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Structure-activity relationship with pyrazoline-based aromatic sulfamates as carbonic anhydrase isoforms I, II, IX and XII inhibitors: synthesis and biological evaluation.

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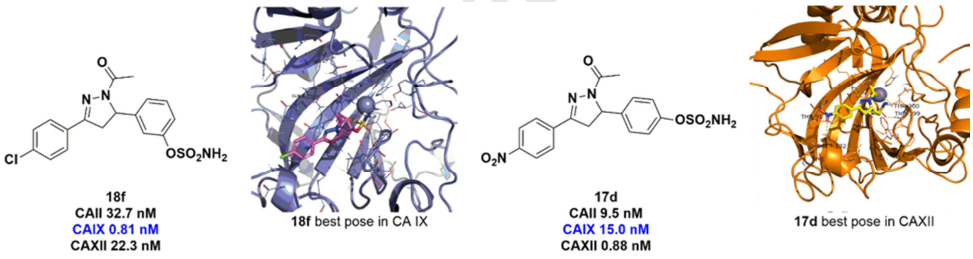
Highlights

* Pyrazoline aryl sulfamates were designed and synthesized.
* Sulfamates were assayed *in vitro* as human Carbonic Anhydrase (CA) isoforms inhibitors.
* Synthesized compound inhibited selectively CAII, CAII, CAIX and CAXII isoforms.
* Sulfamates **8h**, **9e**, **9i**, **17d**, **18e** show CAIX and CAXII K_i in the nanomolar to sub-nanomolar range.
* Docking revealed CA isoform selective inhibition related to distinguish interactions.

Keywords

Carbonic anhydrase, Zinc-binding group, Aromatic sulfamates, Pyrazolines, Enzyme inhibition, Molecular docking, Sulfamoylation

Graphical abstract



Abstract

Four new series of aromatic sulfamates were synthesized and investigated for the inhibition of four human (h) isoforms of zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1), hCA I, II, IX, and XII. The reported derivatives, obtained by a sulfamoylation reaction of the corresponding phenolic precursors, bear 3,5-diarylpyrazoline moieties as spacers between the benzenesulfamate fragment which binds the zinc ion from the active site, and the tail of the inhibitor. Pyrazolines are biologically privileged scaffolds, endowed with versatile biological activity, such as an anti-proliferative action. The derivatives were tested for the inhibition of the cytosolic, hCA I and II (off target isoforms) and the trans-membrane, tumor-associated hCA IX and XII enzymes (anticancer drug targets). Generally, hCA I was not effectively inhibited, whereas many low nanomolar inhibitors were evidenced against hCA II (KIs in the range of 0.42–90.1 nM), IX (KIs in the range of 0.72–63.6 nM), and XII (KIs in the range of 0.88–85.2 nM). The best substitution fragments at the pyrazoline ring included for CA II a 4-sulfamic group on the 3-aryl and halogens on the 5-aryl or a methoxy group on the 3-aryl and a 4-sulfamate group on the 5-aryl; for CA IX and CA XII they included the sulfamic group on the 3- or 4-position of the 5-aryl and an electronwithdrawing group on the 4-postion of the 3-aryl ring.

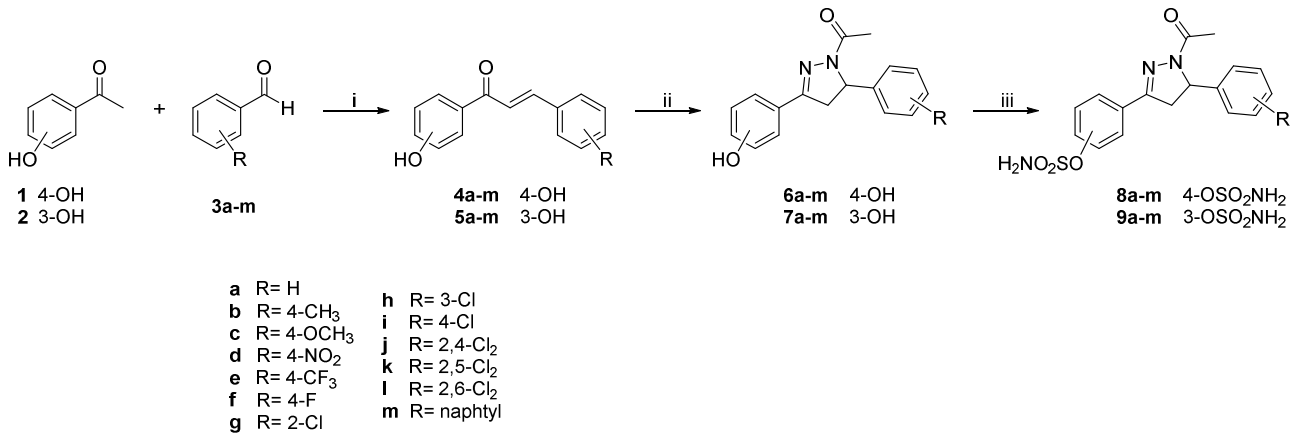
1. Introduction

Pyrazoline ring is a privileged structure in medicinal chemistry because of its wide spectrum of pharmacological activities. A number of pyrazoline derivatives have been reported for their antibacterial [1], antimalarial [2, 3], anti-inflammatory [4], MAO inhibitory [5, 6], antioxidant [7, 8], neuroprotective [9], antidepressant [10] and anticancer activity [11-18]. Furthermore, several series of pyrazoline derivatives displayed carbonic anhydrase (CA, EC 4.2.1.1) inhibitory activity [19-25]. CAs are a superfamily of metalloenzymes that catalyze the CO_2 hydration/dehydration reaction, and are classified into seven genetically distinct families, named α-, β-, γ-, δ-, ζ-, η-, and ɵ-CAs [26-28]. All human (h) CAs are α-class enzymes [28]. Fifteen different human isoforms have been identified and characterized to date, among which twelve are catalytically active (hCAs I-IV, VA, VB, VI, VII, IX, XII-XIV). Human CAs can be further categorized into four different subsets depending on their subcellular localization. Among those identified, hCA I, II, III, VII, VIII, X, XI, XIII are cytosolic proteins, hCA VA and VB are present in the mitochondrial matrix, hCA VI is a secreted enzyme, hCA IV is a glycosylphosphatidylinositol (GPI)-anchored protein and hCA IX, XII and XIV are trans-membrane isoforms [26, 28]. These enzymes are widely distributed in many tissues and organs where they are implicated in a wealth of pivotal physiological processes. Dysregulated expression and/or abnormal activity of hCAs can result into severe pathological conditions [26, 29-32]. hCA IX and XII have been validated as markers of disease progression in many hypoxic tumors and their targeted inhibition has been associated with a significant reduction of the growth of both primary tumors and metastases [26]. In contrast, ubiquitous isoforms hCA I and II are the main off-target isoforms because their promiscuous inhibition might lead to undesired side effects [26]. CA inhibitors (CAIs) can be clustered into several different groups considering their binding mode to the enzyme active site, among which the zinc-binders are the most effective and thus most investigated for drug-design purposes [33]. Within this subset, sulfonamides are the ideal zinc-binding group (ZBG) owing to a peculiar combination of interactions that this moiety can solely establish with the zinc ion and the residues nearby [33]. Pyrazoline sulfonamides were recently reported as CA inhibitors [34] and as dual CA and AchE inhibitors [35-39]. Bioisosteric replacement of the sulfonamide with the sulfamate group, one of sulfonamide most related congeners, produced interesting examples of CAs selective inhibitors [40-43]. These considerations led us to report a small library of pyrazoline sulfamates displaying interesting profile and selectivity as CA inhibitors [44]. Here, we have extended these studies to have full SAR of this class of compounds.

