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Synthesis and carbonic anhydrase I, II, IX and XII inhibition studies of 4-*N*,*N*-disubstituted sulfanilamides incorporating 4,4,4-trifluoro-3-oxo-but-1-enyl, phenacylthiourea and imidazol-2(3*H*)-one/thione moieties

Cenzo Congiu [a,*], Valentina Onnis [a], Gianfranco Balboni [a], Claudiu T. Supuran [b,c,*]

[a] *Department of Life and Environment Sciences, University of Cagliari, via Ospedale 72, Cagliari I-09124, Italy*
[b] *Università degli Studi di Firenze, Laboratorio di Chimica Bioinorganica, Rm. 188, Via della Lastruccia 3, I-50019 Sesto Fiorentino (Firenze), Italy*
[c] *Università degli Studi di Firenze, Polo Scientifico, Dipartimento NEIROFABA, Sezione di Scienze Farmaceutiche e Nutraceutiche, Via Ugo Schiff 6, 50019 Sesto Fiorentino (Firenze), Italy*

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ABSTRACT

A series of sulfonamides incorporating the sulfanilamide (SA) scaffold were prepared. Reaction of the 4-amino moiety of SA with benzyl chlorides or substituted bromoacetophenones afforded the 4-mono-alkylated derivatives which were then reacted with 1,1,1-trifluoro-4-isobutoxybut-3-en-2-one leading to a series of 4-*N*,*N*-disubstituted SAs. The key intermediates were also reacted with ethoxycarbonyl isothiocyanate leading to thioureas or were cyclized in the presence of potassium cyanate/isothiocyanate to the corresponding imidazol-2(3*H*)-one/thiones. The new compounds were tested as inhibitors of four carbonic anhydrase (CA, EC 4.2.1.1) isoforms, the cytosolic CA I and II, and the transmembrane, tumor-associated CA IX and XII. These sulfonamides were ineffective CA I and II inhibitors but were nanomolar CA IX and XII inhibitors, making them of interest as clinical candidates for antitumor/antimetastasis applications.

As CO_2 is a crucial molecule in all life processes, being generated in high amounts in most organisms, specific catalysts evolved for its rapid transformation into bicarbonate.[1–3] These catalysts are the enzymes known as carbonic anhydrases (CAs, EC 4.2.1.1), metalloenzymes which catalyze with very high efficiency the reaction (1):[1–3]

$$CO_2 + H_2O \Longleftrightarrow HCO_3^- + H^+ \qquad (1)$$

However, a range of other hydrolytic processes such as COS and CS_2 hydration,[4,5] cyanamide hydration to urea,[6] as well as ester hydrolysis,[7,8] etc. are catalyzed by some members of this enzyme superfamily. Indeed, by an interesting process of convergent evolution, organisms on earth have developed at least five distinct families of such enzymes, the α-, β-, γ-, δ- and ζ-CAs.[1–3,9,10] They provide a means for the organisms to face the high amounts of CO_2 formed in the metabolic processes but also the possible acid-base disequilibria connecting to this, considering the fact that the products formed in the physiologic reaction catalyzed by the CAs are an ion with strong buffering activity (bicarbonate) as well as acid (H^+ ions).[1–3,9,10] It is thus not surprizing that the CAs are involved in many physiologic processes and that perturbation of some of them lead to disequilibria and disease.[11–13] In fact, the CA inhibitors (CAIs) have pharmacologic applications as diuretics, antiglaucoma, anticonvulsant, antiobesity and anticancer agents/diagnostic tools.[1–3,14,15]

There are at least four classes of CAI targeting enzymes from mammals, which belong to the α-CA class: (i) metal ion binders (inorganic anions; sulfonamides and their isosteres (such as the sulfamates, sulfamides, *N*-hydroxy-sulfonamides));[1–3,14] (ii) compounds which anchor to the zinc-coordinated water molecule/hydroxide ion (phenols, polyamines, sulfocoumarins, etc.);[1–3] (iii) compounds occluding the entrance of the active site (coumarins and their isosteres);[1–3] (iv) compounds which bind in an unknown manner (secondary/tertiary sulfonamides, imatinib, nilotinib, etc).[1–3,14]

