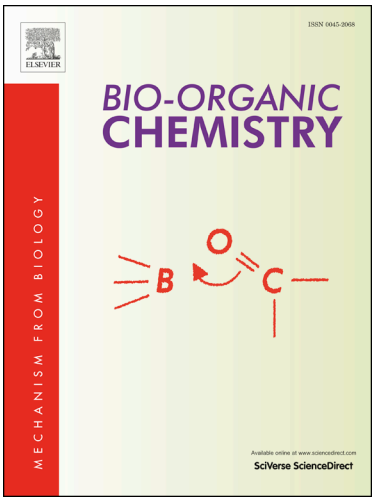

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Synthesis and biological evaluation of novel pyrazoline-based aromatic sulfamates with potent carbonic anhydrase isoforms II, IV and IX inhibitory efficacy.

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Abstract

Herein we report the synthesis of a new series of aromatic sulfamates designed considering the sulfonamide COX-2 selective inhibitors celecoxib and valdecoxib as lead compounds. These latter were shown to possess important human carbonic anhydrase (CA, EC 4.2.1.1) inhibitory properties, with the inhibition of the tumor-associated isoform hCA IX likely being co-responsible of the celecoxib anti-tumor effects. Bioisosteric substitution of the pyrazole or isoxazole rings from these drugs with the pyrazoline one was considered owing to the multiple biological activities ascribed to this latter heterocycle and paired with the replacement of the sulfonamide of celecoxib and valdecoxib with its equally potent bioisoster sulfamate. The synthesized derivatives were screened for the inhibition of four human carbonic anhydrase isoforms, namely hCA I, II, IV, and IX. All screened sulfamates exhibited great potency enhancement in inhibiting isoform II and IV, widely involved in glaucoma (K_Is in the range of 0.4-12.4 nM and 17.7 and 43.3 nM, respectively), compared to the lead compounds, whereas they affected the tumor-associated hCA IX as potently as celecoxib.

Keywords: Carbonic anhydrase, zinc-binding group, aromatic sulfamates, celecoxib, sulfamoylation, sub-nanomolar inhibition.

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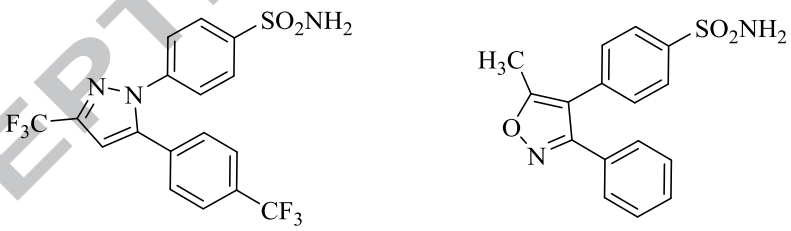
1. Introduction

COX-2 inhibitor or “coxibs” are a class of non-steroidal anti-inflammatory drugs (NSAIDs) which selectively inhibit the inducible isoform of cyclooxygenase.[1] The primary sulfonamide-bearing

coxibs, i.e. celecoxib and valdecoxib, have been shown to also act as potent inhibitors against several isoforms of human (h) carbonic anhydrase (CA, EC 4.2.1.1).[2-4] Abnormal levels or activities of the fifteen α-class CA isozymes identified so far in human have been related to several physiological-pathological processes, awarding to this enzymatic family a growing interest for the design of inhibitors or activators with biomedical applications.[5-7] Isoform CA IX was the main protagonist over the last two decades research on CAs, being a well-established anticancer drug target as well as a marker of the disease progression in many types of hypoxic tumors, with its gene expression strongly upregulated by the hypoxia-inducible factor (HIF-1).[8-10] Recently, its inhibition has been shown to be associated with a significant inhibition of the growth of both primary tumors and metastases.[11] In this context, the anti-proliferative effect on tumor cells shown by celecoxib could be ascribed to the inhibition of the tumor-associated CAs, in addition to COX-2 inhibition which has been displayed to block the COX-2/PGE_2 axis known to stimulate the HIF-1 pathway.[12]

The cytosolic hCA I and II are ubiquitous isoforms and may be both targets for some diseases (e.g. as diuretics or as anti-glaucoma agents) but also off-targets.[5,6] The membrane-associated hCA IV is a drug target for several pathologies, including glaucoma (together with hCA II), retinitis pigmentosa, stroke and rheumatoid arthritis.[5,13]



Celecoxib **Valdecoxib**

Figure 1. Chemical structures of COX-2 selective inhibitors, Celecoxib and Valdecoxib.