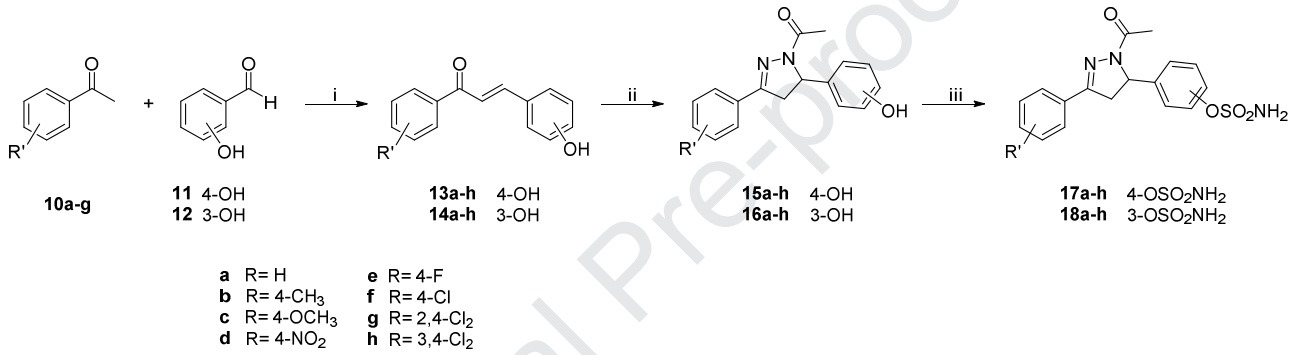
2. Results and discussion

2.1 Chemistry

The synthetic pathway to obtain N^1-acetyl-3,5-diaryl-4,5-dihydropyrazole bearing a sulfamate group at 4- or 3-position on 3-aryl ring (compounds **8a-m** and **9a-m**, Scheme 1) and on 5-aryl ring (compounds **17a-h** and **18 a-h**, Scheme 2) started with the preparation of chalcones (**4**, **5**, **14** and **15**) through the Claisen-Schmidt condensation [45-47] between substituted acetophenone (**1**, **2** and **10**) and substituted benzaldehydes (**3**, **11** and **12**) in methanol in the presence 50% aqueous NaOH. Chalcones were then treated with hydrazine hydrate in boiling AcOH [48] to afford the cyclization into 4,5-dihydropyrazoles (**6**, **7**, **15** and **16**). Finally, the desired compounds were obtained by sulfamoylation of the phenolic hydroxy groups by treatment with freshly prepared sulfamoyl chloride in N,N-dimethylacetamide. Structures were confirmed based on analytical and spectral data which are consistent with results of reported studies [48, 49].



Scheme 1. General synthetic procedure for sulfamates subsets **8** and **9**. Reagents and conditions: (i) MeOH, 50% aqueous NaOH, r.t., 12 h; (ii) $NH_2NH_2{\cdot}H_2O$, AcOH, 3 h, reflux; (iii) $ClSO_2NH_2$, DMA, r.t. 12 h.

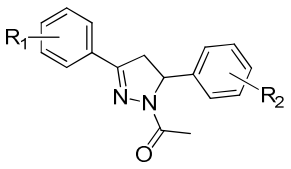


Scheme 2. General synthetic procedure for sulfamates subsets **17** and **18**. Reagents and conditions: (i) MeOH, 50% aqueous NaOH, r.t., 12 h; (ii) $NH_2NH_2{\cdot}H_2O$, AcOH, 3 h, reflux; (iii) $ClSO_2NH_2$, DMA, r.t. 12 h.

2.2. Carbonic anhydrase inhibition

The inhibitory activity against hCA I, hCA II, hCA IX and hCA XII of sulfamate derivatives **8a-m**, **9a-m, 17a-h** and **18 a-h** was tested by a stopped flow CO_2 hydrase assay in the presence of acetazolamide as standard inhibitor [50].

Table 1. Inhibition data of human CA isoforms hCA I, II, IX and XII with sulfamates subsets **8**, **9**, **17** and **18** reported here and the standard sulfonamide inhibitor acetazolamide (AAZ) by a stopped flow CO_2 hydrase assay [50].



Cmpd	R_1	R_2	K_I (nM)[a]			
			CA I	CA II	CA IX	CA XII
8a[b]	4-$OSONH_2$	H	1308.4	8.9	34.1	n.d.
8b	4-$OSONH_2$	4-CH_3	817.1	6.5	7.1	22.3
8c	4-$OSONH_2$	4-OCH_3	1607.4	5.3	25.0	42.6
8d[b]	4-$OSONH_2$	4-NO_2	3208.5	1.2	74.3	n.d.

8e[b]	4-$OSONH_2$	4-CF_3	2741.9	0.83	29.3	n.d.
8f	4-$OSONH_2$	4-F	666.5	1.5	5.9	11.8
8g[b]	4-$OSONH_2$	2-Cl	8253.1	9.0	25.9	n.d.
8h	4-$OSONH_2$	3-Cl	2313.3	0.87	0.72	9.8
8i	4-$OSONH_2$	4-Cl	1137.1	6.0	6.9	27.3
8j	4-$OSONH_2$	2,4-Cl_2	3752.5	9.3	11.2	26.2
8k[b]	4-$OSONH_2$	2,5-Cl_2	4752.2	12.4	25.5	n.d.
8l	4-$OSONH_2$	2,6-Cl_2	4083.0	13.2	33.4	43.7
8m	4-$OSONH_2$	naphthyl	3298.5	27.5	49.0	56.4
9°	3-$OSONH_2$	H	188.4	39.4	29.9	55.1
9b	3-$OSONH_2$	4-CH_3	558.2	71.2	29.4	55.9
9c	3-$OSONH_2$	4-OCH_3	172.2	52.4	8.7	48.6
9d	3-$OSONH_2$	4-NO_2	1458.2	133.9	10.1	20.8
9e	3-$OSONH_2$	4-CF_3	951.8	22.2	3.7	8.9
9f	3-$OSONH_2$	4-F	67.4	38.0	8.4	13.5
9g	3-$OSONH_2$	2-Cl	939.5	90.1	43.6	74.4
9h	3-$OSONH_2$	3-Cl	805.2	56.9	7.3	44.6
9i	3-$OSONH_2$	4-Cl	437.7	14.7	6.9	9.3
9j	3-$OSONH_2$	2,4-Cl_2	1413.2	194.7	13.8	33.8
9k	3-$OSONH_2$	2,5-Cl_2	2035.7	92.6	58.9	34.2
9l	3-$OSONH_2$	2,6-Cl_2	1782.5	223.4	63.6	61.3
9m	3-$OSONH_2$	naphthyl	2244.1	215.7	51.1	462.6
17°	H	4-$OSONH_2$	644.7	44.8	45.1	10.6
17b	4-CH_3	4-$OSONH_2$	2338.4	9.9	22.4	7.7
17c	4-OCH_3	4-$OSONH_2$	2977.2	0.42	22.8	9.5
17d	4-NO_2	4-$OSONH_2$	3818.1	9.5	15.0	0.88
17f	4-Cl	4-$OSONH_2$	970.2	19.5	8.3	29.5
17g	2,4-Cl_2	4-$OSONH_2$	3518.6	5.3	49.9	42.0
17h	3,4-Cl_2	4-$OSONH_2$	2898.4	27.6	7.7	12.9
18°	H	3-$OSONH_2$	755.0	16.5	13.1	22.9
18b	4-CH_3	3-$OSONH_2$	1539.2	25.4	8.9	49.8
18d	4-NO_2	3-$OSONH_2$	2546.8	22.9	9.4	20.5
18e[b]	4-F	3-$OSONH_2$	5225.3	6.5	15.8	n.d.
18f	4-Cl	3-$OSONH_2$	2140.9	32.7	0.81	22.3
18g	2,4-Cl_2	3-$OSONH_2$	6936.0	12.0	48.7	30.8
18h	3,4-Cl_2	3-$OSONH_2$	8174.1	54.8	15.5	85.2
AAZ	-		250	12.5	25	5.7

