5-Arylisothiazol-3(2H)-one-1,(1)-(di)oxides: A new class of selective tumor-associated carbonic anhydrases (hCA IX and XII) inhibitors

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5-Arylisothiazol-3(2*H*)-one-1,(1)-(di)oxides: a new class of selective tumor-associated carbonic anhydrases (hCA IX and XII) inhibitors

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Abstract

Sixteen 5-aryl-substituted isothiazol-3(2*H*)-one-1,(1)-(di)oxide analogs have been prepared from the corresponding 5-chloroisothiazol-3(2*H*)-one-1-oxide or -1,1-dioxide by a Suzuki-Miyaura cross-coupling reaction and screened for their inhibition potency against four human carbonic anhydrase isoenzymes: the transmembrane tumor-associated hCA IX and XII and the cytosolic off-target hCA I and II. Most of the synthesized derivatives inhibited hCA IX and XII isoforms in nanomolar range, whereas remained inactive or modestly active against both hCA I and II isoenzymes. In the *N-tert*-butylisothiazolone series, the 5-phenyl-substituted analog (**1a**) excelled in the inhibition of tumor-associated hCA IX and XII (K_i = 4.5 and K_i = 4.3 nM, respectively) with excellent selectivity against off target hCA I and II isoenzymes (S>2222 and S>2325, respectively). Since the highest inhibition activities were observed with *N-tert*-butyl derivatives, lacking a zinc-binding group, we suppose to have a

new binding mode situated out of the active site. Additionally, three free-NH containing analogs (**3a**, **4a**, **3i**) have also been prepared in order to study the impact of free-NH containing *N*-acyl-sulfinamide- (-SO-NH-CO-) or *N*-acyl-sulfonamide-type (-SO_2-NH-CO-) derivatives on the inhibitory potency and selectivity. Screening experiments evidenced 5-phenylisothiazol-3(2*H*)-one-1,1-dioxide (**4a**), the closest saccharin analog, to be the most active derivative with inhibition constants of K_i = 40.3 nM and K_i = 9.6 nM against hCA IX and hCA XII, respectively. The promising biological results support the high potential of 5-arylisothiazolinone-1,(1)-(di)oxides to be exploited for the design of potent and cancer-selective carbonic anhydrase inhibitors.

Keywords:
5-Arylisothiazol-3(2*H*)-one-1-oxide derivatives
5-Arylisothiazol-3(2*H*)-one-1,1-dioxide derivatives
Suzuki-Miyaura cross-coupling
Anticancer agents
Carbonic anhydrase
Selective hCA IX/ hCA XII inhibitors

1. Introduction

Human carbonic anhydrases (hCAs, EC 4.2.1.1) are ubiquitous metalloenzymes catalyzing the reversible hydration/dehydration of carbon dioxide to bicarbonate ion and a proton. The fifteen isoforms found in humans are involved in a range of physiological functions such as respiration, pH and CO_2 homeostasis, electrolyte secretion in a variety of tissues/organs, bone resorption, calcification and also in biosynthetic pathways (lipogenesis, gluconeogenesis, ureagenesis) [1]. Over the past decades the inhibition of these enzymes has been intensively studied as their dysfunction, and/or over-expression are often associated with diverse pathologies such as mental disorders (CA II, VII, VIII, XIV), obesity (CA VA, VB), oedema (CA I, II), glaucoma (CA II, IV, XII) or cancer (CA IX, XII) [2-4]. Absent in normal tissues, the transmembrane isoenzymes hCA IX and XII have been identified to be over-expressed in several types of tumors, as well as associated to tumor progression, rendering them attractive anticancer drug targets [5-8]. hCA IX [9] is particularly abundant in glioblastoma, colorectal

and breast cancer [10,11], renal [12,13] and prostate carcinoma [14], whilst hCA XII isoform is also over-expressed in gastric, colorectal [15,16] and some breast cancer and in several forms of leukemia [17]. The main difficulty to design selective carbonic anhydrase inhibitors (CAIs) as therapeutic agents [18,19] is related to the high number of isoforms, their diffuse tissue/organ localization, high sequence and structural homology among the various isoenzymes. Inhibition of the cytosolic and catalytically very active hCA II isoform, which is abundant in many tissues/organs with well-defined physiological functions, is found to be responsible for a great part of side effects of the clinically used CAIs.

The most widely investigated CAIs belong to the primary sulfonamide family [20,21], interacting in their deprotonated forms directly with the Zn^{2+} of the active site. Although primary sulfonamides represent the most effective zinc-binding group, the almost identical binding mode and the highly similar active site of CAs render the design of isoenzyme selective sulfonamide-type inhibitors a challenging task in medicinal chemistry [22]. In the search of alternative chemical structures, non-zinc-binding function containing inhibitors have recently appeared with significant isoform selectivity profiles [23]. Thus, a unique inhibition mechanism has been discovered by investigating the CA inhibition of 2-(benzylsulfinyl)benzoic acid [24]. Supported by X-ray experiments, this compound was demonstrated to bind outside the active site blocking the highly flexible His64-mediated proton-shuttle and disrupting the catalytic process. By the fact that the active site entrance varies significantly among isoenzymes, a couple of recently developed new chemotypes displayed non-classical inhibition [25] and likewise high isoform selectivity, especially in favor of the transmembrane, tumor-associated isoforms hCA IX and hCA XII, *vs*. the cytosolic ones, hCA I and hCA II. Various coumarins [26-28], thio- [29] or sulfocoumarins [30] have been reported as new classes of suicide inhibitors with selective hCA IX and XII inhibition profiles.

Despite secondary and tertiary sulfonamides may be considered as poorly effective CA inhibitors, recently different secondary [31,32], tertiary sulfonamides [33,34], sulfamates [35] bearing halogenated or bulky substituents at their NHSOX (X=NH or O) moieties have also emerged as rather selective CAIs. However, the lack of crystallographic evidences does not allow to investigate the inhibition mechanism of these compounds. Saccharin (namely 1,2-benzoisothiazol-3-(2*H*)-one-1,1-dioxide), a cyclic secondary sulfonamide has been reported [36] to display selective hCA IX inhibition properties compared to all other hCA isoenzymes with exception of hCA VII. In order to gain higher hCA IX selectivity, the Zn^{2+}-coordinated

saccharin was functionalized with additional substituents on the benzene ring able to interact with the hydrophobic or the hydrophilic half of the CA active site [37]. *N*-Substituted saccharins [38-40] with alkyl, alkylaryl or alkynyl moieties proved to be potent inhibitors of the tumor-associated (hCA IX and XII) isoforms over the cytosolic ones (hCA I and II).

Pursuing our research towards the design of new and more selective CAIs with anticancer activity [41,42] and initiated by the excellent inhibition potency and selectivity of saccharin derivatives, we focused our attention on the synthesis of new, non-fused arylisothiazol-3(2*H*)-one-1,(1)-(di)oxide derivatives. Indeed, the isothiazol-3(2*H*)-one-1,1-dioxide moiety [43] is the main structural feature of saccharin and its derivatives.

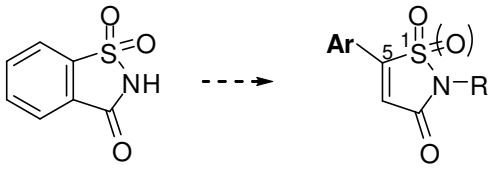


Figure 1. Structure of saccharin and the targeted 5-arylisothiazol-3(2*H*)-one-1,(1)-(di)oxide derivatives as carbonic anhydrase inhibitors.

