.), 6.84 (s, 2H, SO_2NH_2), 7.51–7.74 (m, 7H, arom.), 7.84 (t, J = 7.5 Hz, 1H, arom.), 8.14 (d, J = 7.7 Hz, 1H, H-6, pyrid.), 8.36 (s, 1H, H-2, pyrid.) ppm. Anal. ($C_{20}H_{15}ClN_4O_4S$) C, H, N.

*4.1.4. Procedure for the preparation of N-(chloroacetyl)benzenesulfonamides (**18a–c**) as substrates for the synthesis of 3-pyridinesulfonamide derivatives (**27–29**)*

A solution of the corresponding benzenesulfonamide derivatives (10 mmol) in large excess of 2-chloroacetyl chloride (6 ml) was refluxed with stirring for 16 h. The excess of 2-chloroacetyl chloride was evaporated under reduced pressure. To the solid residue toluene (10 ml) was added and stirred at reflux for 0.5 h. After cooling to room temperature and standing overnight the precipitate was filtered off, washed with toluene (4 × 1.5 ml) and dried. In this manner the following sulfonamides were obtained.

*4.1.4.1. 2-Chloro-N-(chloroacetyl)benzenesulfonamide (**18a**).* Starting from 2-chlorobenzenesulfonamide (1.92 g), the title compound **18a** was obtained (2.3 g, 85%): m.p. 142–143 °C dec; IR (KBr) 3275 (NH), 1715 (C=O), 1350, 1160 (SO_2) cm^{-1}. Anal. ($C_8H_7Cl_2NO_3S$) N.

*4.1.4.2. 4-Chloro-N-(chloroacetyl)benzenesulfonamide (**18b**).* Starting from 4-chlorobenzenesulfonamide (1.92 g), the title compound **18b** was obtained (2.5 g, 93%): m.p. 141–142 °C dec; IR (KBr) 3120 (NH), 1710 (C=O), 1365, 1150 (SO_2) cm^{-1}. Anal. ($C_8H_7Cl_2NO_3S$) N.

*4.1.4.3. N-(Chloroacetyl)-4-nitrobenzenesulfonamide (**18c**).* Starting from 4-nitrobenzenesulfonamide (2.02 g), the mixture of products obtained (2.3 g) was treated with water (20 ml). The resulting suspension was slowly adjusted to pH 8 with 1% solution of NaOH in water. After stirring at room temperature for 3 h, a small amount of insoluble side products was filtered out together with charcoal added. The filtrate (pH-7.5) was acidified with 1% hydrochloric acid to pH 1. The precipitate of title compound **18c** was filtered off, washed with water (5 × 2 ml) and dried at temperatures gradually increasing to 105 °C. Yield 1.5 g (53%): m.p. 171–172 °C dec; IR (KBr) 3255 (NH), 1735, 1715 (C=O), 1535, 1150 (NO_2) 1365, 1350, 1140 (SO_2) cm^{-1}. Anal. ($C_8H_7ClN_2O_5S$) N.

*4.1.5. Procedure for the preparation of N′-(chloroacetyl)-p-toluenesulfonylhydrazide (**19**) and N′-(chloroacetyl)-2-furoichydrazide (**20**) as substrates for the synthesis of 3-pyridinesulfonamides (**30** and **31**)*

To an ice-cold suspension of the corresponding *p*-toluenesulfonylhydrazide (2.8 g, 15 mmol) or 2-furoichydrazide (1.9 g, 15 mmol) in dry 1,4-dioxane (10 ml) was added with stirring a solution of 2-chloroacetyl chloride (2.09 g, 17 mmol) in 1,4-dioxane (5 ml). After 0.5 h ice-bath was removed and the reaction mixture was heated at reflux for 2 h. The solvent was evaporated under normal pressure in part, and the resulting suspension was stirred at room temperature for 6 h. The precipitate was collected by filtration, washed successively with 1,4-dioxane (2 × 2 ml), water (3 × 5 ml) and acetonitrile (2 × 1.5 ml), and dried. Starting from *p*-toluenesulfonylhydrazide, the title compound **19** was obtained (3.5 g, 88%): m.p. 183–184 °C dec; IR (KBr) 3342 (HN–NH), 1705, 1680 (C=O), 1340, 1160 (SO_2) cm^{-1}. Anal. ($C_9H_{11}ClN_2O_3S$) C, H, N.