[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 reference [44].

The following structure–activity relationships (SAR) were gathered from the inhibition data reported in Table 1.

1) CA I: all pyrazoline sulfamates **8a-m**, **9a-m**, **17a-g** and **18a-g** showed low inhibitory activity except for compounds **9a**, **9c** and **9f** that displayed inhibitory values better the reference AAZ.

2) CA II: 5-Aryl substituted pyrazoline sulfamates **8a-l** showed high activity against this CA isoform (K_I in the 0.8-13.2 nM range). Compound **8e** bearing a 4-trifluoromethylphenyl ring showed the best activity (K_I 0.8 nM). The replacement of the 4-trifluoromethyl moiety with a chlorine atom (compound **8i**) led to a reduction in activity. On the contrary the displacement of the chlorine from the 4-position to the 3-position repristinate the activity (compound **8h**, K_I 0.87 nM). The displacement of the chlorine into 2-position (compound **8g**) as well as the introduction of two chlorine atoms (compounds **8j**-**8l**) led to a reduction in activity as compared to the analog **8h**. The comparison of the sulfamates **8** and **9** inhibitory activity revealed that the displacement of the 4-sulfamate group into 3-position produced a reduction in activity. However, sulfamates **9e** and **9i** bearing 4-trifluoromethyl and 4-chlorine moieties maintained high inhibitory activity. In the pyrazoline sulfamates **17** series the inhibitory activity is related to the presence of substituents on the 3-aryl ring; as matter of fact derivatives **17a** showed the lowest activity in this series. The best compound was **17c** bearing a 4-methoxyphenyl group. Interestingly the shift of the methoxy and sulfamate moieties from the 3-aryl to 5-aryl, as can be seen by the comparison of compounds **8c** and **17c** K_Is, generated a different inhibition profile. This trend is confirmed by the other compounds of the series. The presence of 4-methyl, 4-nitro and 2,4-dichloro substituents was favorable for the activity in the pyrazoline **17** series, while the presence of the same groups on the 5-aryl of pyrazolines **8** did not increase the activity. Pyrazoline sulfamates **18** showed high activity as compared to their analogs **9** except for **18f**. As general trend the displacement of the 4-sulfamate group of pyrazolines **17** into 3-position to give pyrazolines **18** led to a slight reduction in activity when substituents are present on the 5-aryl ring.

3) CA IX: Among pyrazoline sulfamates **8** subset, the 3-chlorine derivative **8h** showed the best activity (K_I 0.72 nM). The displacement of the chlorine atom into 4-position (compound **8i**) led to a 10-fold reduction in activity. The replacement of the 4-chlorine of compound **8i** with a methyl group (compound **8b**) maintained the same activity level, while the replacement with a 4-fluorine atom (sulfamate **8f**) produced an increase in activity. On the contrary the presence of two chlorine atoms (compounds **8j**-**8l**) produced a reduction in activity when compared to both **8h** and **8i** analogs. The displacement of the 4-sulfamate group into 3-position to give the isomeric pyrazolines **9** induced similar or better activity in comparison to the corresponding analogs **8** except for compounds **9g**, **9k** and **9l**. In the pyrazoline sulfamates **17** series the best activity was showed by the chlorine substituted compound **17g** whose K_I is very similar to the analog **8i** as well as for the compounds **17a** and **17c** as compared to their analogs **8a** and **8c**. Interestingly, the transposal of the nitro and sulfamate groups on the aryl rings of **8d** to give the isomeric **17d** caused about a 5-fold increase in activity. The shift of the sulfamate moiety of pyrazolines **9** on the 5-aryl ring to give the analogs **18** produced improvement in activity especially in compound **18f** that was the best of the series showing an 8.5-fold higher activity than analog **9i**. The same compound **18f** was about 10-fold more active as compared to the analog **17e**. The same activity trend is showed by the pyrazoline **18a** that was about 3-fold more active than the analog **17a**.

4) CA XII: Pyrazoline sulfamates **8f**, **8h**, **9c**, **9f**, and **9i** confirmed their good inhibitory activity against the other CA isoform expressed in hypoxic tumoral cells. Thus, the presence of a halogen atom or a trifluoromethyl group in 4-position enhanced the activity in the series **9**, while in the series **8** the best activity was correlated to the presence of 4-fluorine or 3-chlorine atom on the 5-aryl ring. The pyrazoline **17d** showed the best activity of all four series (K_I 0.88 nM). The removal of the 4-nitro group of **17d** or its replacement with a methoxy group led to about a 10-fold reduction in activity. Pyrazoline sulfamates of series **18** showed reduced activity as compared to both pyrazolines **17** and **9**.

2.3 Molecular Docking

To better understand the binding patterns of our compounds they were docked in the four CA isoforms evaluated in this study, CA I (PDBID: 3w6h), CA II (PDBID: 4g0c), CA IX (PDBID: 3iai), CA XII (PDBID: 1jd0). These crystal structures were selected for the presence of AAZ as co-crystallized ligand used as a reference for discussing the experimental activities.

A selection of the most interesting compounds **8f**, **8h**, **9e**, **17c**, **17d**, **17g**, **18f** were used to deeply analyze the ligand-protein interactions (the complete docking scores of all compounds can be found on Table 1S of supplementary material).

Just by a first look at the binding site, we can retrieve some useful information. The main difference among the isoforms is the size of the binding sites (Figure S1). The smaller binding site of CA I can explain the lower

activity of the compounds on this isoform. A second evident information is about hydrophobic properties. In fact, CA XII has a more polar binding site in contrast with other isoforms. Finally, another important structural difference between CA I and the other isoforms pertains residue 200, which is a histidine in CA I, while a threonine in CA II, IX and XII. As in most of the poses of the docked compounds there is an interaction between the ligand and Thr200, the Thr/His mutation is likely to induce the ligands lower activity against CA I. Unfortunately, we could not find a clear correlation between the docking score and the in-vitro results. However, some useful information to better understand the reasons behind activity and selectivity can be obtained looking at the best scored poses.