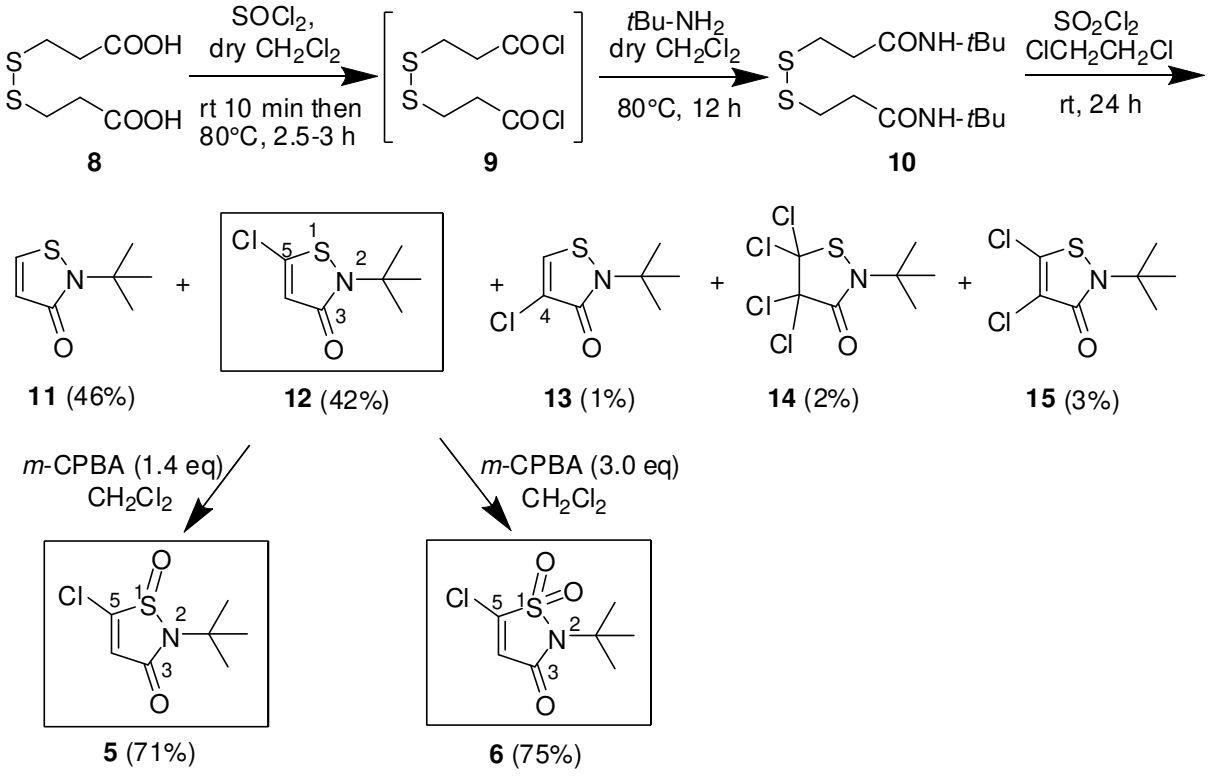
The synthetic strategy consisted in the functionalization of the five-membered ring-system by anchoring a series of lipophilic aromatic/heteroaromatic fragments using a palladium-catalyzed Suzuki-Miyaura cross-coupling reaction. Herein, we report the synthesis of the first examples of new 5-aryl-substituted isothiazol-3(2*H*)-one-1,(1)-(di)oxide derivatives (**1**, **2**, **3, 4**), their preliminary enzyme inhibition profile, measured on isoenzymes hCA I, II, IX and XII and their selectivity for hCA IX and XII over hCA I and II.

2. Results and discussion

2.1. Chemistry

Sixteen 5-aryl substituted isothiazolone(di)oxide derivatives (**1**, **2**, **3, 4**) have been prepared by a general Suzuki-Miyaura cross-coupling reaction between 2-*tert*-butyl-5-chloroisothiazol-3(2*H*)-one-1,(1)-(di)oxide (**5**, **6**) and the corresponding arylboronic acids (**7**). The synthesis of the starting material 5-halogeno partners followed, with some modifications, the previously reported method [44-47] (Scheme 1). The commercially available *bis*(3-thiopropionic acid) **8** was submitted to thionylchloride-mediated chlorination and the intermediate acylchloride **9** was transformed quantitatively into the bisamide **10**. *tert*-Butyl group was selected as *N*-

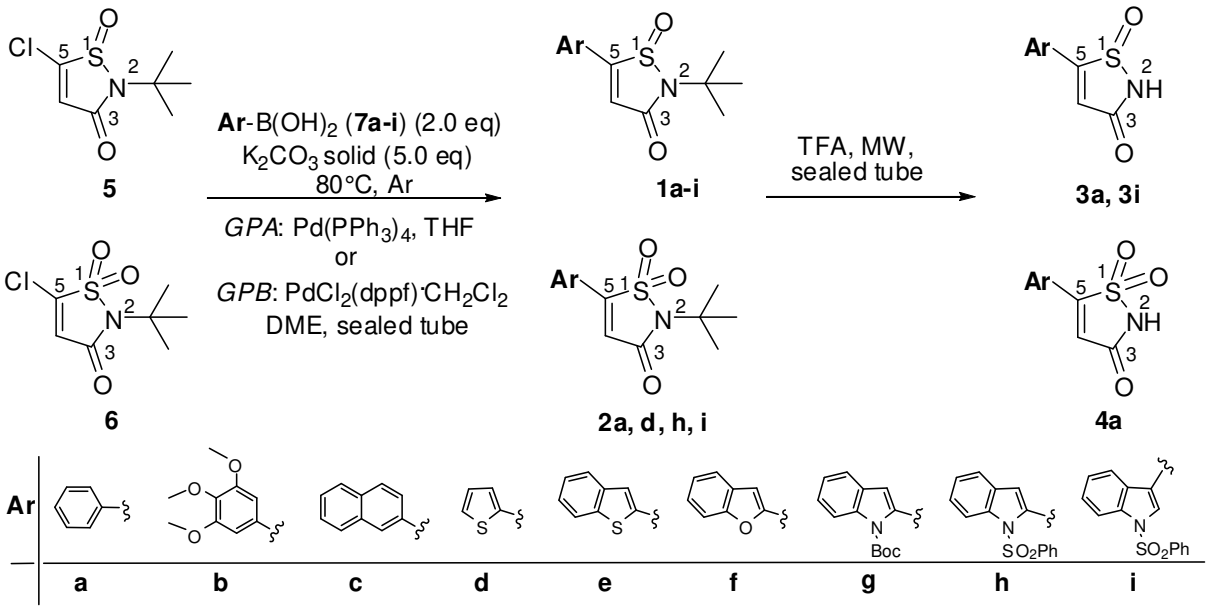
protecting function taking into account its compatibility with the experimental conditions used for the coupling reactions and its possible removal. Bisamide **10** was treated with 4 equivalents of sulfurylchloride in dichloroethane at room temperature for 24 hours to afford isothiazolidinone **11** (46%), its 5-chloro analogue **12** (42%) along with 4-chloro (**13**), 4,5-dichloro (**14**), 4,4,5,5-tetrachloro (**15**) analogs as minor products (1-3%). *S*-Oxidation of **12** was achieved with *m*-CPBA. *S*-Mono-oxidized derivative **5** was obtained with 1.4 equivalents of *m*-CPBA, added in small portions, while *S*-dioxidized analog **6** was prepared with 3 equivalents of *m*-CPBA in long reaction time.