Starting from 2-furoichydrazide, the title compound **20** was obtained (2.5 g, 82%): m.p. 134–135 °C; IR (KBr) 3175, 3135 (HN–NH), 3020 (CH-arom.), 2879 (CH_2), 1695, 1685, 1635 (C=O), 1575, 1560, 1500 (C=C), 1464 (CH_2) cm^{-1}. Anal. ($C_7H_7ClN_2O_3$) C, H, N.

*4.1.6. General procedure for the preparation of 1-sustituted 1,4-dihydro-4-oxo-3-pyridinesulfonamides (**21**–**31**)*

A mixture of 4-methoxy-3-pyridinesulfonamide **1** (0.76 g, 4 mmol) and the corresponding chloride derivatives R–CO–CH_2Cl (4.3 mmol) in dry acetonitrile (20 ml) was stirred at room temperature for 30 h, followed at reflux for 55 h. After cooling to room temperature and standing overnight the precipitate was filtered off, washed with acetonitrile (4 × 1 ml) and dried. In this manner the following 1,4-dihydro-3-pyridinesulfonamides were obtained.

*4.1.6.1. 1,4-Dihydro-1-[(2,6-dimethylphenylcarbamoyl)methyl]-4-oxo-3-pyridinesulfonamide (**21**).* Starting from *N*-chloroacetyl-2,6-dimethylaniline (0.85 g), the title compound **21** was obtained (1.0 g, 74%): m.p. 328–329 °C dec; IR (KBr) 3325, 3270, 3235 (NHC=O, SO_2NH_2), 1700, 1670, 1650 (C=O), 1335, 1160 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.16 (s, 6H, 2 × CH_3), 5.06 (s, 2H, CH_2), 6.38 (d, J = 7.6 Hz, 1H, H-5, pyrid.), 6.84 (s, 2H, SO_2NH_2), 7.07 (s, 3H, H-3, H-4 and H-5, Ph), 7.79 (dd, J_{ortho} = 7.6 Hz, J_{meta} = 2.2 Hz, 1H, H-6, pyrid.), 8.40 (d, J_{meta} = 2.2 Hz, 1H, H-2, pyrid.), 9.69 (s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 18.39, 57.43, 119.65, 126.97, 128.00, 129.83, 134.48, 135.34, 142.80, 143.72, 165,13, 172.66 ppm. Anal. ($C_{15}H_{17}N_3O_4S$) C, H, N.

*4.1.6.2. 1-[(4-Chlorophenylcarbamoyl)methyl]-1,4-dihydro-4-oxo-3-pyridinesulfonamide (**22**).* Starting from *N*-chlorocetyl-4-chloroaniline (0.88 g), the title compound **22** was obtained (1.05 g, 76.8%): m.p. 290–291 °C dec; IR (KBr) 3365, 3380, 3270 (NHC=O, SO_2NH_2), 1700, 1645 (C=O), 1330, 1160 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 4.99 (s, 2H, CH_2), 6.37 (d, J = 7.6 Hz, 1H, H-5, pyrid.), 6.84 (s, 2H, SO_2NH_2), 7.39 (d, J = 8.9 Hz, 2H, H-2, and H-6, Ph), 7.77 (d, J = 8.9 Hz, 2H, H-3 and H-5, Ph), 7.81 (dd, J_{ortho} = 7.6 Hz, J_{meta} = 2.2 Hz, 1H, H-6, pyrid.), 8.37 (d, J_{meta} = 2.2 Hz, 1H, H-2, pyrid.), 10.52 (s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 57.97, 119.52, 120.90, 127.48, 129.13, 129.84, 137.75, 142.94, 143.88, 165.72, 172.69 ppm. Anal. ($C_{12}H_{12}$ ClN_3O_4S) C, H, N.