The sulfamate moiety of all compounds reproduced almost the same interactions with the sulfonamide group of AAZ. In detail the sulfamate group fit deeply into the active site with the negatively charged nitrogen coordinating the Zinc. Moreover, the hydrogen of the sulfamate establish an H-Bond with the T199 oxygen of the hydroxy group and the amidic hydrogen of the same residue binds the S=O oxygens and/or the Ph-O-S oxygen (Figure S2).

About the other portion of the molecules, for what concerns CA I no other important interaction was found except for the compound **9e** which establishes an H-Bond between one fluorine of the trifluoromethyl group and Asn69 side chain.

Speaking about CA II, all selected compounds perform a π-π stacking with Phe131. Moreover, other common H-bonds performed by the compounds are with residues Asn62, Asn67 (**8f**, **8h**, **17f**). Moreover, for the most active compound of the series against this isoform, **17c,** as well as compounds **17d** and **18f**, a H-bond exists between their carbonyl moiety and the hydroxyl group of Thr200 (Figure S3).

Within CA IX active site, the four most active compounds **8f**, **8h**, **9e**, and **18f** show potential H-bond with Asn67 and Gln92 (Figure S4A). Conversely, compounds **17c**, **17d**, and **17f** establish H-bond with Thr200. Moreover, the nitro group **17d** is in a good position to form an H-bond with Asp132 (Figure S4B).

For what concerns CA XII, all compounds except **8f** and **9e** establish H-bonds with Lys67. Other recurrent H-bonds exist with Thr91 and/or Gln92 and varied groups of compounds **8f**, **9e**, **17f** and **18f** (Figure S5A). Moreover, compounds **9e**, **17d**, **17c** and **18f** establish H-bonds by the carbonyl or the pyrazoline nitrogen with Thr200. Finally, compounds **9e**, **17c**, **17d** bearing an H-bond acceptor group in *para* to the non-sulfamate ring establish interaction with Ser132 (Figure S5B).

3. Conclusions

Four new series of aromatic sulfamates were synthesized by a sulfamoylation reaction of the corresponding phenolic precursors. The reported derivatives bearing 3,5-diarylpyrazoline moieties as spacers between the benzenesulfamate fragment which binds the zinc ion from the active site, and the tail of the inhibitor were investigated for the inhibition of four human hCAs, namely the cytosolic hCA I and II (off target isoforms) and the trans-membrane, tumor-associated hCA IX and XII enzymes (anticancer drug targets). The pyrazoline sulfamates did not effectively inhibited hCA I, whereas many low nanomolar inhibitors were evidenced against hCA II (K_Is in the range of 0.42–90.1 nM), IX (K_Is in the range of 0.72–63.6 nM), and XII (K_Is in the range of 0.88–85.2 nM). The best substitution fragments at the pyrazoline ring included for CA II a 4-sulfamic group on the 3-aryl and halogens on the 5-aryl or a methoxy group on the 3-aryl and a 4-sulfamate group on the 5-aryl; for CA IX and CA XII the sulfamic group on the 3- or 4-position of the 5-aryl and an electronwithdrawing group on the 4-postion of the 3-aryl ring. Docking analyses suggested that the selectivity and potency showed by some compounds may be related to the number of hydrogen bonds between the sulfamate compounds and the CA isoforms.

4. Experimental section

4.1. General methods

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. The spectra were recorded in hexadeuteriodimethylsulphoxide (DMSO-d6). 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 with 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 analyser (Yanagimoto, Kyoto, Japan) and the values found were within 0.4% of theoretical values. Pyrazolines **7a** [51], **7b** [52], **7c** [53], **7d** [44], **7i** [52], **7l** [54], **15a** [55], **15b** [55], **15f** [55], **16a** [43], and sulfamates **8a, 8d, 8e, 8g** [44] have been prepared as previously described.

*4.1.1. General procedure for the preparation of 1-acetyl-3,5-diaryl- 4,5-dihydro-1H-pyrazoles **6, 7, 15** and **16**.*

To a solution of chalcone derivative **4, 5, 13, 14** (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.

*4.1.1.1. 1-(5-(4-fluorophenyl)-3-(3-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**7f**)*

Following the general procedure, the title compound was prepared starting from chalcone **5f**. Yield 79%. M.p. 218-219 °C. ^{1}H NMR (DMSO-d_6) 2.33 (s, 3H, CH_3), 2.99 (dd, *J* = 14.0, 3.5 Hz, 1H, CH), 3.93 (dd, *J* = 14.0, 3.0 Hz,1H, CH), 5.73 (dd, *J* = 13.5, 3.5 Hz, 1H, CH), 6.85 (d, *J* = 6.5 Hz, 1H, Ar), 7.05 (d, *J* = 7.0 Hz, 1H, Ar), 7.16 (m, 1H, Ar), 7.21 (d, *J* = 8.0 Hz, 2H, Ar), 7.29 (d, *J* = 8.5 Hz, 2H, Ar), 7.47 (s, 1H, Ar), 9.63 (s, 1H, OH). IR (Nujol) 3272, 1635, 1575 cm^{-1}. m/z 299 (m+H)$^+$. Anal. calcd. for $C_{17}H_{15}FN_2O_2$ (298.31) C, 64.45; H, 5.07; N, 9.39. Found C, 64.49; H, 5.11; N, 9.36.

*4.1.1.2. 1-(5-(2-Chlorophenyl)-3-(3-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**7g**)*

Following the general procedure, the title compound was prepared starting from chalcone **5g**. Yield 84%. M.p. 212-214 °C. ^{1}H NMR (DMSO-d_6) 2.36 (s, 3H, CH_3), 3.01 (dd, *J* = 14.0, 3.0 Hz, 1H, CH), 3.94 (dd, *J* = 14.5, 3.5 Hz,1H, CH), 5.76 (dd, *J* = 13.5, 3.0 Hz, 1H, CH), 6.87 (d, *J* = 7.5 Hz, 1H, Ar), 7.07 (d, *J* = 7.0 Hz, 1H, Ar), 7.16 (m, 1H, Ar), 7.23 (d, *J* = 7.5 Hz, 2H, Ar), 7.31 (d, *J* = 8.0 Hz, 2H, Ar), 7.49 (s, 1H, Ar), 9.64 (s, 1H, OH). IR (Nujol) 3231, 1643, 1573 cm^{-1}. m/z 315 (m+H)$^+$. Anal. calcd. for $C_{17}H_{15}ClN_2O_2$ (314.77) C, 64.87; H, 4.80; N, 8.90. Found C, 64.94; H, 4.78; N, 8.86.

*4.1.1.3. 1-(5-(3-Chlorophenyl)-3-(3-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**7h**)*

Following the general procedure, the title compound was prepared starting from chalcone **5h**. Yield 92%. M.p. 127-128 °C. ^{1}H NMR (DMSO-d_6) 2.31 (s, 3H, CH_3), 3.32 (dd, *J* = 3.0, 14.0 Hz, 1H, CH), 3.84 (dd, *J* = 14.5, 3.5 Hz, 1H, CH), 5.54 (dd, *J* = 13.5, 3.0 Hz, 1H, CH), 6.87 (d, *J* =7.5 Hz, 1H, Ar), 7.18 (d, *J* = 7.0 Hz, 1H, Ar), 7.23 (m, 1H, Ar), 7.27 (d, *J* = 7.5 Hz, 2H, Ar), 7.31 (d, *J* = 8.0 Hz, 2H, Ar), 7.37 (s, 1H, Ar), 9.66 (s, 1H, OH). IR (Nujol) 3172, 1640, 1574 cm^{-1}. m/z 315 (m+H)$^+$. Anal. calcd. for $C_{17}H_{15}ClN_2O_2$ (314.77) C, 64.87; H, 4.80; N, 8.90. Found C, 64.83; H, 4.81; N, 8.93.