Scheme 1. Synthetic pathway for the preparation of 2-*tert*-butyl-5-chloroisothiazol-3(2*H*)-one-1,(1)-(di)oxide **5** and **6**.

With 2-*tert*-butyl-5-chloroisothiazol-3(2*H*)-one-1-oxide (**5**) in hand, the first Suzuki-Miyaura couplings were carried out with phenylboronic acid (**7a**) and naphthylboronic acid (**7c**) using the classical $Pd(PPh_3)_4$ as catalyst and aqueous K_2CO_3 in boiling THF to afford the corresponding 5-phenyl- (**1a**) and 5-naphthyl- (**1c**) substituted isothiazolone-*S*-oxide in low yields (10-20%). Analyzing the reaction mixture we found, apart from deboronated and bis-aryl (biphenyl and binaphthyl) derivatives, often observed as the by-products of Suzuki-Miyaura couplings, other aryl-substituted and non-substituted ring-opened side-products

resulting from solvolyses, promoted by the aqueous alkaline reaction medium. Replacing the aqueous solution with finely powered solid K_2CO_3, we isolated 5-phenyl- (**1a**) and 5-naphthyl-substituted (**1c**) isothiazolone-*S*-oxide in 78 and 58% yields, respectively (Scheme 2, *GPA*) and no ring-opened side-products were observed. In both cases the conversion of the starting halide derivative **5** remained 90% despite keeping the reaction for long time. 5-Trimethoxyphenyl-substituted derivative (**1b**) was also synthesized by this procedure but the relevant chemical yield was lower (46%).



Scheme 2. Synthesis of 2-*tert*-butyl-5-arylisothiazol-3(2*H*)-one-1,(1)-(di)oxides (**1a-i**, **2a**, **d**, **h**, **i**) and 5-arylisothiazol-3(2*H*)-one-1,(1)-(di)oxides (**3a**, **3i** and **4a**).

With the aim to extend the 2-*tert*-butyl-5-chloroisothiazol-3(2*H*)-one-1-oxide (**5**) modulation to heterocyclic scaffolds, best results were obtained when the cross-coupling reaction was carried out in a sealed tube using $PdCl_2(dppf)\cdot CH_2Cl_2$ as catalyst, in the presence of solid K_2CO_3, in dimethoxyethane at 80°C (Scheme 2, *GPB*). Under these optimized conditions various 5-heterocycle-substituted 2-*tert*-butylisothiazol-3(2*H*)-one-1-oxide derivatives (**1d-i**) have been prepared in 39 to 86% yields.

Similarly, 5-aryl-2-*tert*-butylisothiazolone-1,1-dioxide analogs (**2a, d, h, i**), where aryl groups are phenyl (**2a**), 2-thiophenyl (**2d**), 1-phenylsulfonylindol-2-yl (**2h**) and 1-phenylsulfonylindol-3-yl (**2i**) were also prepared in good yields starting from the 2-*tert*-butyl-5-chloroisothiazol-3(2*H*)-one-1,1-dioxide (**6**) and the corresponding arylboronic acids **7** (Scheme 2).

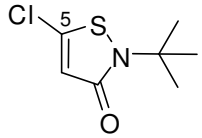
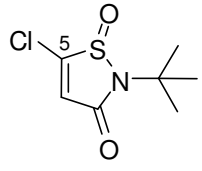
In order to gain more insights in the structure-activity relationship studies within this series, we prepared three *N*-deprotected analogs (**3a, 3i, 4a**) by TFA-mediated *N*-*tert*-butyl group cleavage reactions in a sealed tube, under microwave activation (Scheme 2). All synthesized compounds have been fully characterized by spectral (IR, ^{1}H NMR, ^{13}C NMR, MS, HRMS) data (see, Supplementary data, sections S3 and S4).

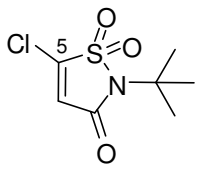
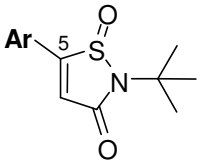
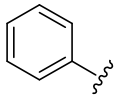
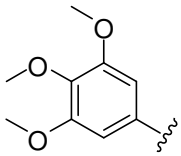
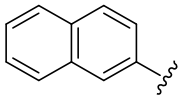
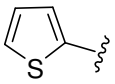
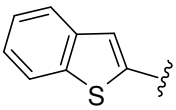
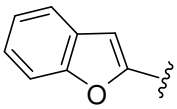
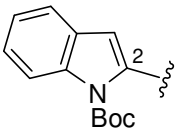
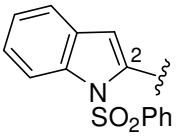
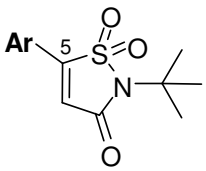
3. Biological evaluation

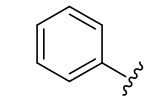
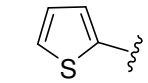
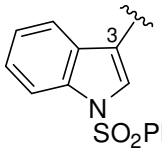
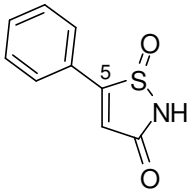
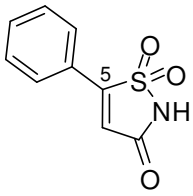
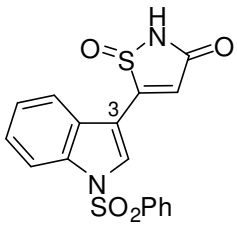
3.1. Carbonic anhydrase inhibition

The synthesized 5-arylisothiazol-3(2*H*)-one-1-oxide (**1**), 5-arylisothiazol-3(2*H*)-one-1,1-dioxide (**2**) and their free NH group containing analogs (**3**) and (**4**), respectively were screened for their inhibitory potency against four carbonic anhydrase isoenzymes, namely the widely distributed off-target cytosolic hCA I and II, as well as the tumor-associated transmembrane hCA IX and XII. In order to investigate the impact of 5-aryl substitution on the inhibition potency and selectivity in favor of hCA IX and XII isoenzymes, 2-*tert*-butyl-5-chloroisothiazol-3(2*H*)-one (**12**), 2-*tert*-butyl-5-chloroisothiazol-3(2*H*)-one-1-oxide (**5**) and its 1,1-dioxide (**6**) analog have also been tested. The results of these preliminary pharmacological screening, obtained by a stopped-flow CO_2 hydrase assay [48], were compared to the inhibition profile of acetazolamide (AAZ), a clinically used non-selective CAI (Table 1).

Table 1. hCA I, II, IX and XII isoforms inhibitory activity and selectivity of 2-*tert*-butyl-5-chloroisothiazol-3(2*H*)-one (**12**), its 1-oxide (**5**), 1,1-dioxide (**6**) analogs, 5-aryl-2-*tert*-butylisothiazol-3(2*H*)-one-1-oxide (**1a-i**), 5-aryl-2-*tert*-butylisothiazol-3(2*H*)-one-1,1-dioxide (**2a, d, h, i**), 5-arylisothiazol-3(2*H*)-one-1,(1)-(di)oxide (**3a, 3i**, **4a**) derivatives and acetazolamide (**AAZ**) as standard, by a stopped-flow CO_2 hydrase assay.