*4.1.6.3. 1-[(4-Chloro-3-nitrophenylcarbomoyl)methyl]-1,4-dihydro-4-oxo-3-pyridinesulfonamide (**23**).* Starting from *N*-chloroacetyl-4-chloro-3-nitroaniline (1.07 g), the title compound **23** was obtained (1.3 g, 84%): m.p. 284–285 °C dec; IR (KBr) 3360, 2265, 3175 (NHC=O, SO_2NH_2), 1715, 1651 (C=O), 1335, 1165 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 5.02 (s, 2H, CH_2), 6.37 (d, J = 7.8 Hz, 1H, H-5, pyrid.), 6.82 (s, 2H, SO_2NH_2), 7.73–7.79 (m, 3H, arom.) 8.37 (s, 1H, H-2, Ph), 8.39 (s, 1H, H-2, pyrid.), 10.94 (s, 1H, NH) ppm. Anal. ($C_{13}H_{11}$ ClN_4O_6S) C, H, N.

*4.1.6.4. 1,4-Dihydro-1-[(3-nitrophenylcarbamoyl)methyl]-4-oxo-3-pyridinesulfonamide (**24**).* Starting from *N*-chloracetyl-3-nitroaniline (0.8 g), the title compound **24** was obtained (1.0 g, 71%): m.p. 267–269 °C dec; IR (KBr) 3360, 3325, 3225 (NHC=O, SO_2NH_2), 1705, 1655 (C=O), 1350, 1160 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 5.05 (s, 2H, CH_2), 6.38 (d, J = 7.6 Hz, 1H, H-5, pyrid.), 6.85 (s, 2H, SO_2NH_2), 7.64 (t, J = 8.1 Hz, 1H, H-5, Ph), 7.81 (dd, J_{ortho} = 7.6 Hz, J_{meta} = 2.1 Hz, 1H, H-6, pyrid.), 7.87–7.97 (m, 2H, H-4 and H-5, Ph), 8.40 (d, J_{ortho} = 2.1 Hz, 1H, H-2, pyrid.), 8.60 (t, J_{meta} = 2.0 Hz, 1H, H-2, Ph), 10.91 (s, 1H, NH), ppm. Anal. ($C_{13}H_{12}N_4O_6S$) C, H, N.

*4.1.6.5. 1,4-Dihydro-1-[(2-methoxcarbonylphenylcarbamoyl)methyl]-4-oxo-3-pyridinesulfonamide (**25**).* Starting from methyl *N*-chloracetylanthranilate (0.98 g), the title compound **25** was obtained (1.2 g, 82.1%): m.p. 254–255 °C dec; IR (KBr) 3330, 3280, 3175 (NHC=O, SO_2NH_2), 1700, 1690, 1650 (C=O), 1335, 1170 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.86 (s, 3H, CO_2CH_3), 5.11 (s, 2H, CH_2), 6.40 (d, J = 7.6 Hz, 1H, H-5, pyrid.), 6.85 (s, 2H, SO_2NH_2), 7.22–7.30 (m, 1H, Ph), 7.59–7.68 (m, 1H, Ph), 7.80 (dd, J_{ortho} = 7.6 Hz, J_{meta} = 2.2 Hz, 1H, H-6, pyrid.), 7.92 (dd, J_{ortho} = 7.8 Hz, J_{meta} = 1.4 Hz, 1H, H-3, Ph), 8.11 (d, J = 8.3 Hz, 1H, H-6, pyrid.), 8.38 (d, J_{meta} = 2.2 Hz, 1H, H-2, pyrid.), 10.79 (s, 1H, NH) ppm. Anal. ($C_{15}H_{15}$ N_3O_6S) C, H, N.

*4.1.6.6. 1,4-Dihydro-1-[(4-sulfamoylphenylcarbamoyl)methyl]-4-oxo-3-pyridinesulfonamide (**26**).* Starting from 4-(chloracetylamino) benzenesulfonamide (1.07 g), the title compound **26** was obtained (1.2 g, 77.6%): m.p. 310–311 °C dec; IR (KBr) 3365, 3315, 3300, 3260, 3225 (NHCO, SO_2NH_2), 1695, 1650 (C=O), 1365, 1340, 1185, 1168 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 5.03 (s, 2H, CH_2), 6.38 (d, J = 7.4 Hz, 1H, H-5, pyrid.), 6.84 (s, 2H, Py-SO_2NH_2), 7.29 (s, 3H, $PhSO_2NH_2$ and H-6, pyrid.), 7.72–7.83 (m, 4H, Ph), 8.39 (s, 1H, H-2, pyrid.), 10.79 (s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 59.49, 119.38, 129.66, 142.06, 142.92, 143.99, 164.48, 164.99, 165.67, 171.67, 172.74 ppm. Anal. ($C_{13}H_{14}$ $N_4O_6S_2$) C, H, N.