*4.1.1.4. 1-(5-(2,4-Dichlorophenyl)-3-(3-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**7j**)*

Following the general procedure, the title compound was prepared starting from chalcone **5j**. Yield 92%. M.p. 162-163 °C. ^{1}H NMR (DMSO-d_6) 2.34 (s, 3H, CH_3), 3.02 (dd, *J* = 4.0, 14.0 Hz, 1H, Ch), 3.90 (dd, *J* = 13.5, 3.5 Hz, 1H, CH), 5.71 (dd, *J* = 13.5, 3.0 Hz, 1H, CH), 6.87 (d, *J* =7.5 Hz, 1H, Ar), 7.08 (d, *J* = 7.0 Hz, 1H, Ar), 7.22 (m, 2H, Ar), 7.66 (m, 2H, Ar), 7.72 (s, 1H, Ar), 9.65 (s, 1H, OH). IR (Nujol) 3264, 1644, 1574 cm^{-1}. m/z 349 (m+H)$^+$. Anal. calcd. for $C_{17}H_{14}Cl_2N_2O_2$ (349.21) C, 58.47; H, 4.04; N, 8.02. Found C, 58.42; H, 4.02; N, 8.05.

*4.1.1.5. 1-(5-(2,5-Dichlorophenyl)-3-(3-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**7k**)*

Following the general procedure, the title compound was prepared starting from chalcone **5k**. Yield 91%. M.p. 231-233 °C. ^{1}H NMR (DMSO-d_6) 2.35 (s, 3H, CH_3), 3.25 (dd, *J* = 4.0, 14.0 Hz, 1H, CH), 4.07 (dd, *J* = 13.5, 3.5 Hz, 1H, CH), 5.68 (dd, *J* = 13.5, 3.0 Hz, 1H, CH), 6.93 (d, *J* = 7.5 Hz, 1H, Ar), 7.35 (d, *J* = 7.0 Hz, 1H, Ar), 7.56 (m, 2H, Ar), 7.73 (m, 2H, Ar), 8.06 (s, 1H, Ar), 10.1 (s, 1H, OH). IR (Nujol) 3320, 1636, 1571 cm^{-1}. m/z 349 (m+H)$^+$. Anal. calcd. for $C_{17}H_{14}Cl_2N_2O_2$ (349.21) C, 58.47; H, 4.04; N, 8.02. Found C, 58.41; H, 4.05; N, 8.06.

*4.1.1.6. 1-(3-(3-Hydroxyphenyl)-5-(naphthalen-1-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**7m**)*

Following the general procedure, the title compound was prepared starting from chalcone **5m**. Yield 71%. M.p. 180-181 °C. ^{1}H NMR (DMSO-d6) 2.44 (s, 3H, CH_3), 2.98 (dd, *J* = 14.0, 4.0 Hz, 1H, CH), 4.22 (dd, *J* = 13.5,

4.0 Hz, 1H, CH), 6.17 (dd, *J* = 13.0, 3.5 Hz, 1H, CH), 6.62 (d, *J* = 8.0 Hz, 2H, Ar), 7.08 (d, *J* = 8.5 Hz, 2H, Ar), 7.62 (m, 3H, Ar), 7.70 (m, 2H, Ar), 7.92 (m, 2H, Ar), 8.15 (s, 1H, Ar), 9.42 (s, 1H, OH). IR (Nujol) 3212, 1643 cm^{-1}. m/z 331 $(m+H)^+$. Anal. calcd. for $C_{21}H_{18}N_2O_4$ (330.38) C, 76.34; H, 5.49; N, 8.48. Found C, 76.41; H, 5.50; N, 8.45.

*4.1.1.7. 1-(5-(4-Hydroxyphenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**15d**)*

Following the general procedure, the title compound was prepared starting from chalcone **13d**. Yield 41%. M.p. 130-131 °C. ^{1}H NMR (DMSO-d_6) 2.29 (s, 3H, CH_3), 3.11 (dd, *J* = 13.0, 3.0 Hz, 1H, CH), 3.88 (dd, *J* = 14, 4.0 Hz, 1H, CH), 5.61 (dd, *J* = 13.0, 3.5 Hz, 1H, CH), 6.77 (d, *J* = 8.5 Hz, 2H, Ar), 7.14 (d, *J* = 8.0 Hz, 2H, Ar), 8.22 (d, *J* = 7.0 Hz, 2H, Ar), 8.30 (d, *J* = 7.5 Hz, 2H, Ar), 9.66 (s, 1H, OH). IR (Nujol) 3259, 1702, 1606 cm^{-1}. m/z 326 $(m+H)^+$. Anal. calcd. for $C_{17}H_{15}N_3O_4$ (325.32) C, 62.76; H, 4.65; N, 12.92. Found C, 62.70; H, 4.66; N, 12.96.

*4.1.1.8. 1-(3-(2,4-Dichlorophenyl)-5-(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**15g**)*

Following the general procedure, the title compound was prepared starting from chalcone **13g**. Yield 62%. M.p. 127-128°C. ^{1}H NMR (DMSO-d_6) 2.28 (s, 3H, CH_3), 3.13 (dd, *J* = 14.0, 4.0 Hz, 1H, CH), 3.81 (s, 2H, NH_2), 3.86 (dd, *J* = 13.5, 3.5 Hz, 1H, CH), 5.44 (dd, *J* = 13.5, 3.0 Hz, 1H, CH), 6.73 (d, *J* = 8.5 Hz, 2H, Ar), 7.02 (d, *J* = 8.0 Hz, 2H, Ar), 7.61 (d, *J* = 7.5 Hz, 1H, Ar), 7.80 (d, *J* = 8.0 Hz, 1H, Ar), 7.81 (s, 1H, Ar), 9.35 (s, 1H, OH). IR (Nujol) 3318, 1645, 1587 cm^{-1}. m/z 349 $(m+H)^+$. Anal. calcd. for $C_{17}H_{14}Cl_2N_2O_2$ (349.21) C, 58.47; H, 4.04; N, 8.02. Found C, 58.43; H, 4.05; N, 8.06.

*4.1.1.9. 1-(3-(3,4-Dichlorophenyl)-5-(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**15h**)*

Following the general procedure, the title compound was prepared starting from chalcone **13h**. Yield 84%. M.p. 122-123°C. ^{1}H NMR (DMSO-d6) 2.31 (s, 3H, CH_3), 3.09 (dd, *J* = 14.0, 4.5 Hz, 1H), 3.88 (dd, *J* = 13.5, 3.5 Hz, CH), 5.52 (dd, *J* = 13.0, 3.0 Hz, 1H), 6.73 (d, *J* = 8.0 Hz, 2H, Ar), 7.01 (d, *J* = 8.0 Hz, 2H, Ar), 7.76 (d, *J* = 7.5 Hz, 1H, Ar), 7.89 (d, *J* = 8.0 Hz, 1H, Ar), 7.94 (s, 1H, Ar), 9.33 (s, 1H, OH). IR (Nujol) 3250, 1646, 1595 cm^{-1}. m/z 349 $(m+H)^+$. Anal. calcd. for $C_{17}H_{14}Cl_2N_2O_2$ (349.21) C, 58.47; H, 4.04; N, 8.02. Found C, 58.52; H, 4.03; N, 7.99.