The zinc binders coordinate the metal from the center of the CA active site in a tetrahedral or trigonal bipyramydal geometries of the metal ion.[1–3,14] Sulfonamides, sulfamates, sulfamides, which are the main class of clinically used such pharmacologic agents, bind as anions, with the nitrogen atom of the sulfamoyl moiety coordinated to the Zn(II) ion, which is a tetrahedral geometry.[1–3] The scaffold of the inhibitor also participates in various other

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[*] Corresponding authors. Tel.: +39 055 457 3005; fax: +39 055 4573385.
E-mail addresses: ccongiu@unica.it (C. Congiu), claudiu.supuran@unifi.it (C.T. Supuran).

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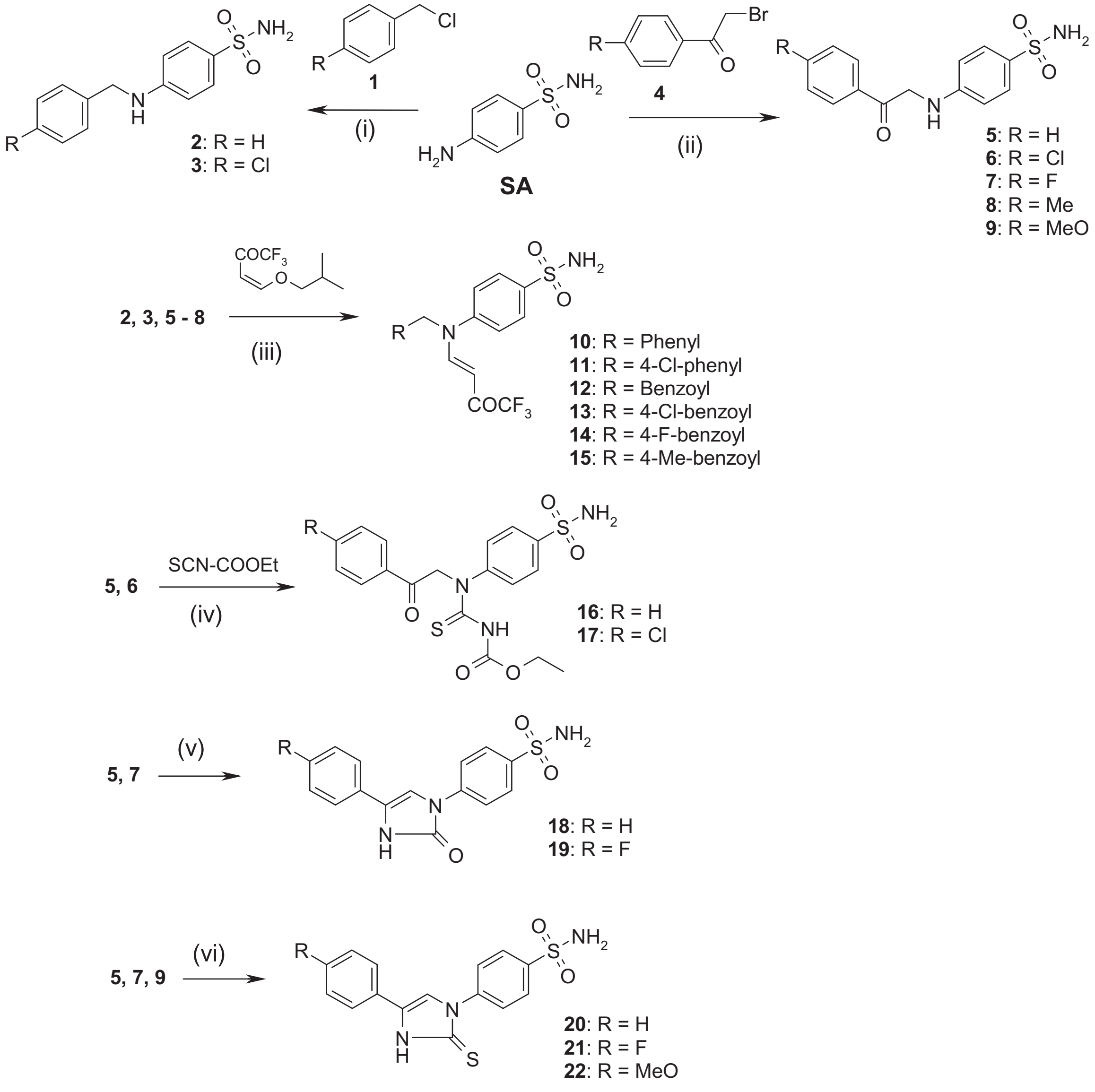
favorable interactions with the hydrophilic and/or hydrophobic halves of the active site, as well as ordered water molecules present within it, as shown by extensive X-ray crystallographic work on adducts of various CAs with many representatives of all these classes of inhibitors.[1–3]