*4.1.6.7. 1-[(2-Chlorobenzenesulfonamidocarbonyl)methyl]-1,4-dihydro-4-oxo-3-pyridinesulfonamide (**27**).* Starting from 2-chloro-*N*-chlorocetylbenzenesulfonamide **18a** (1.15 g), the title compound **27** was obtained (1.3 g, 80%): m.p. 271–272 °C dec; IR (KBr) 3395, 3235 (SO_2NH_2 and SO_2NH), 1725, 1645 (C=O) 1365, 1340, 1330, 1160, 1155 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 4.95 (s, 2H, CH_2), 6.30 (d, J = 7.6 Hz, 1H, H-5, pyrid.), 6.77 (br s, 3H, SO_2NH_2 and SO_2NH), 7.58 (dd, J_{ortho} = 7.6 Hz, J_{meta} = 2.2 Hz, 1H, H-6, pyrid.), 7.67–7.72 (m, 3H, H-3, H-4 and H-5, Ph), 8.09 (d, J = 7.5 Hz, H-6, Ph), 8.33 (d, J_{meta} = 2.2 Hz, 1H, H-2, pyrid.) ppm; ^{13}C NMR (DMSO-d_6) δ 57.50, 119.54, 127.98, 129.89, 131.27, 132.13, 132.32, 135.62, 136.52, 142.87, 143.67, 166.81, 172.59 ppm. Anal. ($C_{13}H_{12}$ $ClN_3O_6S_2$) C, H, N.

*4.1.6.8. 1-[(4-Chlorobenzenesulfonamidocarbonyl)methyl]-1,4-dihydro-4-oxo-3-pyridinesulfonamide (**28**).* Starting from 4-chloro-*N*-chlorocetylbenzenesulfomide **18b** (1.15 g), the title compound **28** was obtained (1.25 g, 77%): m.p. 285–286 °C dec; IR (KBr) 3405, 3185 (SO_2NH_2 and SO_2NH), 1740, 1645 (C=O), 1345, 1175, 1150 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 4.88 (s, 2H, CH_2), 6.31(d, J = 7.5 Hz, 1H, H-5, pyrid.), 6.81 (br s, 3H, SO_2NH_2 and SO_2NH), 7.65 (dd, J_{ortho} = 7.5 Hz, J_{meta} = 2.1 Hz, 1H, H-6, pyrid.), 7.73 (d, J = 8.5 Hz, 2H, 4-ClPh), 7.94 (d, J = 8.5 Hz, 2H, 4-ClPh), 8.30 (d, J_{meta} = 2.1 Hz, 1H, H-2, pyrid.) ppm; ^{13}C NMR (DMSO-d_6) δ 57.55, 119.53, 129.67, 129.91, 138.14, 139.18, 142.82, 143.68, 166.11, 166.89, 172.61 ppm. Anal. ($C_{13}H_{12}$ $ClN_3O_6S_2$) C, H, N.

*4.1.6.9. 1,4-Dihydro-1-[(4-nitrobenzenesulfonamidocarbonyl)methyl]-4-oxo-3-pyridinesulfonamide (**29**).* Starting from *N*-chloracetyl-4-nitrobenzenesulfonamide **18c** (1.2 g), the title compound **29** was obtained (1.3 g, 78%): m.p. 275–276 °C dec; IR (KBr) 3370, 3270 (SO_2NH_2 and SO_2NH), 1735, 1650 (C=O), 1365, 1350, 1170, 1160 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 4.90 (s, 2H, CH_2), 6.32 (d, J = 7.6 Hz, 1H, H-5, pyrid.), 6.79 (br s, 3H, SO_2NH_2 and SO_2NH), 7.67 (dd, J_{ortho} = 7.6 Hz, J_{meta} = 2.1 Hz, 1H, H-6, pyrid.), 8.18 (d, J = 8.9 Hz, 2H, 4-O_2NPh), 8.29 (d, J_{meta} = 2.1 Hz, 1H, H-2, pyrid.), 8.45 (d, J = 8.9 Hz, 2H, 4-O_2NPh) ppm. Anal. ($C_{13}H_{12}N_4O_8S_2$) C, H, N.