*4.1.1.10. 1-(5-(3-Hydroxyphenyl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**16b**)*

Following the general procedure, the title compound was prepared starting from chalcone **14b**. Yield 83%. M.p. 98-100 °C. ^{1}H NMR (DMSO-d6) 2.32 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 2.80 (dd, *J* = 13.0, 3.5 Hz, 1H, CH), 3.41 (dd, *J* =13.5, 3.5 Hz, 1H, CH), 4.73 (dd, *J* = 14.5, 3.0 Hz, 1H, CH), 6.65 (d, *J* = 8.5 Hz, 1H, Ar), 6.79 (d, *J* = 8.5 Hz, 1H, Ar), 7.20 (m, 1H, Ar), 7.52 (d, *J* = 7.0 Hz, 2H, Ar), 7.68 (d, *J* = 7.0 Hz, 2H, Ar), 9.32 (s, 1H, OH). IR (Nujol) 3328, 1595 cm^{-1}. m/z 295 $(m+H)^+$. Anal. calcd. for $C_{18}H_{18}N_2O_2$ (294.35) C, 73.45; H, 6.16; N, 9.52. Found C, 73.51; H, 6.18; N, 9.47.

*4.1.1.11. 1-(5-(3-hydroxyphenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**16d**)*

Following the general procedure, the title compound was prepared starting from chalcone **14d**. Yield 71%. M.p. 139-140 °C. ^{1}H NMR (DMSO-d_6) 2.19 (s, 3H, CH_3), 3.15 (dd, *J* = 3.0, 14.0 Hz, 1H, CH), 3.98 (dd, *J* =14.5, 3.5 Hz, 1H, CH), 5.63 (dd, *J* = 13.5, 3.0 Hz, 1H, CH), 6.89 (d, *J* = 7.5 Hz, 1H, Ar), 7.12 (d, *J* = 7.0 Hz, 1H, Ar), 7.21 (m, 1H, Ar), 7.54 (d, *J* = 8.5 Hz, 2H, Ar), 7.69 (d, *J* = 8.0 Hz, 2H, Ar), 9.33 (s, 1H, OH). IR (Nujol) 3347, 1604 cm^{-1}. m/z 326 $(m+H)^+$. Anal. calcd. for $C_{17}H_{15}N_3O_4$ (325.32) C, 62.76; H, 4.65; N, 12.92. Found C, 62.70; H, 4.66; N, 12.89.

*4.1.1.12. 1-(3-(4-Chlorophenyl)-5-(3-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**16e**)*

Following the general procedure, the title compound was prepared starting from chalcone **14e**. Yield 67%. M.p. 109-110 °C. ^{1}H NMR (DMSO-d_6) 2.22 (s, 3H, CH_3), 3.13 (dd, *J* = 14.0, 3.0 Hz, 1H), 3.96 (dd, *J* = 14.5, 3.5 Hz, CH), 5.01 (dd, *J* = 13.5, 3.0 Hz, 1H), 6.81 (d, J = 7.5 Hz, 1H, Ar), 6.98 (d, J = 7.0 Hz, 1H, Ar), 7.22 (m, 1H, Ar), 7.46 (d, J = 7.5 Hz, 2H, Ar), 7.58 (d, J = 8.0 Hz, 2H, Ar), 9.36 (s, 1H, OH). IR (Nujol) 3322, 1652, 1591 cm^{-1}. m/z 315 $(m+H)^+$. Anal. calcd. for $C_{17}H_{15}ClN_2O_4$ (314.77) C, 64.87; H, 4.80, N, 8.90. Found C, 64.82; H, 4.79; N, 8.95.

*4.1.1.13. 1-(3-(2,4-Dichlorophenyl)-5-(3-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**16g**)*

Following the general procedure, the title compound was prepared starting from chalcone **14g**. Yield 51%. M.p. 124-125 °C. ^{1}H NMR (DMSO-d_6) 2.31 (s, 3H, CH_3), 3.17 (dd, *J* = 14.0, 4.0 Hz, 1H, CH), 3.97 (dd, *J* =13.5, 3.5 Hz,1H, CH), 5.03 (dd, *J* = 13.5, 3.0 Hz, 1H, CH), 6.67 (d, *J* = 7.5 Hz, 1H, Ar), 6.91 (d, *J* = 7.0 Hz, 1H, Ar), 7.11 (m, 1H, Ar), 7.19 (d, *J* = 7.5 Hz, 1H, Ar), 7.53 (d, *J* = 8.0 Hz, 1H, Ar), 7.79 (s, 1H, Ar), 9.12 (s, 1H, OH). IR (Nujol)

3330, 1672, 1585 cm^{-1}. m/z 349 $(m+H)^+$. Anal. calcd. for $C_{17}H_{14}Cl_2N_2O_2$ (349.21) C, 58.47; H, 4.04; N, 8.02. Found C, 58.52; H, 4.05; N, 7.98.

*4.1.1.14. 1-(3-(3,4-Dichlorophenyl)-5-(3-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**16h**)*

Following the general procedure, the title compound was prepared starting from chalcone **14h**. Yield 73%. M.p. 114-116 °C. ^{1}H NMR (DMSO-d_6) 2.33 (s, 3H, CH_3), 3.18 (dd, *J* = 14.0, 4.0 Hz, 1H, CH), 4.01 (dd, *J* = 13.5, 3.5 Hz, 1H, CH), 5.13 (dd, *J* = 13.5, 3.0 Hz, 1H, CH), 6.52 (d, *J* = 7.5 Hz, 1H, Ar), 6.81 (d, *J* = 7.0 Hz, 1H, Ar), 7.31 (m, 1H, Ar), 7.44 (d, *J* = 7.5 Hz, 1H, Ar), 7.58 (d, *J* = 8.0 Hz, 1H, Ar), 7.89 (s, 1H, Ar), 9.12 (s, 1H, OH). IR (Nujol) 3407, 1669, 1590 cm^{-1}. m/z 349 $(m+H)^+$. Anal. calcd. for $C_{17}H_{14}Cl_2N_2O_2$ (349.21) C, 58.47; H, 4.04; N, 8.02. Found C, 58.42; H, 4.02; N, 8.05.

*4.1.2. General procedure for the preparation of 1-acetyl-3,5-diaryl-4,5-dihydro-1H-pyrazole sulfamates **8a-m, 9a-m, 17a-h, 18a-h*.* To an ice-cooled stirred solution of pyrazolines **6**, **7**, **15**, **16** (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 2 h, then the formed precipitate was filtered off and dried.