Among the five-membered heterocycles, pyrazolines have received significant attention in recent years due to their wide range of pharmacological and biological activities. [14] Specifically, 2-pyrazolines were shown to possess cytotoxic,[15] antidepressant,[16] antimicrobial,[17] antimalarial,[18] amine oxidase inhibitory,[19] anti-inflammatory,[20] neuroprotective activity.[21] Furthermore, several series of pyrazolines bearing benzenesulfonamide moieties as well as additional aromatic moieties were recently reported to act as potent CA inhibitors.[22]

Herein we further explore celecoxib analogues as CAIs by reporting the synthesis, characterization and CA inhibition evaluation of a new series of N^1-acetylpyrazolines bearing phenylsulfamate moieties at the 3 or 5-position of the 4,5-dihydropyrazole ring.

2. Results and discussion

2.1. Chemistry

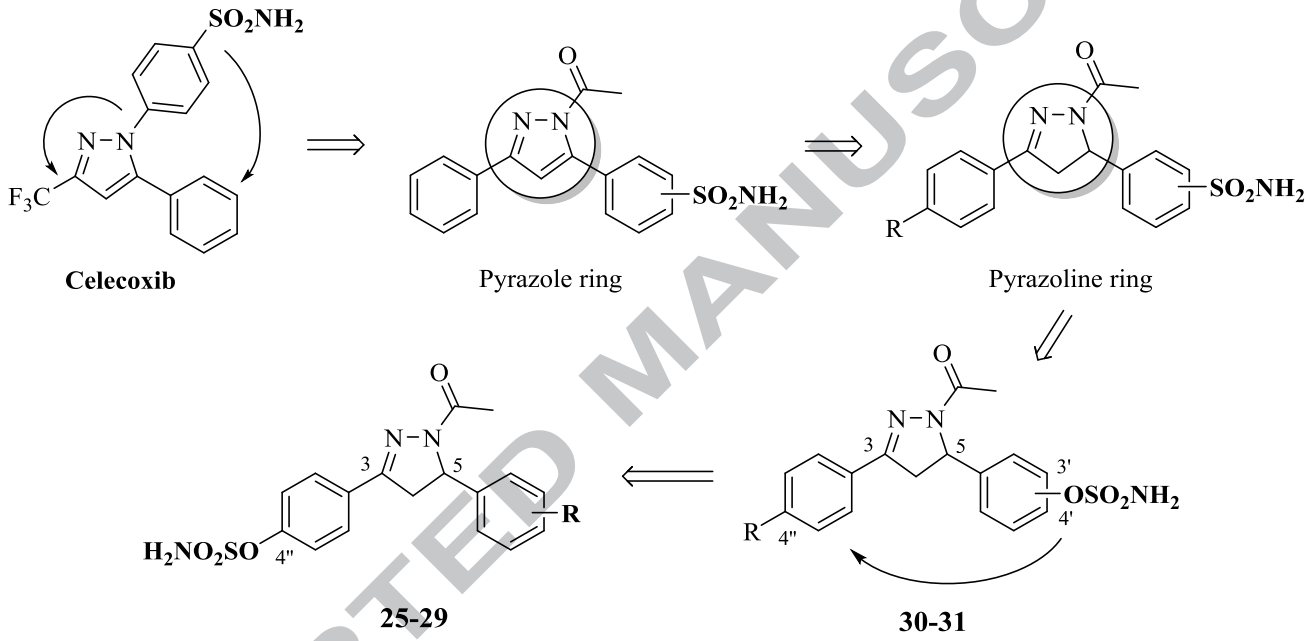
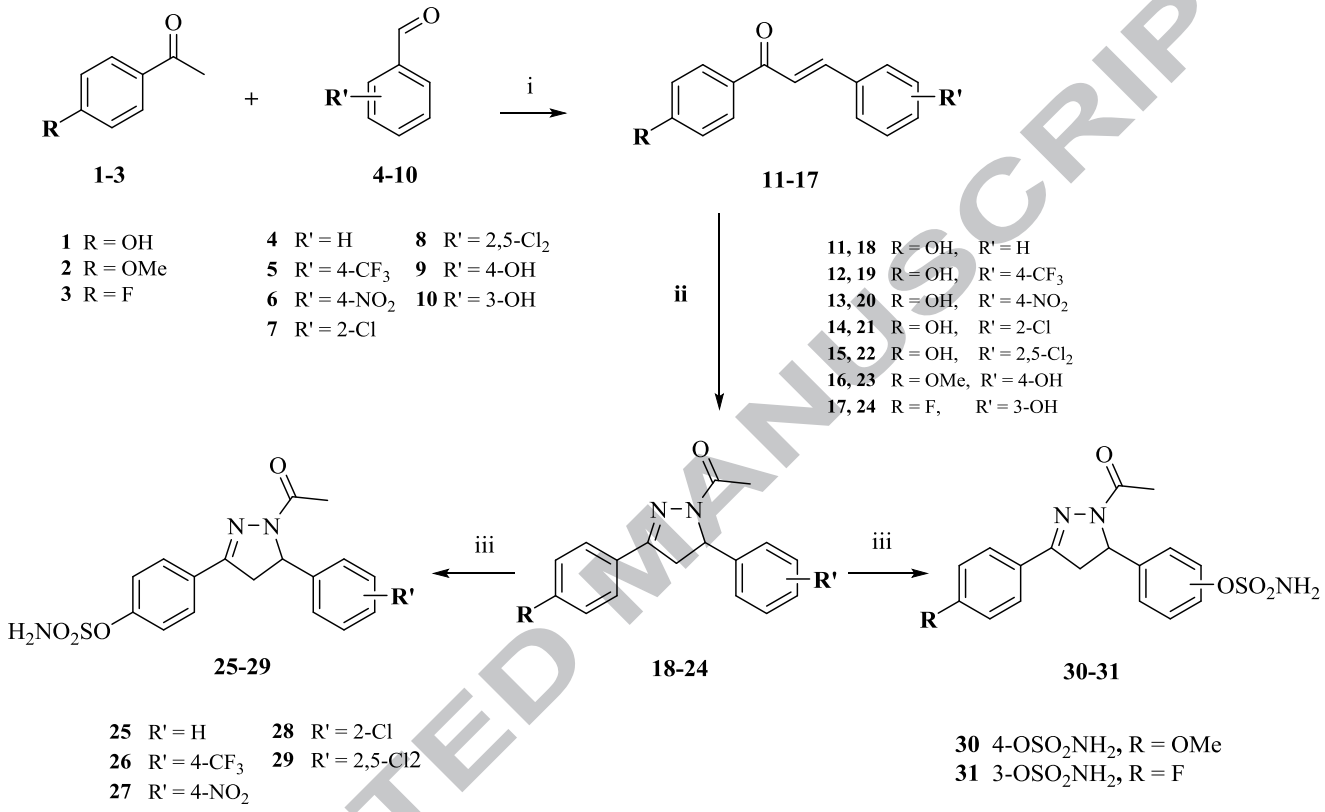