Sulfanilamide (SA), the first sulfonamide with clinical applications as a bacteriostatic,[1] was frequently used as a lead molecule for preparing CAIs due to the fact that it is reactive (at the 4-amino moiety), being easily acylated, alkylated or sulfonylated, leading thus to compounds with effective CA inhibitory properties.[15–18] In fact many such N-4 mono-substituted derivatives were reported as effective and sometimes isoform-selective CAIs.[15,18] However the 4-*N*,*N*-disubstituted such compounds were much less investigated.[15]

Here we report s aseries of such 4-*N*,*N*-disubstituted SAs as well as compounds in which the 4-nitrogen was incorporated into a five-membered heterocycles. We investigated these new sulfonamides as inhibitors[19] of four mammalian (human, h) CA isoforms involved in several pathologies, hCA I and II (antiglaucoma target)[1,14] as well as hCA IX and XII (anti-tumor targets).[12,13]

The rationale for obtaining the new sulfonamides reported here is based on the fact that many mono-*N*-4-substituted SAs, incorporating 4-*N*-acyl- or 4-*N*-arylsulfonyl moieties in their molecules, showed good inhibitory activity against hCAs of pharmacological interest, such as hCA VA/B, hCA VII, hCA IX and/or hCA XII.[18,20] Recently we also reported a small series of *N*,*N*-disubstituted SAs which were also effective tumor-associated CAIs, with a good selectivity for the inhibition of the transmembrane (hCA IX and XII) over the cytosolic (hCA I and II) isoforms.[15] Thus, we decided to investigate alternative such substitution patterns at the N4 atom from SA.

Reaction of SA with substituted benzyl chlorides **1** afforded the first intermediates **2** and **3** as reported in the literature (Scheme 1).[16] In a similar manner, alkylation of SA with substituted bromoacetophenones **4** afforded the remaining key intermediates **5**–**9**[15,16]—Scheme 1 (see Supporting information for the detailed chemistry and characterization of the intermediates and new compounds reported here).[21] We incorporated halogens (F, Cl) and lipophilic (Me, MeO) not very bulky moieties in the key intermediates **2**, **3** and **5**–**9**, in order to investigate the structure–activity relationship (SAR) for the newly prepared CAIs reported here.



Scheme 1. Reagents and conditions: (i) H_2O, $CaCO_3$, reflux 3 h; (ii) MeOH, reflux 4 h; (iii) MeCN, reflux 16 h; (iv) MeCN, reflux 6 h; (v) AcOH, potassium cyanate, 60–65 °C, 1 h; and (vi) 10% HCl, ammonium thiocyanate, reflux 1,5 h.