Compound		K_i [nM]				Selectivity ratio			
		hCA I	hCA II	hCA IX	hCA XII	I/IX	II/IX	I/XII	II/XII
12		1291.3	>10000	98.5	18.1	13.1	>101.5	71.3	>552.5
5		454.3	>10000	171.1	53.6	2.6	>58.4	8.5	>186.6

6		2508.6	>10000	226.9	40.0	11.1	>44.1	62.7	>250
	 **Ar**								
1a		>10000	>10000	4.5	4.3	>2222	>2222	>2325	>2325
1b		>10000	>10000	236.7	59.7	>42.2	>42.2	>167.5	>167.5
1c		4142.4	>10000	16	62.4	258.9	>625	66.4	>160.2
1d		>10000	>10000	48.6	791.7	>205.7	>205.7	>12.6	>12.6
1e		1834.7	>10000	133	49.8	13.8	>75.2	36.8	>200.8
1f		4484.3	>10000	250.5	7.3	17.9	>39.9	614.3	>1370
1g		>10000	>10000	47.7	42.3	>209.6	>209.6	>236.4	>236.4
1h		>10000	>10000	40.3	323.4	>248.1	>248.1	>30.9	>30.9
1i		>10000	2106	8.8	0.76	>1136	239.3	>13158	2771
	 **Ar**								

2a		>10000	1566	28.4	0.97	>352.1	55.1	>10309	1614
2d		>10000	>10000	220.8	560	>45.3	>45.3	>17.8	>17.8
2h		>10000	>10000	347.3	60.6	>28.8	>28.8	>165	>165
2i		>10000	81.8	4.3	0.94	>2325	19	>10638	87
3a		>10000	>10000	45.5	86.8	>219.8	>219.8	>115.2	>115.2
4a		>10000	>10000	40.3	9.6	>248.1	>248.1	>1041	>1041
3i		>10000	>10000	100.6	66.4	>99.4	>99.4	>150.6	>150.6
	AAZ	250	12	25	5.7	10	0.5	49.3	2.1

2-*tert*-Butyl-5-chloroisothiazol-3(2*H*)-one (**12**), 2-*tert*-butyl-5-chloroisothiazol-3(2*H*)-one-1-oxide (**5**) and the corresponding *S,S*-dioxide (**6**) proved to be inactive against hCA II but inhibited the other off-target isoform hCA I in low micromolar/nanomolar ranges, whereas all displayed two/three-digit nanomolar potency towards cancer-related hCA IX and hCA XII isoenzymes. However, inhibition selectivity profile in favor of hCA IX / XII *vs*. hCA I remained rather poor.

Concerning the 5-aryl-substituted isothiazolinone derivatives (**1**), a major part of the screened compounds was inactive against ubiquitous off-target hCA I and II isoforms, while all of them showed to inhibit tumor-associated hCA IX and XII in nanomolar concentration (K_i = 0.76 -

792 nM). 2-*tert*-Butyl-5-phenylisothiazol-3(2*H*)-one-1-oxide (**1a**) was the first and the simplest aryl derivative displaying excellent inhibitory potency and selectivity in favor of hCA IX and XII isoforms. By introduction of bulkier and also lipophilic naphthyl group a loss of inhibition towards cancer-related isoenzymes was observed for derivative **1c**, with a 3.5 and 14.5 fold reduced efficiency on isoforms hCA IX and hCA XII, respectively. Trimethoxyphenyl-substituted compound **1b** displayed no activity against off-target hCA I and hCA II isoenzymes, while its inhibition in favor of tumor-related hCA IX isoform remained modest (K_i = 236.7 nM).

When the phenyl group was replaced with the bioisosteric thiophene ring, an important loss of hCA XII inhibition (K_i = 792 nM) was observed even if the activity of **1d** against the other cancer-associated hCA IX isoenzyme remained relatively good (K_i = 48.6 nM). This negative impact on the hCA XII inhibition might be attributed to a slight conformational change of the dihedral angle between thiophene and isothiazolinone ring sytems owing to an interaction between lone-pair electrons of the sulfur atom and the C=C double bond of isothiazolinone. Benzothiophene- (**1e**) and benzofuran- (**1f**) functionalized analogs inhibited cytosolic hCAI enzyme in micromolar range (K_i = 1.8 μM and 4.5 μM, respectively), while both compounds showed good to excellent selectivity in hCA XII *vs*. hCA II enzyme inhibition assays (S = 200 and 1370, respectively). Toward the other cancer-related isoform hCA IX they were slightly less potent with K_i = 133.5 nM *vs*. 49.8 nM for benzothiophene derivative **1e** and K_i = 250.5 nM *vs*. 7.3 nM for the benzofuran analog **1f**. Cancer-related isoenzyme inhibition potency of **1c** and **1f** comparing to 5-naphthyl analog **1c** may be the result of the electronic effect of the heteroatoms with different electronegativity.

In the indole-substituted series, regioisomers and the nature of *N*-protecting groups seemed to play a significant role in enzyme inhibition. Indeed, *N*-Boc protected indole-containing compound **1g** displayed acceptable inhibition potency against both transmembrane hCA IX and XII isoforms (K_i = 47.7 nM and 42.3 nM, respectively), while its 1-phenylsulfonylindole-substituted derivative **1h** kept a similar hCA IX inhibition behavior (K_i = 40.3 nM) with an 8 fold less inhibition potency against the other cancer-associated isoenzyme hCA XII (K_i = 323.4 nM). Changing the functionalization from C-2 to C-3 carbon of the indole ring, we observed a drastic improvement of hCA XII inhibition for 2-*tert*-butyl-5-[1-(phenylsulfonyl)-1*H*-indol-3-yl]isothiazol-3(2*H*)-one-1-oxide (**1i**), affording the best inhibition constant (K_i = 0.76 nM) of the whole compound collection. In addition, one of the highest selectivity was displayed for cancer-associated isoenzymes hCA IX and hCA XII *vs*. off-target hCA I and in

a lesser extent *vs*. hCA II. The high potency and selectivity towards hCA IX and XII of **1i**, comparing to **1h** might be the origin of a greater rotational freedom, associated to supplementary lipophilic and/or electronic interactions of the $PhSO_2$ group.

In order to gain a better insight into the fine relations between isothiazolinone structure and hCA inhibition, some 5-aryl-isothiazolinone-*S*,*S*-dioxide derivatives (**2**) have also been prepared and screened. To this end, we selected the 5-phenyl- (**2a**), 5-thiophenyl- (**2d**), 5-(1-phenylsulfonylindol-2-yl)- (**2h**), and 5-(1-phenylsulfonylindol-3-yl)- (**2i**) substituted 2-*tert*-butylisothiazol-3(2*H*)-one-1,1-dioxide-type derivatives. Such compounds may be considered as the structurally closest ones to *N*-alkyl saccharins, reported as selective inhibitors of tumor-assisted hCA XII [38]. All the four analogs were inactive against hCA I isoenzyme, while their inhibition profile was more contrasting *vs*. the other off-target hCA II isoform: 2-*tert*-butyl-5-phenylisothiazol-3(2*H*)-one-1,1-dioxide (**2a**) and a higher extent 2-*tert*-butyl-5-(1-phenylsulfonylindol-3-yl)isothiazol-3(2*H*)-one-1,1-dioxide (**2i**) displayed respectively micromolar (K_i = 1.56 μM) and nanomolar (K_i = 82 nM) activities. In both cases, replacing the sulfinamide function by a sulfonamide moiety brought about an enhanced hCA II inhibition (comparison of K_i > 10000 nM *vs.* K_i = 1.56 μM for 5-phenyl-substituted analogs **1a** and **2a,** respectively or K_i = 2106 nM *vs.* K_i = 82 nM for 5-(1-phenylsulfonylindol-3-yl)-function containing analogs **1i** and **2i**, respectively). On the other hand, all of the *S*,*S*-dioxidized analogs were more efficient inhibitors of tumor-associated hCA IX and hCA XII isoforms comparing to the cytosolic ones (hCA I and hCA II). A fine analysis of their hCA IX inhibitory potency showed that *S*,*S*-dioxidized compounds, except for the 5-(1-phenylsulfonylindol-3-yl)-function containing analog (**2i**), became 4.5 to 8.6 fold less active comparing to their *S*-mono-oxidized counterparts. This loss of activity may be attributed to a slightly different interaction/position of the *S*,*S*-dioxidized compounds on the hCA IX enzyme. Conversely, the hCA XII inhibition properties of 5-(1-phenylsulfonylindol-2-yl)-function containing compounds (**1h** *vs*. **2h**) showed an opposite tendency when sulfinamide moiety was replaced by sulfonamide function. Thus, 2-*tert*-butyl-5-[1-(phenylsulfonyl)-1*H*-indol-2-yl]isothiazol-3(2*H*)-one-1,1-dioxide (**2h**) appeared 5.3 fold more active than its *S*-mono-oxidized analog (**1h**) (K_i = 323.4 nM *vs.* K_i = 60.6 nM). Outstanding results for the inhibition of hCA XII were reached with 5-phenyl- (**2a**) and 5-(1-phenylsulfonylindol-3-yl)-(**2i**) substituted derivatives (K_i = 0.97 nM and K_i = 0.94 nM) though the selectivity of this latter for the transmembrane tumor-associated hCA XII over the cytosolic hCA II remained modest (S = 87).