Figure 2. Design approach to pyrazoline derivatives **25-31**.

The sulfonamide group (R-SO_2NH_2) is the most classical and largely used zinc binding group (ZBG) for the design of CA inhibitors, with the sulfamate (R-OSO_2NH_2) group (as well as sulfamide, R-$NHSO_2NH_2$) being the closest congeners and bioisosters.[23] Sulfamates possess an additional electron-withdrawing oxygen atom directly attached to the sulfamoyl function, enabling the formation of a more complex hydrogen bond network than that formed by the classical sulfonamide inhibitors nearby the zinc ion present at the bottom of the binding site cavity. Indeed, sulfamates were reported to possess highly effective inhibitory properties against all known mammalian CA isoforms.[5,23]

Considering the above, in the present study we explored the bioisosteric substitution of the pyrazole of celecoxib with the pyrazoline one, namely 4,5-dihydropyrazole, incorporating in the main scaffold a phenylsulfamate moiety as ZBG portion, with the benzenesulfonamide having already been investigated in a similar context.[22]

The two aromatic portions were placed at the 3 and 5 positions of the pyrazoline, at greater distance than the two adjacent carbons in the lead compounds, whereas the sulfamate group being appended alternatively on one of the benzene rings. An acetyl group was appended at N^1 position of the heterocycle as mimic of the methyl and trifluoromethyl group present in the lead.



Scheme 1. Reagents and conditions: i) MeOH, 50% aqueous NaOH, r.t., 12 h; ii) $NH_2NH_2 \cdot H_2O$, AcOH, 3h, reflux; iii) $ClSO_2NH_2$, DMA, r.t. 12 h.

1-Acetyl-3,5-diaryl-4,5-dihydropyrazole sulfamates **25-31** were easily prepared by the synthetic pathway shown in Scheme 1. The starting materials for the synthesis of pyrazoline derivatives **18-24** were chalcones **11-17**, in turn prepared through the well-established Claisen–Schmidt condensation [24] between 4-substituted acetophenones **1-3** and substituted benzaldehydes **4-10** in methanol and in presence of NaOH.

Chalcones **11-17** were then reacted with hydrazine hydrate in boiling acetic acid [25] to afford the cyclization into pyrazolines **18-24** in 65-80% yields. Sulfamoylation of phenol pyrazoline derivatives **18-24** upon treatment with sulfamoyl chloride in *N*,*N*-dimethylacetamide (DMA) furnished the sulfamates **25-31**. Their structures were assigned on the basis of analytical and spectral data (see Experimental for details).

2.2 Carbonic anhydrase inhibition.

The CA inhibitory properties of sulfamate derivatives **25-31** against human isoforms CA I, II, IV and IX were measured, using acetazolamide (**AAZ**) as standard inhibitor, by a stopped flow CO_2 hydrase assay.[26] Inhibitory activities were displayed in comparison to those of the lead compounds celecoxib and valdecoxib. The following structure–activity relationships (SAR) were gathered from the inhibition data reported in Table 1.