*4.1.6.10. 1,4-Dihydro-1-[(p-toluenesulfonylhydrazinocarbonyl)methyl]-4-oxo-3-pyridinesulfonamide (**30**).* Starting from *N′*-(chloroacetyl)-*p*-toluenesulfonylhydrazide **19** (1.12 g), the title compound **30** was obtained (1.17 g, 73,9%): m.p. 270–271 °C dec; IR (KBr) 3365, 3300, 3245 (HN–NH), 1705, 1685, 1650 (C=O), 1340, 1160 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.36 (s, 3H, CH_3), 4.73 (s, 2H, CH_2), 6.31 (d, J = 7.6 Hz, 1H, H-5, pyrid.), 6.82 (s, 2H, SO_2NH_2), 7.35 (d, J = 8.1 Hz, 2H, H-3 and H-5, Ph), 7.54 (dd, J_{ortho} = 7.6 Hz, J_{meta} = 2.2 Hz, 1H, H-6, pyrid.), 7.69 (d, J = 8.1 Hz, 2H, H-2 and H-6, Ph), 8.15 (d, J_{meta} = 2.2 Hz, 1H, H-2, pyrid.), 9.95 (s, 1H, SO_2NH–N), 10.48 (s, 1H, CO–NH–N) ppm; ^{13}C NMR (DMSO-d_6) δ 21.31, 55.89, 119.60, 127.90, 129.74, 129.88, 135.73, 142.51, 143.31, 143.89, 165.63, 172.58 ppm. Anal. ($C_{14}H_{16}N_4O_6S_2$) C, H, N.

*4.1.6.11. 1,4-Dihydro-1-[(2-fuorylhydrazinocarbonyl)methyl]-4-oxo-3-pyridinesulfonamide (**31**).* Starting from *N′*-(chloroacetyl)-2-furoichydrazide **20** (0.87 g), the title compound **31** was obtained (0.95 g, 69.7%): m.p. 234–235 °C dec; IR (KBr) 3405, 3335, 3235 (SO_2NH_2 and HN–NH), 1710, 1680, 1650 (C=O), 1320, 1180, 1160 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.95 (s, 2H, CH_2), 6.37 (d, $J = 7.6$ Hz, 1H, H-5, pyrid.), 6.66 (t, $J = 1.6$ Hz, 1H, H-4, furan), 6.84 (s, 2H, SO_2NH_2), 7.23 (d, $J = 3.3$ Hz, 1H, H-5, furan), 7.72 (dd, $J_{ortho} = 7.6$ Hz, $J_{meta} = 1.8$ Hz, 1H, H-6, pyrid.), 7.90 (s, 1H, H-3, furan), 8.30 (d, $J_{meta} = 1.8$ Hz, 1H, H-2, pyrid.), 10.41 and 10.44 (2s, O=C–*H*N–N*H*–C=O) ppm; ^{13}C NMR (DMSO-d_6) δ 56.08, 112.20, 115.13, 119.75, 129.96, 142.66, 143.49, 146.12, 146.19, 157.14, 166.29, 172.63 ppm. Anal. ($C_{12}H_{12}N_4O_6S_2$) C, H, N.

4.2. CA inhibition assay

An Applied Photophysics (Oxford, UK) stopped-flow instrument has been used for assaying the CA-catalyzed CO_2 hydration activity [37]. Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 10 mM Hepes (pH 7.5) as buffer, 0.1 M Na_2SO_4 (for maintaining constant the ionic strength), following 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 (1 mM) were prepared in distilled-deionized water with 10–20% (v/v) DMSO (which is not inhibitory at these concentrations) and dilutions up to 0.1 nM were done thereafter with distilled-deionized water. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of E–I complex. The inhibition constants were obtained by non-linear last-squares methods using PRISM 3, as reported earlier [38–40], and represent the mean from at least three different determinations.

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