Finally, three of the most potent and selective tumor-associated enzyme (hCA IX and XII) inhibitors (**1a**, **2a**, **1i**) were deprotected and screened in order to study the impact of free NH containing *N*-acyl-sulfinamide- (-SO-NH-CO-) or *N*-acyl-sulfonamide-type (-SO_2-NH-CO-) derivatives (**3a**, **4a**, **3i**) on the inhibitory potency and selectivity. It is important to note that these compounds may be considered as close analogs of saccharin with non-fused aryl groups of greater conformational mobility. All of the three free-NH containing compounds (**3a**, **4a**, **3i**) were inactive against the off-target hCA I and hCA II isoenzymes, whilst inhibited both cancer-associated hCA IX and XII isoenzymes in nanomolar concentration (from K_i = 9.6 nM for **4a** to K_i = 100.6 nM for **3i**). Interestingly, similar tendency was observed when the inhibition potency and selectivity of saccharin and its *N*-alkyl (Et, *n*-Pr, *n*-Bu, *n*-pentyl) analogs were evaluated [40].

Comparing the three free-NH containing derivatives (**3a**, **4a**, **3i**) to their *N-tert*-Bu analogs (**1a**, **2a**, **1i**), we observed a loss of inhibitory potency against both hCA IX and XII. This drop of inhibition was more pronounced on hCA XII isoenzyme (from 9.9 to 87.3 fold) *vs.* hCA IX (from 1.42 to 11.4 fold). These results support the involvement of the *N-tert*-Bu group in a supplementary lipophilic interaction with both cancer-related isoenzymes (hCA IX and XII).

However, 5-phenylisothiazol-3(2*H*)-one-1,1-dioxide (**4a**), the closest saccharin analog, proved to be the most active derivative among the free-NH function containing compounds with inhibition constants of K_i = 40.3 nM and K_i = 9.6 nM against hCA IX and hCA XII, respectively.

4. Conclusions

As part of our research aiming at the design of selective tumor-associated CA inhibitors we have synthesized a series of 5-aryl-substituted isothiazol-3(2*H*)-one-1,(1)-(di)oxide analogs (**1**, **2**, **3**, **4**) starting from the corresponding 5-chloro derivatives (**5**, **6**) by a Suzuki-Miyaura cross-coupling reaction. Their CA inhibition activities were tested against four human CAs: the two widespread, off-target, cytosolic isoforms hCA I and hCA II, and the two cancer-related isoenzymes hCA IX and hCA XII. Most of the prepared compounds had no or modest (micromolar) affinity for hCA I and hCA II, while displayed nanomolar inhibition potency toward tumor-associated hCA IX and hCA XII isoforms. On the basis of this preliminary study we found that the highest inhibition activities were observed with no zinc-binding *N-tert*-butyl derivatives suggesting a binding mode outside the active site. Nevertheless, further in-depth mechanistic studies, supported by X-crystallography are required to shed light on

drug-enzyme interaction and consequently on the origin of the high selectivity of these 5-aryl-substituted isothiazol-3(2*H*)-one-1,(1)-(di)oxide analogs. The excellent inhibition potency and selectivity in favor of tumor-associated isoforms (hCA IX and XII) make this class of compounds attractive leads for the development of new antitumor agents.

5. Experimental protocols

5.1. Chemistry

General

All solvents were of reagent grade and, when necessary, purified and dried by standard methods. Reagents were purchased from Sigma-Aldrich, Alfa Aesar, and Strem Chemicals and used without further purification. Reactions and products were monitored by thin layer chromatography (TLC) on silica gel (KIESELGEL 60 F254, Merck). Flash column chromatography purifications were performed on CHROMAGEL 60 ACC (40-63 μm) silica gel. Melting points were determined on a Stuart SMP3 melting point apparatus (capillary tube). IR spectra were measured on a Perkin-Elmer Spectrum BX FTIR instrument. NMR spectra were recorded on a Bruker AC-300 spectrometer (^{1}H at 300 MHz and ^{13}C at 75 MHz) at 298 K or on a Bruker Avance III-600 spectrometer equipped with a cryoprobe (^{1}H at 600 MHz and ^{13}C at 150 MHz), using $CDCl_3$ or DMSO-d_6 as solvents. All ^{1}H NMR and ^{13}C NMR spectra are reported in δ units (ppm) using TMS as internal standard. Coupling constants *J* are expressed as s, brs, d, dd, ddd, td, t, dt, q, and m and correspond to singlet, broad singlet, doublet, doublet of doublets, doublet of doublet of doublets, triplet of doublets, triplet, doublet of triplets, quarter, and multiplet, respectively. Mass spectra were recorded on a GCT Waters apparatus using electron impact ionization (EI, HRMS). Microwave activated reactions were carried out in a CEM Discover® (300 W) apparatus. Purity of the final products (**1a-i**, **2a**, **2d**, **2h**, **2i**, **3a**, **3i** and **4a)** was checked by HRMS (±10 ppm of the theoretical values) and by NMR spectra (Supporting Information, sections S2, S3 and S4).

Synthesis of N,N'-di(tert-butyl)-dithiodipropionamide **(10**)

N,N'-di(*tert*-Butyl)-dithiodipropionamide **10** was prepared according to the described procedure [45].

Synthesis of 2-tert-butylisothiazol-3(2H)-one (**11**), *2-tert-butyl-5-chloroisothiazol-3(2H)-one* (**12**), *2-tert-butyl-4-chloroisothiazol-3(2H)-one* (**13**), *2-tert-butyl-4,5-dichloroisothiazol-3(2H)-one* (**14**) *and 2-tert-butyl-4,4,5,5-tetrachloroisothiazolidin-3(2H)-one* (**15**)

Compounds **11**, **12**, **13**, **14** and **15** were prepared according to the modified procedures described by Lewis *et al.* [45], Combs *et al.* [46] and Yue *et al*. [47] for the synthesis of **11** and **12**. These modified conditions allowed the synthesis of three new compounds **13**, **14** and **15**.