Table 1: Inhibition data of human CA isoforms hCA I, II, IV and IX with sulfamates **25-31** reported here celecoxib, valdecoxib, and the standard sulfonamide inhibitor acetazolamide (**AAZ**) by a stopped flow CO_2 hydrase assay.[26]

			K_I (nM)			
Compound	**R**	**R'**	hCA I	hCA II	hCA IV	hCA IX
25	-OSO_2NH_2	H	2460.2	8.9	27.1	34.1
26	-OSO_2NH_2	4'-CF_3	2741.9	0.8	24.1	29.3
27	-OSO_2NH_2	4'-NO_2	3208.5	1.2	30.2	18.2
28	-OSO_2NH_2	2'-Cl	8253.1	9.0	25.9	14.8
29	-OSO_2NH_2	2',5'-Cl_2	4752.2	12.4	17.7	25.5
30	-OMe	4'-OSO_2NH_2	3142.8	0.4	20.5	25.0
31	-F	3'-OSO_2NH_2	5225.3	6.5	43.3	15.8
Celecoxib[b]	-	-	50000	21	290	16
Valdecoxib[b]	-	-	54000	43	340	27
AAZ	-	-	250	12	74	25

[a] Mean from 3 different assays, by a stopped flow technique (errors were in the range of ± 5-10 % of the reported values).
[b] From ref. 2.

(i) The bioisosteric and positional substitutions applied to design derivatives **25-31** generally enhanced their hCA I inhibitory properties in comparison to lead compounds independently from the positions of the phenylsulfamate moiety on the pyrazoline ring. Indeed, inhibition constants (K_Is) of derivative **25-31** against the ubiquitous isoform were measured to range between 2460.2 and 8253.1 nM, whereas celecoxib and valdecoxib did not inhibit hCA I below 50 µM.

(ii) Sub-nanomolar and low nanomolar inhibition against the cytosolic isoform II were achieved by all reported sulfamates (K_Is spanning between 0.4-12.4 nM), with the 5-(4'-OMe-phenyl)-bearing compound **30** being the most active in the series. Remarkably, even an opposite incorporation of the

ZBG and *para*-substituents as in compounds **26** (4'-CF_3) and **27** (4'-NO_2) led to sub- or low-nanomolar inhibitory profiles (K_Is of 0.8 and 1.2 nM, respectively). Hence, it could be speculated a putative superimposition of the two regio-isomer types (**25-29** and **30-31**) within the CA binding site pockets. Incorporation of two chlorine atoms in 2' and 5' position of the 5-phenyl ring (**29**) elicited the least effective hCA II inhibition herein reported, with a K_I of 12.4 nM, being anyhow **29** almost two or four-fold more potent than celecoxib and valdecoxib, respectively.

(iii) Inhibition of the membrane-bound hCA IV was the most affected by the considered modifications at the celecoxib scaffold. Indeed, while the lead compounds were high nanomolar hCA IV inhibitors (K_Is of 290 and 340 nM), all reported sulfamates exhibited low nanomolar inhibition against this isozyme, with K_Is ranging between 17.7 and 43.3 nM. hCA IV is mostly expressed on the surface of neurons and glial cells, being involved in the nutrition of the latter in the brain hypoxic environment, thus of interest for the design of tools targeting the central nervous system.[5,13] In order to shed light on this potency enhancement, it should be considered that hCA IV lacks the α-helix containing residues 126−136, positioned straightforwardly to the zinc-coordination system, which stabilize many compounds orientation within hCA I, II or IX binding sites through hydrogen bonds and van der Waals interactions.[5,9] Additional pockets present in hCA IV binding cavity can exert a similar role, but need to be reached by proper angles and connections within the inhibitor structure. The additional torsion angles furnished by the C-O-S connection of the aromatic sulfamate and the substituent at the 5 position of the pyrazoline ring, which lack aromaticity, thus likely contribute to an enhanced set of interactions within the hCA IV binding cavity. The switch of the sulfamate from the *para* to the *meta* position of the scaffold (**31**) indeed slightly worsened the inhibitory potency.

(iv) The tumor-associated hCA IX was potently inhibited by all sulfamates **25-31** within a rather flat range, spanning between 14.8 and 34.1 nM. It is worth noting the comparable inhibitory efficacy with the lead derivatives, indication of the fact that the incorporated modifications did not affect the inhibition of such isozyme.

(v) The strong enhancement of potency displayed by most derivatives against hCA II and IV in comparison to celecoxib and valdecoxib could be seen with interest for the design of novel anti-glaucoma agents, with the two isoforms being upregulated in the eyes of glaucomatous patients. The non-selectivity over hCA IX should not be seen negatively owing to the tumor-associated isoform low expression levels in normal cells.

