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Carbonic anhydrase inhibitors. The β-carbonic anhydrases from the fungal pathogens *Cryptococcus neoformans* and *Candida albicans* are strongly inhibited by substituted-phenyl-1*H*-indole-5-sulfonamides

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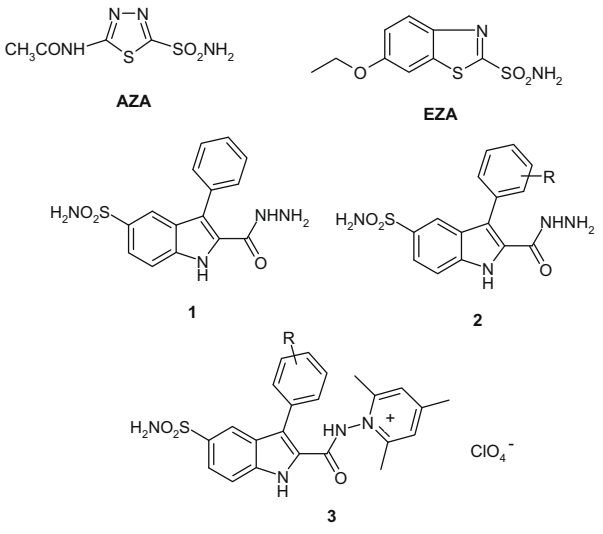
ABSTRACT

A series of 2-(hydrazinocarbonyl)-3-substituted-phenyl-1*H*-indole-5-sulfonamides and 1-({[5-(aminosulfonyl)-3-phenyl-1*H*-indol-2-yl]carbonyl}amino)-2,4,6 trimethylpyridinium perchlorates possessing various 2-, 3- or 4-substituted phenyl groups with methyl-, halogeno- and methoxy-functionalities, as well as the perfluorophenyl moiety, have been evaluated as inhibitors of the β-carbonic anhydrases (CAs, EC 4.2.1.1) from the pathogenic fungi *Cryptococcus neoformans* (Can2) and *Candida albicans* (CaNce103). Both enzymes were potently inhibited by these sulfonamides, K_Is in the range of 4.4–118 nM against Can2, and of 5.1–128 against CaNce103, respectively. Minor structural changes in the 3-substituted phenyl moiety contribute significantly to the inhibitory activity. Some of the investigated sulfonamides showed promising selectivity ratios for inhibiting Can2 over the host, human enzymes CA I and II.

In previous work[1] from this laboratory we have reported that 2-(hydrazinocarbonyl)-3-substituted-phenyl-1*H*-indole-5-sulfonamides **1** and **2** possessing various 2-, 3- or 4-substituted phenyl groups, show interesting inhibitory activity against some isoforms of the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1).[2] Indeed, CAs are widespread enzymes in organisms all over the phylogenetic tree, as they catalyze the interconversion of carbon dioxide and bicarbonate, a simple but physiologically crucial reaction.[2–4] Whereas mammals contain only α-class CAs (in the form of 16 different isoforms),[2] genetically distinct β-, γ-, δ- and ξ-CAs are present, among others, in bacteria, archaea, fungi, plants, diatoms and other such simpler organisms.[2–4] The five classes of CAs are thus a perfect example of convergent evolution, as their active sites, 3D folds of the protein backbone and catalytic mechanisms are very different among the diverse enzyme classes.[2–6]

Sulfonamides represent the main class of clinically used CA inhibitors (CAIs).[2] Members of this class including acetazolamide **AZA** or ethoxzolamide **EZA** among others, and are clinically employed for the management of a variety of disorders connected to CA disbalances, such as glaucoma;[2,7] in the treatment of edema due to congestive heart failure,[8] or for drug-induced edema; as mountain sickness drugs,[8] whereas other agents of this pharmaco-



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logical class show applications as anticonvulsants,[9] antiobesity[2] or antitumor drugs/tumor diagnostic agents.[2,10] As there are few isoform-selective inhibitors to date, new sulfonamides are continuously reported to find derivatives with better inhibition profiles as compared to the promiscuous, first generation inhibitors such as **AZA** or **EZA**.[2] Furthermore, enzymes belonging to the β- and γ-CA classes, some of which present in pathogenic bacteria or fungi,[2,5,6,11,12] have also been investigated recently for their interaction with this class of derivatives, in the search for antiinfectives possessing a different mechanism of action compared to the classical antibiotic/antifungal drugs.[11,12]