*4.1.2.1 4-(1-Acetyl-5-(p-tolyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl sulfamate (**8b**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **6b**. Yield 22%. M.p. 140-142 °C. ^{1}H NMR (DMSO-d_6) 2.15 (s, 3H, CH_3), 3.11 (dd, *J* = 14.0, 4-0 Hz, 1H, CH), 3.36 (dd, *J* = 13.0, 3.5 Hz, 1H, CH), 3.61 (s, 3H, CH_3), 4.22 (s, 2H, NH_2), 5.47 (dd, *J* = 13.5, 4.0 Hz, 1H, CH), 6.83 (d, *J* = 8.0, 2H, Ar), 7.09 (d, *J* = 8.5 Hz, 2H, Ar), 7.62 (d, *J* = 7.0 Hz, 2H, Ar), 7.87 (d, *J* = 7.5 Hz, 2H, Ar). IR (Nujol) 3155, 1629, 1516 cm^{-1}. m/z 374 $(m+H)^+$. Anal. calcd. for $C_{18}H_{19}N_3O_4S$ (373.43) C, 57.89; H, 5.13; N, 11.25. Found C, 57.93; H, 5.15; N, 11.21.

*4.1.2.2. 4-(1-Acetyl-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl sulfamate (**8c**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **6c**. Yield 61%. M.p. 119-120 °C. ^{1}H NMR (DMSO-d_6) 2.15 (s, 3H, CH_3), 3.09 (dd, *J* = 14.0, 4.0 Hz, 1H, CH), 3.68 (dd, *J* = 13.0, 3.5 Hz, 1H, CH), 3.72 (s, 3H, OCH_3), 3.87 (s, 2H, NH_2), 5.44 (dd, *J* = 13.5, 4.0 Hz, 1H, CH), 6.59 (d, *J* = 8.0 Hz, 2H, Ar), 6.84 (d, *J* = 8.5 Hz, 2H, Ar), 7.66 (d, *J* = 7.0 Hz, 2H, Ar), 7.85 (d, *J* = 7.5 Hz, 2H, Ar). IR (Nujol) 3198, 1611 cm^{-1}. m/z 390 $(m+H)^+$. Anal. calcd. for $C_{18}H_{19}N_3O_5S$ (389.43) C, 55.52; H, 4.92; N, 10.79. Found C, 55.48; H, 4.93; N, 10.83.

*4.1.2.3. 4-(1-Acetyl-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl sulfamate (**8f**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **6f**. Yield 95%. M.p. 134-135°C. ^{1}H NMR (DMSO-d_6) 2.33 (s, 3H, CH_3), 3.23 (dd, *J* = 13.0, 5.0, Hz, 1H, CH), 3.92 (dd, *J* = 12.0, 4.5 Hz, 1H, CH), 4.16 (s, 2H, NH_2), 5.66 (dd, *J* = 13.0, 5.0 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). IR (Nujol) 3265, 1652, 1513 cm^{-1}. m/z 378 $(m+H)^+$. Anal. calcd. for $C_{17}H_{16}FN_3O_4S$ (377.39) C, 54.10; H, 4.27; N, 11.37. Found C, 54.15; H, 4.26; N, 11.34.

*4.1.2.4. 4-(1-Acetyl-5-(3-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl sulfamate (**8h**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **6h**. Yield 91%. M.p. 118-120 °C. ^{1}H NMR (DMSO-d_6) 2.28 (s, 3H, CH_3), 3.13 (dd, *J* = 14.0, 3.0 Hz, 1H, CH), 3.76 (dd, *J* = 14.5, 3.5 Hz, 1H, CH), 3.82 (s, 2H, NH_2), 5.51 (dd, *J* = 13.5, 3.0 Hz, 1H, CH), 6.82 (d, *J* = 7.5Hz, 1H, Ar), 6.86 (d, *J* = 7.0 Hz, 1H, Ar), 7.32 (m, 1H, Ar), 7.46 (s, 1H, Ar), 7.62 (d, *J* = 7.5 Hz, 2H, Ar), 7.88 (d, *J* = 8.0 Hz, 2H, Ar). IR (Nujol) 3222, 1610 cm^{-1}. m/z 394 $(m+H)^+$. Anal. calcd for $C_{17}H_{16}ClN_3O_4S$ (393.84) C, 51.84; H, 4.09; N, 10.67. Found C, 51.89; H, 4.07; N, 10.63.

*4.1.2.5. 4-(1-Acetyl-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl sulfamate (**8i**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **6i**. Yield 31%. M.p. 123-124°C. ^{1}H NMR (DMSO-d_6) 2.28 (s, 3H, CH_3), 3.15 (dd, *J* = 14.0, 3.5 Hz, 1H, CH), 3.82 (dd, J = 13.0, 3.5 Hz, 1H, CH), 4.67 (s, 2H, NH_2), 5.55 (dd, *J* = 13.5, 3.0 Hz, 1H, CH), 6.85 (d, *J* = 8.0 Hz, 2H, Ar), 7.21 (d, *J* = 8.0 Hz, 2H, Ar), 7.63 (d, *J* = 7.5 Hz, 2H, Ar), 7.88 (d, *J* = 8.0 Hz, 2H, Ar). IR (Nujol) 3329, 1634, 1609 cm^{-1}. m/z 394 $(m+H)^+$. Anal. calcd. for $C_{17}H_{16}ClN_3O_4S$ (393.84) C, 51.84; H, 4.09; N, 10.67. Found C, 51.79; H, 4.10; N, 10.70.

*4.1.2.6. 4-(1-Acetyl-5-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl sulfamate (**8j**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **6j**. Yield 49%. M.p. 183-184 °C. ^{1}H NMR (DMSO-d_6) 2.32 (s, 3H, CH_3), 3.14 (dd, *J* = 14.0, 4.0 Hz, 1H, CH), 3.88 (s, 2H, NH_2), 3.95 (dd, *J* = 13.5, 3.5 Hz, 1H, CH), 5.68 (dd, *J* = 13.5, 3.0 Hz, 1H, CH), 6.83 (d, J = 8.5Hz, 2H, Ar), 7.07 (d, *J* =

8.0 Hz, 2H, Ar), 7.62 (d, *J* = 7.5 Hz, 1H, Ar), 7.85 (d, *J* = 8.0 Hz, 1H, Ar), 8.09 (s, 1H, Ar). IR (Nujol) 3296, 1630, 1599 cm^{-1} m/z 428 $(m+H)^+$. Anal. calcd. for $C_{17}H_{15}Cl_2N_3O_4S$ (428.29) C, 47.67; H, 3.53; N, 9.81. Found C, 47.71; H, 3.52; N, 9.84.

*4.1.2.7. 4-(1-Acetyl-5-(2,5-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl sulfamate (**8k**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **6k**. Yield 50%. M.p. 174–176°C. ^1H NMR (DMSO-d_6) 2.31 (dd, *J* = 16.0, 4.5 Hz, 1H, CH), 2.35 (s, 3H, CH_3), 3.94 (m, 1H, CH), 5.74 (dd, *J* = 12.5, 4.5 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) 3308, 1637, 1603 cm^{-1}. m/z 428 $(m+H)^+$. Anal. calcd. for $C_{17}H_{15}Cl_2N_3O_4S$ (428.29) C, 47.67; H, 3.53; N, 9.81. Found C, 47.62; H, 3.54; N, 9.78.