3. Conclusions

In the present article, we reported the first incorporation of sulfamate moieties, as sulfonamide bioisoster, in celecoxib analogues, to afford a novel series of potent carbonic anhydrase inhibitors. Indeed, the sulfonamide COX-2 selective inhibitors, celecoxib and valdecoxib, taken as lead compounds, were shown to possess important human carbonic anhydrase inhibitory properties, with the inhibition of the tumor-associate isoform hCA IX likely being co-responsible of the celecoxib anti-tumor effect. Bioisosteric substitution of the pyrazole or isoxazole rings with the pyrazoline one was considered owing to the multiple biological activities reported for such eterocycle and paired with the replacement of the sulfonamide with its equally potent sulfamate bioisoster. The synthesised derivatives were screened for the inhibition of four human isoforms of zinc enzyme carbonic anhydrase, namely hCA I, II, IV, and IX. All screened sulfamates exhibited great potency enhancement in inhibiting isoform II and IV, widely involved in glaucoma (K_Is in the range of 0.4-12.4 nM and 17.7 and 43.3 nM, respectively), in comparison to the lead compounds, whereas affected the tumor-associated hCA IX as potently as celecoxib.

4. Experimental Section

4.1 Chemistry

All commercially available solvents and reagents were used without further purification. NMR spectra were recorded on an Inova 500 spectrometer (Varian, Palo Alto, CA, USA). The chemical shifts (δ) are reported in part per million downfield from tetramethylsilane (TMS), which was used as internal standard, and the spectra were recorded in hexadeuteriodimethylsulphoxide (DMSO-d_6). Infrared spectra were recorded on a Vector 22 spectrometer (Bruker, Bremen, Germany) in Nujol mulls. The main bands are given in cm^{-1}. Positive-ion electrospray ionization (ESI) mass spectra were recorded on a double-focusing MAT 95 instrument (Finnigan, Waltham, MA, USA) with BE geometry. Melting points (mp) were determined on a SMP1 Melting Point apparatus (Stuart Scientific, Stone, UK) and are uncorrected. All products reported showed ^{1}H NMR spectra in agreement with the assigned structures. The purity of the tested compounds was determined by combustion elemental analyses conducted by the Microanalytical Laboratory of the Chemistry Department of the University of Ferrara with a MT-5 CHN recorder elemental analyzer (Yanagimoto, Kyoto, Japan) and the values found were within 0.4% of theoretical values.

General procedure for the preparation of 1-acetyl-3,5-diaryl-4,5-dihydro-1H-pyrazoles 18-24

To a solution of chalcone derivative **11-17** (1 mmol) in acetic acid (3 mL) hydrazine hydrate (0.3 mL, 6 mmol) was added. The mixture was refluxed under stirring for 3 h, and then poured onto

crushed ice. The precipitate was filtered off, washed with cold water, and crystallized from methanol to give the titled pyrazolines.

1-(3-(4-Hydroxyphenyl)-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethanone (18)

Obtained from chalcone **11:** 69% yield, mp 170-172 °C. (lit. 170-173 °C [27]). ^{1}H NMR: δ (ppm) 2.27 (s, 3H, CH_3), 2.97 (dd, J=4.0, 17.0 Hz, 1H, CH), 3.80 (m, 1H, CH), 5.37 (dd, J=4.0, 12.5 Hz, 1H, CH), 6.84 (d, J=7.5 Hz, 2H, Ar), 7.23 (m, 3H, Ar), 7.42 (m, 2H, Ar), 7.68 (d, J=8.0 Hz, 2H, Ar), 9.63 (s, 1H, OH). IR (Nujol: ν (cm^{-1}) 3080, 1619, 1593. ESI-MS m/z 281 $(M + H)^+$. Anal. $C_{17}H_{16}N_2O_2$ (280.32) Calcd. %C 72.84; %H 5.75; %N 9.99. Found %C 72.91; %H 5.74; %N 10.03.

1-(3-(4-Hydroxyphenyl)-5-(4-(trifluoromethyl)phenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (19)

Obtained from chalcone **12:** 80% yield, mp 145-147 °C. ^{1}H NMR: δ (ppm) 2.29 (s, 3H, CH_3), 3.10 (dd, J= 5.0, 18.0 Hz, 1H, CH), 3.83 (dd, J=12.5, 18.5 1H, CH), 5.58 (dd, 5.0, 12.5 Hz, 1H, CH), 6.83 (d, J=9.0 Hz, 2H, Ar), 7.41 (d, J=7.5 Hz, 2H, Ar), 7.62 (d, J=9.0 Hz, 2H, Ar), 7.69 (d, J=8.0 Hz, 2H, Ar), 9.97 (s, 1H, OH). IR (Nujol: ν (cm^{-1}) 3139, 1624, 1520. ESI-MS m/z 349 $(M + H)^+$. Anal. $C_{18}H_{15}F_3N_2O_2$ (348.32) Calcd. %C 62.07; %H 4.34; %N 8.04. Found %C 62.14; %H 4.33; %N 8.02.

1-(3-(4-Hydroxyphenyl)-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (20).