We have recently shown[11a] that the β-CAs from the fungal pathogens *Cryptococcus neoformans* (Can 2)[5] and *Candida albicans* (CaNce103)[6] are inhibited by sulfonamides. Up to now we have investigated only simple sulfonamide scaffolds as well as the 20 clinically used sulfonamides, among which acetazolamide **AZA** and ethoxzolamide **EZA**, for the inhibition of these enzymes, finding very few effective inhibitors against the fungal enzymes.[11a] Indeed, **AZA** was a good Can2 inhibitor (with a K_I of 10 nM) whereas it had a more modest activity against CaNce103 (with a K_I of 132 nM).[11a] **EZA** was even less effective, with K_Is of 87 nM against Can2 and 1070 nM against CaNce103, respectively. Thus, in the search for more effective sulfonamide compounds targeting the fungal pathogenic enzymes Can2 and CaNce103, we report here an inhibition study with a series of 2-(hydrazinocarbonyl)-3-substituted-phenyl-1*H*-indole-5-sulfonamides **1**, **2** and 1-({[5-(aminosulfonyl)-3-phenyl-1*H*-indol-2-yl]carbonyl}amino)-2,4,6 trimethylpyridinium perchlorates **3,** which have been described earlier for their interaction with the mammalian α-CAs.[1,2]

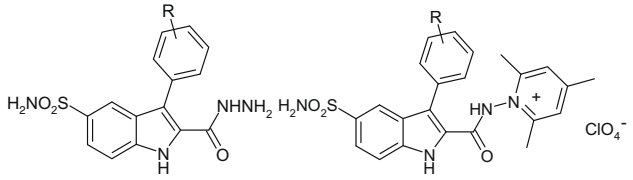
We have included sulfonamides **1**–**3** in this study as they constitute a class of CA inhibitors (CAIs) of potential interest.[1,12e] In fact we have observed recently an excellent activity against two of the three β-CAs from the bacterial pathogen *Mycobacterium tuberculosis* for the 2-(pyridiniumaminocarbonyl)-3-substituted-phenyl-1*H*-indole-5-sulfonamides **3**.[12e] The presence of various substituents at the 3-phenyl group of the indole rings of derivatives **2** may increase lipo- or hydrosolubility of these compounds, and also interact in a positive/negative manner with amino acid residues present in the CA active site allowing thus to obtain structure activity relationship (SAR) insights for the inhibition of these enzymes.[12a] Thus, incorporation of 2-, 3- or 4-substituted phenyl groups possessing methyl-, halogeno- and methoxy-functionalities in the 3-position of the indolesulfonamides **2**, has been contemplated in order to explore as many as possible scaffolds for detecting high affinity and possibly selective inhibitors for the fungal (Can2, CaNce103) over host enzymes (hCA I and hCA II).

Inhibition data with sulfonamides **1**–**3** as well as the standard drugs **AZA** and **EZA** against Can2 and CaNce103 (β-class enzymes) as well as the ubiquitous,[2] human CA isoforms hCA I and II (α-CAs)[1a] (off-targets) are shown in Table 1.[13,14]

The following SAR can be drawn by considering data of Table 1:

(i) Against Can2, the indolesulfonamides **1** and **2** showed good inhibitory activity, with K_Is in the range of 7.2–118 nM, making this entire sulfonamide class among the best sulfonamide Can2 inhibitors detected so far. Thus, the lead compound **1**, and derivatives **2i–2l** and **2n** showed medium-high potency as Can2 inhibitors, with inhibition constants in the range of 62–118 nM. These derivatives incorporate the 4-chloro, 2-, 3- and 4-bromo as well as pentafluorophenyl moieties (together with the lead **1**). In contrast, the remaining derivatives, incorporating methyl-, fluoro-, 2-/3-chloro- and methoxy-substituted phenyl moieties in the position 3 of the indole ring, of types **2a–2h**, and **2m**, showed a much stronger Can2 inhibitory effect, with K_Is in the range of 7.2–10.5 nM. Thus, the nature of the group substituting the 3-phenyl ring present in compounds **1** and **2** strongly influences the Can2 inhibitory activity, with the methyl-, methoxy-, fluoro- and chloro-substituted derivatives showing a better activity (around 10-fold) compared to the lead **1** or the bromosubstituted compounds **2j–2l**. The position of the substituent of the phenyl ring is somehow less influential on the inhibitory activity (except for the chloro-derivatives **2g–2i**, case in which the 4-chlorosubstituted compound **2i** was around 8.7–9.4 times a weaker inhibitor compared to the 2- or 3-chlorosubstituted isomers **2g** and **2h**). Indeed, the 2-, 3- or 4-methyl-substituted compounds **2a–2c**, or the 2-, 3- or 4-fluoro-substituted compounds **2d–2f**, respectively, showed comparable Can2 inhibitory activities (Table 1). The pyridinium-substituted sulfonamides **3** showed similar activity with the corresponding series of sulfonamides **2** (from which they have been prepared). However they were better Can2 inhibitors compared to the corresponding carbohydrazides **2**, with inhibition constants in the range of 4.4–60.1 nM. Again the

Table 1
Inhibition of human α-CA (hCA) isozymes I and II and fungal β-CAs from *C. neoformans* (Can2) and *C. albicans* (CaNce103) with sulfonamides **1, 2a–n, 3a–p**, acetazolamide (**AZA**) and ethoxzolamide (**EZA**) as standards[13]



1, 2 **3**

Inhibitor	R	K_I[#] (nM)			
		hCA I[a]	hCA II[a]	Can2[b]	CaNce103[b]
1	H	7.5	7.2	72	85
2a	2-Me	107	11.6	9.2	78
2b	3-Me	730	48.4	9.1	43
2c	4-Me	104	60.5	10.5	52
2d	2-F	621	36.0	7.9	47
2e	3-F	116	8.6	7.2	54
2f	4-F	108	15.5	8.6	61
2g	2-Cl	640	38.8	8.0	45
2h	3-Cl	311	9.2	7.4	52
2i	4-Cl	112	11.6	70	55
2j	2-Br	110	48.5	62	81
2k	3-Br	510	54.1	93	87
2l	4-Br	659	40.8	118	94
2m	3-OMe	342	7.4	12.0	128
2n	F_5	110	7.0	103	7.5
3a	H	9.0	71	16.5	42
3b	2-F	8.5	91	16.2	19
3c	3-F	11.3	3380	8.3	24
3d	4-F	7.6	65	8.7	21
3e	2-Cl	25.1	100	15.4	33
3f	3-Cl	113	1800	4.4	31
3g	4-Cl	3.2	77	3.1	29
3h	2-Br	43.4	38	12.1	45
3i	3-Br	30.8	74	14.3	48
3j	4-Br	12.3	85	10.9	53
3k	2-Me	10.5	106	6.5	62
3l	3-Me	110	104	6.1	64
3m	4-Me	5.1	68	5.9	41
3n	3-OMe	8.6	2840	8.3	119
3p	F_5	9.7	0.93	60.1	5.1
AZA	—	250	12	10	132
EZA	—	25	8	87	1070

[#] Errors in the range of ±5% of the reported data from three different assays by a stopped-flow CO_2 hydration method.[13]
[a] From Ref. 1a.
[b] This work.

best Can2 inhibitor was a chlorine-substituted derivative, **3f**, whereas the least effective one the pentafluorophenyl derivative **3p**. Overall, this subseries of positively-charged sulfonamides showed excellent inhibitory capacity against the fungal enzyme Can2. It should be also mentioned that the most active compounds among the indolesulfonamides investigated here showed a better activity than acetazolamide **AZA**, the most effective Can2 inhibitor detected before this study.[11a]