To a suspension of *N,N'*-di(*tert*-butyl)-dithiodipropionamide **10** (5.0 g, 15.6 mmol, 1.0 eq.) in dichloroethane (30 mL), placed in an ice bath at 0°C was dropwise added SO_2Cl_2 (5 mL, 62.4 mmol, 4.0 eq.). The reaction mixture was stirred at 0°C for 5 minutes then at room temperature for 24 h. After evaporation of the solvent, the crude product was diluted in CH_2Cl_2 (50 mL) and washed at 0°C with a saturated aqueous solution of K_2CO_3 (40 mL). Aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated. The obtained brown oil was purified by flash column chromatography on silica gel (eluent: petroleum ether / ethyl acetate) to isolate successively **14** (93 mg, 2%), **15** (106 mg, 3%), **12** (1.26 g, 42%), **13** (30 mg, 1%) and **11** (1.13 g, 46%) in growing polarity.

Melting points and missing analytical data (IR, ^{13}C NMR, MS (EI), HRMS) are given for the previously described compounds **11** [45,49-51] (Supporting Information, section S1; section S4 for NMR spectra) and **12** [46,49] (Supporting Information, section S4 for NMR spectra).

2-tert-Butyl-5-chloroisothiazol-3(2H)-one (**12**)

Eluent used for purification: petroleum ether / ethyl acetate: 99/1, Yield: 42%; White solid; M.p. 112 °C; IR (KBr): ν_{max} 3175, 2966, 2925, 1649 (CO), 1558, 1549, 1365, 1220 cm^{-1}; ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.2 (*C*O), 144.6 (C-5), 116.6 (CH-4), 59.8 (*C*$(CH_3)_3$), 28.3 (C(*C*$H_3)_3$); MS (EI) *m/z* (%): 193 (15, $[^{37Cl}M]^{+\bullet}$), 191 (41, $[^{35Cl}M]^{+\bullet}$), 138 (39), 137 (31, $[^{37Cl}M^{+\bullet} - C(=CH_2)(CH_3)_2]$), 136 (100), 135 (71, $[^{35Cl}M^{+\bullet} - C(=CH_2)(CH_3)_2]$), 57 (48); HRMS (EI) calculated for $C_7H_{10}NOS^{35}Cl_2$ 191.0172, found 191.0174 [$M^{+\bullet}$].

Melting points and analytical data (IR, ^{1}H NMR, ^{13}C NMR, MS (EI), HRMS) of compounds **13**, **14** and **15** are given in the Supporting Information, section S1. NMR spectra are given in Supporting Information, section S4.

*Synthesis of 2-tert-butyl-5-chloroisothiazol-3(2H)-one-1-oxide (**5**) and 2-tert-butyl-5-chloroisothiazol-3(2H)-one-1,1-dioxide* (**6**)

Compounds **5** and **6** were prepared according to respectively modified procedures of Lewis *et al.* [44], Ghizzoni *et al.* [43], Combs *et al.* [46]and Yue *et al*. [47].

*S-monooxidation of **12***: to a solution of 2-*tert*-butyl-5-chloroisothiazol-3(2*H*)-one (**12**) (2.31 g, 12.0 mmol, 1.0 eq.) in distilled CH_2Cl_2 (80 mL), placed in an ice bath at 0°C was portionwise added *m*-CPBA (70% purity, 3.77 g, 16.8 mmol, 1.4 eq.). The obtained orange suspension was stirred at 0°C for 5 minutes then at room temperature for 48 h. The obtained yellow suspension was filtered off under reduced pressure. The filtrate was washed with a 5% aqueous solution of $NaHCO_3$ (5 × 40 mL) and the obtained organic layer was dried over $MgSO_4$, filtered and evaporated. The crude product was purified by flash column chromatography on silica gel to obtain 2-*tert*-butyl-5-chloroisothiazol-3(2*H*)-one-1,1-dioxide (**6**), as a white crystalline solid (eluent: petroleum ether / ethyl acetate, 99/1, 402 mg, 15%), and 2-*tert*-butyl-5-chloroisothiazol-3(2*H*)-one-1-oxide (**5**), as a yellow oil (eluent: petroleum ether / ethyl acetate, 98/2, 1.78 g, 71%).

*S-dioxidation of **12***: to a solution of 2-*tert*-butyl-5-chloroisothiazol-3(2*H*)-one (**12**) (1.43 g, 7.46 mmol, 1.0 eq.) in distilled CH_2Cl_2 (40 mL), placed in an ice bath at 0°C was portionwise added *m*-CPBA (70% purity, 5.2 g, 22.4 mmol, 13.0 eq.). The obtained orange suspension was stirred at 0°C for 5 minutes then at room temperature for 48 h. After the reaction completion, the obtained yellow suspension was filtered off under reduced pressure. The filtrate was washed with a 5% aqueous solution of $NaHCO_3$ (5 × 20 mL) and the obtained organic layer was dried over $MgSO_4$, filtered and evaporated. The crude product was purified by flash column chromatography on a silica gel to isolate 2-*tert*-butyl-5-chloroisothiazol-3(2*H*)-one-1,1-dioxide (**6**), as a white crystalline solid (eluent: petroleum ether / ethyl acetate, 99/1, 1.25 g, 75%).

Since compound **5** [43] and compound **6** [46,49] have already been described, their melting point and missing analytical data (**5**: IR, MS (EI), HRMS; **6**: IR, ^{13}C NMR, MS (EI), HRMS) are given. NMR spectra are given in Supporting Information, section S4.

2-tert-Butyl-5-chloroisothiazol-3(2H)-one-1-oxide (**5**)

IR (KBr): ν_{max} 3068, 2973, 2926, 1700 (CO), 1610, 1364, 1257, 1194, 1107 (SO), 940 cm^{-1}; MS (EI) *m/z* (%): 209 (2, $[^{37Cl}M]^{+\bullet}$), 207 (5, $[^{35Cl}M]^{+\bullet}$), 194 (44, $^{37Cl}[M]^{+\bullet} - CH_3$), 192 (100,

$^{35Cl}[M]^{+\bullet} - CH_3$), 154 (41), 152 (95), 146 (31), 144 (84), 57 (81); HRMS (EI) calculated for $C_7H_{10}NO_2S^{35}Cl_2$ 207.0121, found 207.0124 [$M^{+\bullet}$].

2-tert-Butyl-5-chloroisothiazol-3(2H)-one-1,1-dioxide (**6**)

M.p. 112-114 °C; IR (KBr): ν_{max} 3078, 2987, 2930, 1719 (CO), 1335 (SO_2), 1257, 1220, 1182, 1156 (SO_2), 955 cm^{-1}; ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.1 (*C*O), 143.6 (C-5), 123.7 (CH-4), 62.4 (*C*(CH_3)$_3$), 27.7 (C(*C*H_3)$_3$); MS (EI) *m/z* (%): 223 (0.1, [$^{35Cl}M]^{+\bullet}$), 210 (39, [$^{37Cl}M^{+\bullet} - 15$]), 208 (100, [$^{35Cl}M^{+\bullet} - 15$]), 170 (13), 168 (35), 56 (46); HRMS (EI) calculated for $C_7H_{10}NO_3S^{35}Cl$ 223.0070, found 223.0069 [$^{35Cl}M^{+\bullet}$]; HRMS (EI) calculated for $C_6H_7NO_3S^{35}Cl$ 207.9835, found 207.9839 [$^{35Cl}M^{+\bullet} - CH_3$].