Obtained from chalcone **13:** 75% yield, mp 232-234 °C. ^{1}H NMR: δ (ppm) 2.31 (s, 3H, CH_3), 3.13 (dd, J= 4.0, 18.0 Hz, 1H, CH), 3.85 (dd, J=5.5, 17.5 Hz, 1H, CH), 5.66 (m, 1H, CH), 6.85 (d, J=7.5 Hz, 2H, Ar), 7.48 (d, J=8.0 Hz, 2H, Ar), 7.63 (d, J=7.5 Hz, 2H, Ar), 8.21 (d, J=8.0 Hz, 2H, Ar), 10.00 (s, 1H, OH). IR (Nujol: ν (cm^{-1}) 2675, 1610, 1568. ESI-MS m/z 349 $(M + H)^+$. Anal. $C_{17}H_{15}N_3O_4$ (325.32) Calcd. %C 62.76; %H 4.65; %N 12.92. Found %C 62.70; %H 4.66; %N 12.95.

1-(5-(2-Chlorophenyl)-3-(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (21)

Obtained from chalcone **14:** 80% yield, mp 213-214 °C (lit. 214-215 °C [28]). ^{1}H NMR: δ (ppm) 2.29 2.33 (s, 3H, CH_3), 2.98 (dd, J=4.5, 17.5 Hz, 1H, CH), 3.89 (m, 1H, CH), 5.72 (dd, J=4.5, 12.0 Hz, 1H, CH), 6.81 (d, J=6.5 Hz, 2H, Ar), 7.04 (m, 1H, Ar), 7.29 (m, 2H, Ar), 7.48 (m, 1H, Ar), 7.61 (d, J=7.0 Hz, 2H, Ar), 9.95 (s, 1H, OH). IR (Nujol: ν (cm^{-1}) 3064, 1621, 1590. ESI-MS m/z 315 $(M + H)^+$, 317 $(M+2+H)^+$. Anal. $C_{17}H_{15}ClN_2O_2$ (314.08) Calcd. %C 64.87; %H 4.80; %N 8.90. Found %C 64.93; %H 4.81; %N 8.87.

1-(5-(2,5-Dichlorophenyl)-3-(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (22)

Obtained from chalcone **15:** 70% yield, mp 218-220 °C. ^{1}H NMR: δ (ppm) 2.33 (s, 3H, CH_3), 3.03 (dd, J=4.0, 18.0 Hz, 1H, CH), 3.90 (dd, J=12.5, 17.5 Hz, 1H, CH), 5.74 (m, 1H, CH), 6.84 (d, J = 8.5 Hz, 2H, Ar), 7.08 (d, J = 8.5 Hz, 2H, Ar), 7.38 (d, J = 8.0 Hz, 1H, Ar), 7.62 (d, J = 8.5 Hz, 2H, Ar), 7.67 (s, 1H, Ar), 9.98 (s, 1H, OH). IR (Nujol: ν (cm^{-1}) 3068, 1598. ESI-MS m/z 350 $(M + H)^+$, 352 $(M+ H+2)^+$, 354 $(M+H+4)^+$. Anal. $C_{17}H_{14}Cl_2N_2O_2$ (349.21) Calcd. %C 58.47; %H 4.04; %N 8.02. Found %C 58.52; %H 4.03; %N 8.04.

1-(5-(4-Hydroxyphenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (23)

Obtained from chalcone **16:** 65% yield, mp 150-152 °C. ^{1}H NMR: δ (ppm) 2.29 (s, 3H, CH_3), 3.07 (dd, J=4.5, 18.0 Hz, 1H, CH), 3.81 (m, 4H, CH and OCH_3), 5.42 (dd, J=4.5, 11.5 Hz, 1H, CH), 6.64 (d, J=6.5 Hz, 2H, Ar), 7.10 (m, 3H, Ar), 7.23 (s, 1H, Ar), 7.72 (d, J=8.5 Ar, 2H, Ar), 9.34 (s, 1H, OH). IR (Nujol: ν (cm^{-1}) 3070, 1602. ESI-MS m/z 349 $(M + H)^+$. Anal. $C_{18}H_{18}N_2O_3$ (310.35) Calcd. %C 69.66; %H 5.85; %N 9.03. Found %C 69.60; %H 5.87; %N 9.06.

1-(3-(4-Fluorophenyl)-5-(3-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (24)

Obtained from chalcone **17:** 75% yield, mp 183-185 °C. ^{1}H NMR: δ (ppm) 2.31 (s, 3H, CH_3), 3.13 (dd, J= 4.0, 18.0 Hz, 1H, CH), 3.82 (dd, J=12.0, 18.0 Hz, 1H, CH), 5.45 (m, 1H, CH), 6.56 (s, 1H, Ar), 6.63 (t, J= 7.0 Hz, 2H, Ar), 7.11 (t, J= 7.0 Hz, 1H, Ar), 7.32 (t, J= 8.0 Hz, 2H, Ar), 7.83 (t, J= 7.0 Hz, 2H, Ar), 9.36 (s, 1H, OH). IR (Nujol: ν (cm^{-1}) 3072, 2783, 1663, 1583. ESI-MS m/z 299 (M + H)$^+$. Anal. $C_{17}H_{15}FN_2O_2$ (298.31) Calcd. %C 68.45; %H 5.07; %N 9.39. Found %C 68.39; %H 5.08; %N 9.42.