(ii) CaNce103 was slightly less susceptible to be inhibited by the indolesulfonamides **1**–**3**, compared to Can2, a situation already observed with other classes of sulfonamides.[11a] Thus, derivatives **1, 2** showed K_Is of 7.5–128 nM for the inhibition of CaNce103, being more effective CAIs compared to **AZA** (K_I of 132 nM, the best sulfonamide inhibitor detected before this study) or **EZA** (K_I of 1070 nM). The 3-methoxy-substituted derivative **2m** was the least effective CaNce103 inhibitor in this series, with a K_I of 128 nM, whereas the pentafluorophenyl-substituted one, **2n**, the most effective inhibitor (K_I of 7.5 nM). This is one of the best CaNce103 inhibitors detected so far, with efficiency 17.6 times better than that of **AZA**, and also possessing a quite hydrophobic character due to the presence of the pentafluorophenyl moiety. This is a positive feature indeed, as sulfonamides are normally insufficiently lipophilic to penetrate the cell walls and membranes of some bacteria or fungi,[12f] a fact which is attributed to the highly polar nature of the SO_2NH_2 group. The remaining derivatives, of types **1** and **2a–2l**, showed a rather flat SAR, and were only moderately inhibitory against CaNce103, with inhibition constants of 43–94 nM. Both the substitution pattern and the nature of the 3-substituted-phenyl moieties influence the CaNce103 inhibitory activity of this series of indolesulfonamides. Thus, the lead **1** was moderately active (K_I of 85 nM) and all substitution patterns at the 3-phenyl moiety (except the bromophenyl ones, present in **2j–2l**) lead to an increase of the CaNce103 inhibitory power. Actually, the bromophenyl derivatives **2j–2l** showed similar (**2j** and **2k**) or slightly diminished (**2l**) CaNce103 inhibitory activity compared to **1**. For the halogenosubstituted compounds, the 2-halogeno derivative was a better CaNce103 inhibitor compared to the corresponding 3-halogeno substituted compound, which in turn was a better inhibitor compared to the 4-halogeno substituted derivative. For the methyl substituted compounds, the best CaNce103 inhibitor was the 3-substituted compound **2b**. Thus, minor structural changes in the scaffold of compounds **2** strongly influence the CaNce103 inhibitory activity for this series of derivatives. The compounds **3**, bearing the trimethylpyridinium moiety instead of the terminal amino one present in **2**, were also effective CaNce103 inhibitors, with inhibition constants in the range of 5.1–119 nM (Table 1). SAR for these positively-charged derivatives was rather similar to the corresponding carbohydrazides from which they were prepared, with the pentafluorophenyl derivative **3p** being the most effective CaNce103 inhibitor reported so far (K_I of 5.1 nM) and the 3-methoxy-substituted one **3n** the least effective (K_I of 119 nM). Generally, all the positively-charged, trimethylpyridinium derivatives **3** were better CaNce103 inhibitors compared to the corresponding non-charged derivatives **2**.

(iii) the investigated sulfonamides **2, 3** were generally less effective hCA I inhibitors (K_Is of 110–730 nM) except for the lead **1**, which is a very potent hCA I inhibitor (K_I of 7.5 nM) but most of them were highly effective hCA II inhibitors (K_Is of 7.2–60.5 nM). However, some interesting selectivity ratios for the inhibition of the fungal over the host enzymes have been observed for some of the investigated sulfonamides. Thus, compound **2b** had a selectivity ratio of 5.1 for inhibiting Can2 over hCA II, and of 80.2 for inhibiting Can2 over hCA I. This is the first example of a fungal pathogenic CA-selective inhibitor (over the off-target hCA II). Similar features were also observed for **2g**, with selectivity ratios of 4.8 (Can2 over hCA II) and 80 (Can2 over hCA I). However, no CaNce103 selective inhibitors (over hCA II) have been detected so far, in this or other studies.[11a]

In conclusion, in this Letter we investigated a series of 2-(hydrazinocarbonyl)-3-substituted-phenyl-1*H*-indole-5-sulfonamides possessing various 2-, 3- or 4-substituted phenyl groups with methyl-, halogeno- and methoxy-functionalities, as well as the perfluorophenyl moiety, for the inhibition of two β-CAs from the fungal pathogens *C. neoformans* (Can 2) and *C. albicans* (CaNce103). Both enzymes were potently inhibited by these sulfonamides, with inhibition constants in the range of 4.4–118 nM against Can2, and of 5.1–128 against CaNce103, respectively. SAR was rather well defined, with minor structural changes in the 3-substituted phenyl moiety being the main contributors to the enzyme inhibitory activity. Some of the investigated sulfonamides also showed acceptable selectivity ratios for inhibiting Can2 over the host, human enzymes hCA I and II.

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