*General Procedures for the synthesis of 2-tert-butyl-5-arylisothiazol-3(2H)-one-1-oxides (**1a-i**) and 2-tert-butyl-5-arylisothiazol-3(2H)-one-1,1-dioxides* (**2a, 2d, 2h, 2i**)

2-*tert*-Butyl-5-arylisothiazol-3(2*H*)-one-1-oxides (**1a**-**c**) and 2-*tert*-butyl-5-phenylisothiazol-3(2*H*)-one-1,1-dioxide (**2a**) were prepared using the Suzuki-Miyaura cross-coupling reaction of 2-*tert*-butyl-5-chloroisothiazol-3(2*H*)-one-1-oxide (**5**) or 2-*tert*-butyl-5-chloroisothiazol-3(2*H*)-one-1,1-dioxide (**6**) with boronic acids **7a**-**c** using $Pd(PPh_3)_4$ as catalyst (*General Procedure A (GPA)*, Supporting Information, section S2). In a typical procedure, **5** (90 mg, 0.43 mmol, 1.0 eq.) or **6** (90 mg, 0.40 mmol, 1.0 eq.), **7a**-**c** (0.80-0.96 mmol, 2.0 eq.), potassium carbonate (276 mg, 2.0 mmol or 297 mg, 2.15 mmol, 5.0 eq.) and $Pd(PPh_3)_4$ (69 mg, 0.06 mmol or 75 mg, 0.065 mmol, 15 mol%) were successively added in a two-necked round-bottomed flask under an inert atmosphere of argon. Dry THF (10 mL) was added and the resulting suspension was degassed with argon and stirred at 80 °C. After cooling to room temperature, the suspension was filtered over a Celite® pad and concentrated to dryness. The crude solids were purified by two successive flash column chromatographies on silica gel with petroleum ether / ethyl acetate as eluent (Supporting Information, section S2).

2-*tert*-Butyl-5-arylisothiazol-3(2*H*)-one-1-oxides (**1a, 1d**-**i**) and 2-*tert*-butyl-5-arylisothiazol-3(2*H*)-one-1,1-dioxides (**2a**, **2d**, **2h**, **2i**) were prepared following a modified procedure of Yue *et al.* [47] for the Suzuki-Miyaura cross-coupling reaction of 2-*tert*-butyl-5-chloroisothiazol-3(2*H*)-one-1-oxide (**5**) or 2-*tert*-butyl-5-chloroisothiazol-3(2*H*)-one-1,1-dioxide (**6**) with boronic acids **7a**, **7d-i** using $PdCl_2(dppf)\cdot CH_2Cl_2$ as catalyst (*General Procedure B (GPB)*, Supporting Information, section S2). In a typical procedure, **5** (83-118 mg, 0.40-0.57 mmol,

1.0 eq.) or **6** (96 mg, 0.43 mmol, 1.0 eq.), boronic acids **7a**, **7d**-**i** (0.80-1.14 mmol, 2.0 eq.) and potassium carbonate (276-394 mg, 2.0-2.85 mmol, 5.0 eq.) were successively added in a sealed tube under argon atmosphere. DME was added (7 mL), the resulting suspension was degassed with argon and $PdCl_2(dppf)\cdot CH_2Cl_2$ (49-70 mg, 0.06-0.085 mmol, 15% mol) was introduced keeping the tube under argon atmosphere. The tube was sealed and the mixture was stirred at 80°C in a heat-insulating Erlenmeyer flask containing oil. After cooling to room temperature, the suspension was filtered over a Celite® pad and concentrated to dryness. The crude oily mixtures were purified by two successive flash column chromatographies on silica gel with: petroleum ether / ethyl acetate for the first column and petroleum ether / ethyl acetate or petroleum ether / CH_2Cl_2 as eluent for the second one. (Supporting Information, section S2).

Chemical yields, analytical data of the phenyl series **1a**, **2a** are given as typical examples; data of compounds **1b**-**i**, **2d**, **2h**, **2i** and copies of ^1H and ^{13}C NMR spectra are given in Supporting Information, sections S2 and S4, respectively.

Since compound **1a** [52] has already been described, its melting point and missing analytical data (IR, MS (EI), HRMS) are given. NMR spectra of molecules **1a** and **2a** are given in Supporting Information, section S4.

2-tert-Butyl-5-phenylisothiazol-3(2H)-one-1-oxide (**1a**)

Following *GPA* or *GPB*: purification with petroleum ether / ethyl acetate: 99/1, then petroleum ether / ethyl acetate : 99/1→98/2, Yield: 78%; Beige solid; M.p. 63 °C; IR (KBr): ν_{max} 3059, 2959, 2931, 2834, 1693 (CO), 1678, 1092 (SO) cm^{-1}; MS (EI) *m/z* (%): 249 (11, $[M^{+\bullet}]$), 234 (27, $[M^{+\bullet} - CH_3]$), 194 (58), 193 (96, $[M^{+\bullet} - C(=CH_2)(CH_3)_2]$), 147 (45), 125 (58), 102 (100); HRMS (EI) calculated for $C_{13}H_{15}NO_2S$ 249.0840, found 249.0824 $[M^{+\bullet}]$.

2-tert-Butyl-5-phenylisothiazol-3(2H)-one-1,1-dioxide (**2a**)

Following *GPA*: purification with petroleum ether / ethyl acetate: 99/1→98/2, then petroleum ether / ethyl acetate: 98/2→97/3, Yield: 86%; Following *GPB*: purification with petroleum ether / ethyl acetate: 99/1→98/2, then petroleum ether / ethyl acetate : 98/2→97/3, Yield: 76%; White solid; M.p. 85 °C; IR (KBr): ν_{max} 3421, 3070, 2972, 2920, 1711 (CO), 1326 (SO_2), 1272, 1246, 1184, 1150 (SO_2) cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 7.76-7.79 (m, 2H, 2 × Ar-H), 7.54-7.57 (m, 1H, Ar-H), 7.50-7.53 (m, 2H, 2 × Ar-H), 6.65 (s, 1H, H-4), 1.74 (s, 3 × 3H, $C(CH_3)_3$); ^{13}C NMR (150 MHz, $CDCl_3$): δ 160.4 (*C*O), 150.7 (C-5), 132.5 (CH),

129.6 (2 × CH), 127.9 (2 × CH), 124.4 (C-1′), 119.5 (CH-4), 61.5 (*C*(CH_3)$_3$), 27.8 (C(*C*H_3)$_3$); MS (EI) *m/z* (%): 265 (11, [$M^{+\bullet}$]), 250 (74, [$M^{+\bullet} - CH_3$]), 209 (39, [$M^{+\bullet} - C(=CH_2)(CH_3)_2$]), 210 (91), 105 (74), 102 (100), 56 (38); HRMS (EI) calculated for $C_{13}H_{15}NO_3S$ 265.0773, found 265.0782 [$M^{+\bullet}$].

*General Procedure for the synthesis of 5-arylisothiazol-3(2H)-one-1-oxides (**3a**, **3i**) and 5-phenylisothiazol-3(2H)-one-1,1-dioxide* (**4a**)

5-Arylisothiazol-3(2*H*)-one-1-oxides **3a, 3i** and 5-arylisothiazol-3(2*H*)-one-1,1-dioxide **4a** were prepared according to the slightly modified condition of Miambo *et al.* [53] by using a TFA-mediated *N-tert*-butyl group cleavage of 2-*tert*-butyl-5-arylisothiazol-3(2*H*)-one-1-oxides **1a**, **1i** or 2-*tert*-butyl-5-phenylisothiazol-3(2*H*)-one-1,1-dioxide (**2a**) under microwave irradiation. In a typical procedure, a solution of **1a** (41 mg, 0.16 mmol), **1i** (45 mg, 0.11 mmol) or **2a** (41 mg, 0.15 mmol) in TFA (3 mL) was stirred in a sealed tube heated under microwave irradiation at 200W over a 3 min run time period until 100 °C (**2a**), 120°C (**1i**) or 130°C (**1a**). The reaction mixture was then heated during a hold time period of 9 min at 100°C (**4a**) or 21 min at 120°C (**3i**) or 42 min at 130°C (**3a**). After cooling to room temperature, **3a** was isolated by evaporation of the crude mixture under reduced pressure, at 30°C in the presence of methanol (4 × 0.5 mL) followed by crystallization in diethylether (1-2 mL). **3i** and **4a** were isolated by filtration after cooling the crude mixture in an ice bath during 5–10 min.