General procedure for the preparation of 1-acetyl-3,5-diaryl-4,5-dihydro-1H-pyrazole sulfamates 25-31

To an ice-cooled stirring solution of pyrazolines **18-24** (1 mmol) in anhydrous DMA (10 mL), freshly prepared sulfamoyl chloride (0.81 g, 7 mmol) in DMA (5 mL) was added dropwise in 30 min. The obtained mixture was stirred at room temperature overnight, then water (30 mL) was added. The mixture was stirred for additional 2h, then the formed precipitate was filtered off and dried.

4-(1-Acetyl-5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl sulfamate (25)

Obtained from pyrazoline **18:** 96% yield, mp 186-187 °C. ^{1}H NMR: δ (ppm) 2.31 (s, 3H, CH_3), 3.13 (dd, J= 5.5, 19.0 Hz, 1H, CH), 3.87 (m, 1H, CH), 5.59 (m, 1H, CH), 7.28 (m, 5H, Ar), 7.70 (m, 2H, Ar), 7.88 (d, J=8, 2H, Ar), 7.97 (s, 2H, NH_2). IR (Nujol: ν (cm^{-1}) 3310, 3182, 1640, 1595. ESI-MS m/z 360 $(M + H)^+$. Anal. $C_{17}H_{17}N_3O_4S$ (359.40) Calcd. %C 56.81; %H 4.77; %N 11.69. Found %C 56.87; %H 4.76; %N 11.66.

4-(1-Acetyl-5-(4-(trifluoromethyl)phenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl sulfamate (26)

Obtained from pyrazoline **19:** 50% yield, mp 194-195 °C. ^{1}H NMR: δ (ppm) 2.33 (s, 3H, CH_3), 3.23 (dd, J= 5.0, 18.0 Hz, 1H, CH), 3.92 (dd, J=12.0, 18.0 Hz, 1H, CH), 5.66 (dd, J= 5.0, 12.0 Hz, 1H, CH), 7.37 (d, J=8.5 Hz, 2H, Ar), 7.43 (d, J=7.5 Hz, 2H, Ar), 7.71 (d, J=8.0 Hz, 2H, Ar), 7.87 (d, J=9.0 Hz, 2H, Ar), 8.09 (s, 2H, NH_2). IR (Nujol: ν (cm^{-1}) 3301, 1636, 1600. ESI-MS m/z 428 $(M + H)^+$. Anal. $C_{18}H_{16}F_3N_3O_4S$ (427.40) Calcd. %C 50.58; %H 3.77; %N 9.83. Found %C 50.63; %H 3.76; %N 9.86.

4-(1-Acetyl-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl sulfamate (27)

Obtained from pyrazoline **20:** 25% yield, mp 169-170 °C. ^{1}H NMR: δ (ppm) 2.34 (s, 3H, CH_3), 3.24 (dd, J= 4.5, 18.0 Hz, 1H, CH), 3.95 (m, 1H, CH), 5.70 (dd, J=4.5, 12.0 Hz, 1H, CH), 7.38 (d, J=8.0 Hz, 2H, Ar), 7.50 (d, J=8.5 Hz, 2H, Ar), 7.88 (d, J=8.0 Hz, 2H, Ar), 8.11 (s, 2H, NH_2), 8.22 (d, J=6.5 Hz, 2H, Ar). IR (Nujol: ν (cm^{-1}) 3311, 3039 1641, 1599. ESI-MS m/z 405 $(M + H)^+$. Anal. $C_{17}H_{16}N_4O_6S$ (404.40) Calcd. %C 50.49; %H 3.99; %N 13.85. Found %C 50.54; %H 3.98; %N 13.88.

4-(1-Acetyl-5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl sulfamate (28)

Obtained from pyrazoline **21:** 30% yield, mp 174-175 °C. ^{1}H NMR: δ (ppm) 2.36 (s, 3H, CH_3), 3.08 (dd, J=4.0, 18.0 Hz, 1H, CH), 3.96 (m, 1H, CH), 5.77 (dd, J=4.5, 11.5 Hz, 1H, CH), 7.07 (m, 2H, Ar), 7.31 (d, J=7.0 Hz, 1H, Ar), 7.35(d, J=7.0 Hz, 1H, Ar), 7.49 (m, 3H, Ar), 7.86 (d, J=6.5 Hz, 1H, Ar), 8.08 (s, 2H, NH_2). IR (Nujol: ν (cm^{-1}) 3312, 1636, 1598. ESI-MS m/z 394 $(M + H)^+$. Anal. $C_{17}H_{16}ClN_3O_4S$ (393.84) Calcd. %C 51.84; %H 4.09; %N 10.67. Found %C 51.78; %H 4.08; %N 10.72.