Chemical yields, analytical data of the phenyl series (compounds **3a** and **4a**) are given as typical examples. Since compound **4a** [54,55] has already been described, its melting point and missing analytical data (^{13}C NMR, HRMS) are given. ^{1}H and ^{13}C NMR spectra are given in Supporting Information, section S4.

Analytical data and ^{1}H and ^{13}C NMR spectra of compound **3i** are given in Supporting Information, sections S3 and S4 respectively.

5-Phenylisothiazol-3(2H)-one-1-oxide (**3a**)

Yield: 65%, White solid; M.p. 146 °C; IR (KBr): ν_{max} 3383, 3196, 3063, 1703 (CO), 1612, 1448, 1314, 1254, 1069 (SO) cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d$_6$): δ 7.81-7.85 (m, 2H, 2 × Ar-H), 7.54-7.58 (m, 3H, 3 × Ar-H), 7.16 (s, 1H, H-4); ^{13}C NMR (150 MHz, DMSO-d$_6$): δ 171.2 (*C*O), 164.7 (C-5), 131.8, 129.8 (2 × CH), 129.0, 128.3 (2 × CH), 121.3; MS (EI) *m/z*

(%): 193 (7, [$M^{+\bullet}$]), 177 (95), 147 (92), 129 (100), 102 (79), 77 (30); HRMS (EI) calculated for $C_9H_7NO_2S$ 193.0198, found 193.0195 [$M^{+\bullet}$].

5-Phenylisothiazol-3(2H)-one-1,1-dioxide (**4a**)

Yield: 73%; White solid; M.p. 251 °C; ^{13}C NMR (150 MHz, DMSO-d_6): δ 164.7 (*C*O) (observed *via* the HMBC heteronuclear bidimensional NMR experiment, Supporting Information, section S4), 152.9 (C-5), 133.3 (CH), 129.9 (2 × CH), 128.4 (2 × CH), 125.9 (C-1′), 121.9 (*C*H-4); HRMS (EI) calculated for $C_9H_7NO_3S$ 209.0147, found 209.0147 [$M^{+\bullet}$].

5.2. CA Inhibition assay

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity [48]. Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 10 mM Hepes (pH 7.4), 10 mM Tris-HCl, and 0.1 M 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 sec. 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 noncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (10 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 15 min at room temperature prior to assay in order to allow for the formation of the E−I complex. As sulfonamides act as noncompetitive inhibitors with CO_2 as substrate [56,2,19] the inhibition constants were obtained by nonlinear least-squares methods using PRISM 3 and the Cheng−Prusoff equation as reported earlier [48] and represent the mean from at least three different determinations. All CAs were recombinant proteins obtained as reported earlier by these groups [57-59].

Acknowledgements

Financial support of CNRS, University of Reims is gratefully acknowledged. B. Cornelio is grateful for financial support from Italian Ministry of Education, University and Research

(MIUR, PRIN 2010-2011 prot. 2010N3T9M4), Italian-French University (Vinci 2011), and University of Chieti-Pescara. R.F. Miambo thanks the French Ministry of Foreign Affairs for a PhD Fellowship. We thank Ms Gabriela Garncarz (Medical University of Warsaw, Poland) for her skillful technical assistance. B. Borisova (Univ. Sofia), M. De Grandis, R. Riccioni (Univ. Chieti-Pescara) and G. Garncarz (Univ. Warsaw) thank the EU for Erasmus+ Fellowship. We thank A. Martinez, V. Gonçalves, and D. Harakat for spectroscopic measurements.

Appendix A. Supplementary data

Supplementary data to this article can be found online at

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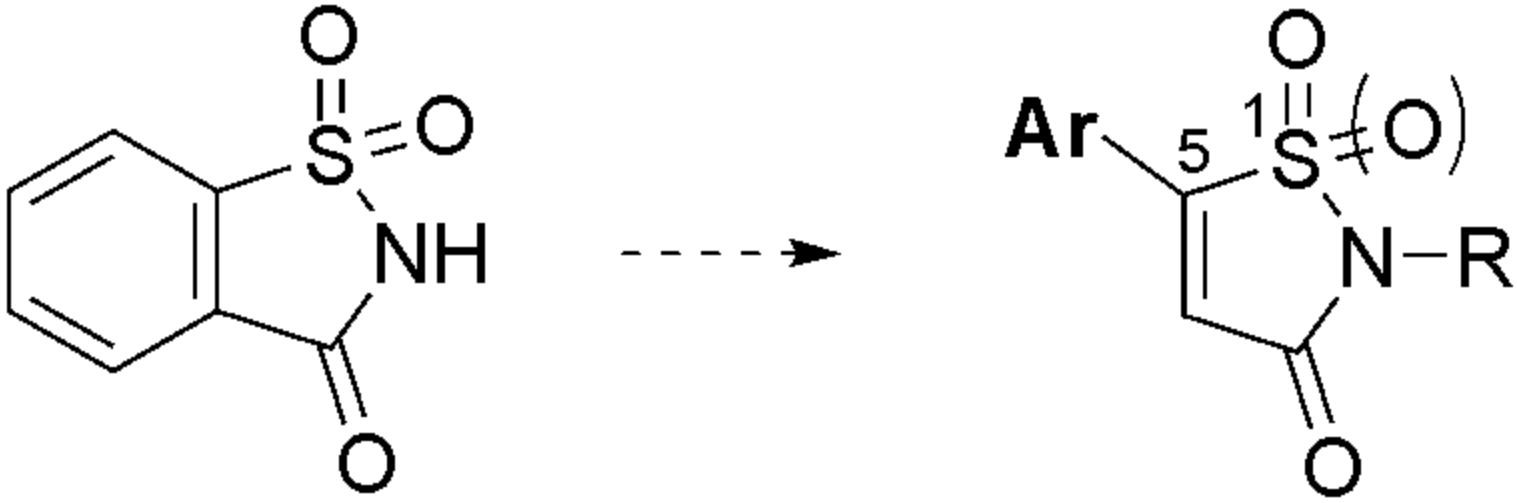
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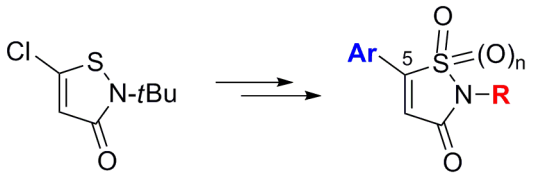

Ar	R	n	K_i [nM] hCA I	hCA II	hCA IX	hCA XII
Ph	*t*Bu	0	>10^4	>10^4	4.5	4.3
		1	>10^4	1566	28.4	0.97
Ph	H	0	>10^4	>10^4	45.5	86.8
		1	>10^4	>10^4	40.3	9.6

Ar: phenyl, 2-naphthyl, 2-thienyl, 2-benzo[*b*]furanyl, 2-benzo[*b*]thiophenyl, 1-phenylsulfonylindol-2-yl, 1-phenylsulfonylindol-3-yl...