4-(1-Acetyl-5-(2,5-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl sulfamate (29)

Obtained from pyrazoline **22:** 50% yield, mp 174-176 °C. ^{1}H NMR: δ (ppm) 2.31 (dd, J=4.5, 16.0 Hz, 1H, CH), 2.35 (s, 3H, CH_3), 3.94 (m, 1H, CH), 5.74 (dd, J=4.5, 12.5 Hz, 1H, CH), 7.11 (m, 1H, Ar), 7.36 (m, 3H, Ar), 7.66 (s, 1H, Ar), 7.85 (m, 2H, Ar), 8.09 (s, 2H, NH_2). IR (Nujol: ν (cm^{-1}) 3308, 1637, 1603. ESI-MS m/z 429 $(M + H)^+$. Anal. $C_{17}H_{15}Cl_2N_3O_4S$ (428.29) Calcd. %C 47.67; %H 3.53; %N 9.81. Found %C 47.62; %H 3.54; %N 9.84.

4-(1-Acetyl-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenyl sulfamate (30)

Obtained from pyrazoline **23**: 30% yield, mp 177-178 °C. ^{1}H NMR: δ (ppm) 2.31 (s, 3H, CH_3), 3.11 (dd, J=4.5, 17.5 Hz, 1H, CH), 3.84 (m, 4H, CH and OCH_3), 5.56 (d, J=4.5, 11.5 Hz, 1H, CH), 7.02 (d, J=8.5 Hz, 2H, Ar), 7.23 (d, J=9.0 Hz, 2H, Ar), 7.27 (d, J=8.5 Hz, 2H, Ar), 7.73 (d, J=9.0 Hz, 2H, Ar), 7.98 (s, 2H, NH_2). IR (Nujol: ν (cm^{-1}) 3321, 3167, 1637, 1568. ESI-MS m/z 390 $(M + H)^+$. Anal. $C_{18}H_{19}N_3O_5S$ (389.10) Calcd. %C 55.52; %H 4.92; %N 10.79. Found %C 55.58; %H 4.90; %N 10.76.

4-(1-Acetyl-3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenyl sulfamate (31)

Obtained from pyrazoline **24:** 40% yield, mp 190-191 °C. ^{1}H NMR: δ (ppm) 2.32 (s, 3H, CH_3), 3.18 (dd, J= 4.0, 18.0 Hz, 1H, CH), 3.88 (dd, J=13.5, 16.5 Hz, 1H, CH), 5.59 (m, 1H, CH), 7.11 (s, 1H, Ar),7.16 (t, J= 7.5 Hz, 2H, Ar), 7.32 (t, J= 7.5 Hz, 1H, Ar), 7.42 (t, J= 7.5 Hz, 2H, Ar), 7.85 (m, 2H, Ar), 7.99 (s, 2H, NH_2). IR (Nujol: ν (cm^{-1}) 3325, 1638, 1608. ESI-MS m/z 378 $(M + H)^+$. Anal. $C_{17}H_{16}FN_3O_4S$ (377.39) Calcd. %C 54.10; %H 4.27; %N 11.13. Found %C 54.05; %H 4.28; %N 11.17.

4.2 Carbonic anhydrase inhibition

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity.[26] Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mMHepes (pH 7.5) as buffer, and 20 mM Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10–100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled-deionized water and dilutions up to 0.01 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E-I complex. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3 and the Cheng–Prusoff equation, as reported earlier,[29] and represent the mean from at least three different determinations. All CA isofoms were recombinant ones obtained in-house as reported earlier.[30,31]

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Conflict of interest

The authors declare no conflict of interest.

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Highlights

Synthesis and biological evaluation of novel pyrazoline-based aromatic sulfamates with potent carbonic anhydrase isoforms II, IV and IX inhibitory efficacy.

Alessio Nocentini, [1,*] Davide Moi,[2] Gianfranco Balboni,[2] Severo Salvadori,[3] Valentina Onnis,[2,*] Claudiu T. Supuran[1]

- A novel series pyrazoline-based sulfamates, inspired by the lead celecoxib was reported.
- Bioisosteric substitution of the pyrazole ring of the lead with the pyrazoline one was considered.
- The derivatives were investigated as hCA I, II, IV and IX inhibitors.
- The derivatives exhibited the best inhibitory activity against hCA II and hCA IV.
- Promising anti-glaucoma scaffolds were reported.

Graphical abstract

Synthesis and biological evaluation of novel pyrazoline-based aromatic sulfamates with potent carbonic anhydrase isoforms II, IV and IX inhibitory efficacy.

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