*4.1.2.8. 4-(1-Acetyl-5-(2,6-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl sulfamate (**8l**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **6l**. Yield 75%. M.p. 138-140°C. ^1H NMR (DMSO-d_6) 2.24 (s, 3H, CH_3), 3.16 (dd, *J* = 14.0, 4.0 Hz, 1H, CH), 3.78 (dd, *J* = 13.0, 3.5 Hz, 1H, CH), 3.88 (s, 2H, NH_2), 6.05 (dd, *J* = 13.5, 4.0 Hz, 1H, CH), 6.85 (d, *J* = 8.0 Hz, 2H, Ar), 7.31 (m, 2H, Ar), 7.39 (m, 2H, Ar), 7.88 (d, *J* = 7.5 Hz, 2H, Ar). IR (Nujol) 3387, 1638, 1602 cm^{-1}. m/z 428 $(m+H)^+$. Anal. calcd. for $C_{17}H_{15}Cl_2N_3O_4S$ (428.29) C, 47.67; H, 3.53; N, 9.81. Found C, 47.70; H, 3.54; N, 9.83.

*4.1.2.9. 4-(1-Acetyl-5-(naphthalen-1-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl sulfamate (**8m**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **6m**. Yield 71%. M.p. 116-118 °C. ^1H NMR (DMSO-d_6) 2.39 (s, 3H, CH_3), 3.10 (dd, *J* = 14.0, 4.0 Hz, 1H, CH), 3.44 (s, 2H, NH_2), 4.13 (dd, *J* = 13.5, 4.0 Hz, 1H, CH), 6.30 (dd, *J* = 13.0, 3.5 Hz, 1H, CH), 7.14 (d, *J* = 8.0 Hz, 2H, Ar), 7.32 (d, *J* = 8.5 Hz, 2H, Ar), 7.44 (m, 3H, Ar), 7.62 (m, 2H, Ar), 7.87 (m, 2H, Ar). IR (Nujol) 3285, 1646 cm^{-1}. m/z 410 $(m+H)^+$. Anal. calcd. for $C_{21}H_{19}N_3O_4S$ (409.46) C, 61.60; H, 4.68; N, 10.26. Found C, 61.55; H, 4.69; N, 10.29.

*4.1.2.10. 3-(1-Acetyl-5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl sulfamate (**9a**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **7a**. Yield 28%. M.p. 103-105 °C. ^1H NMR (DMSO-d_6) 2.33 (s, 3H, CH_3), 3.08 (dd, *J* = 13.0, 3.5 Hz, 1H, CH), 3.88 (dd, *J* = 14.5, 3.5 Hz, 1H, CH), 5.54 (s, 2H, NH_2), 5.57 (dd, *J* = 14.5, 3.0 Hz, 1H, CH), 6.88 (d, *J* = 8.0 Hz, 1H, Ar), 6.83 (d, *J* = 8.5 Hz, 1H, Ar), 7.26 (m, 1H, Ar), 7.55 (m, 3H, Ar), 7.88 (m, 2H, Ar), 8.05 (s, 1H, Ar). IR (Nujol) 3261, 1615 cm^{-1}. m/z 360 $(m+H)^+$. Anal. calcd. for $C_{17}H_{17}N_3O_4S$ (359.40) C, 56.81; H, 4.77; N, 11.69. Found C, 56.86; H, 4.75; N, 11.72.

*4.1.2.11. 3-(1-Acetyl-5-(p-tolyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl sulfamate (**9b**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **7b**. Yield 88%. M.p. 117-118 °C. ^1H NMR (DMSO-d_6) 2.17 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 3.17 (dd, *J* = 13.0, 3.5 Hz, 1H, CH), 3.89 (dd, *J* = 13.5, 3.5 Hz, 1H, CH), 5.21 (s, 2H, NH_2), 5.33 (dd, *J* = 14.5, 3.0 Hz, 1H, CH), 6.70 (d, *J* = 8.5 Hz, 1H, Ar), 6.88 (d, *J* = 8.5 Hz, 1H, Ar), 7.12 (m, 1H, Ar), 7.41 (d, J = 7.0 Hz, 2H, Ar), 7.84 (d, J = 7.0 Hz, 2H, Ar), 8.09 (s, 1H, Ar). IR (Nujol) 3246, 1614 cm^{-1}. m/z 374 $(m+H)^+$. Anal. calcd. for $C_{18}H_{19}N_3O_4S$ (373.43) C, 57.89; H, 5.13; N, 11.25. Found C, 57.93; H, 5.11; N, 11.22.

*4.1.2.12. 3-(1-Acetyl-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl sulfamate (**9c**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **7c**. Yield 42%. M.p. 116-118°C. ^1H NMR (DMSO-d_6) 2.28 (s, 3H, CH_3), 3.12 (dd, *J* = 13.0, 3.5 Hz, 1H, CH), 3.72 (s, 3H, OCH_3), 3.79 (dd, *J* = 13.5, 3.5 Hz, 1H, CH), 3.90 (s, 2H, NH_2), 5.49 (dd, *J* = 14.5, 3.0 Hz, 1H, CH), 6.88 (d, *J* = 8.5 Hz, 1H, Ar), 7.04 (d, *J* = 8.5 Hz, 1H, Ar), 7.12 (m, 1H, Ar), 7.27 (d, *J* = 7.0 Hz, 2H, Ar), 7.71 (d, *J* = 7.0 Hz, 2H, Ar), 8.06 (s, 1H, Ar). IR (Nujol) 3246, 1614 cm^{-1}. m/z 390 $(m+H)^+$. Anal. calcd. for $C_{18}H_{19}N_3O_5S$ (389.43) C, 55.52; H, 4.92; N, 10.79. Found C, 55.59; H, 4.91; N, 10.77.

*4.1.2.13. 3-(1-Acetyl-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl sulfamate (**9d**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **7d**. Yield 47%. M.p. 203-205°C. ^1H NMR (DMSO-d_6) 2.32 (s, 3H, CH_3), 3.11 (dd, *J* = 13.0, 3.5 Hz, 1H, CH), 3.17 (dd, *J* = 13.5, 3.5 Hz, 1H, CH), 3.87 (s, 2H, NH_2), 5.68 (dd, *J* = 14.5, 3.0 Hz, 1H, CH), 6.89 (d, *J* = 8.5 Hz, 1H, Ar), 7.17 (d, *J* = 8.5 Hz, 1H, Ar), 7.25 (m, 1H, Ar), 7.49 (d, *J* = 7.0 Hz, 2H, Ar), 7.57 (d, *J* = 7.0 Hz, 2H, Ar), 8.20 (s, 1H, Ar). IR (Nujol) 3239, 1634 cm^{-1}. m/z 405 $(m+H)^+$. Anal. calcd. for $C_{17}H_{16}N_4O_6S$ (404.40) C, 50.49; H, 3.99; N, 13.85. Found C, 50.56; H, 4.01; N, 13.81.

*4.1.2.14. 3-(1-Acetyl-5-(4-(trifluoromethyl)phenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl sulfamate (**9e**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **7e**. Yield 40%. M.p. 114-115 °C. ^{1}H NMR (DMSO-d_6) 2.34 (s, 3H, CH_3), 3.04 (dd, *J* = 14.0, 3.0 Hz, 1H, CH), 3.59 (dd, *J* = 14.5, 3.5 Hz, 1H, CH), 3.98 (s, 2H, NH_2), 5.69 (dd, *J* = 13.5, 3.0 Hz, 1H, CH), 6.88 (d, *J* = 7.5 Hz, 1H, Ar), 7.17 (d, *J* = 7.0 Hz, 1H, Ar), 7.28 (m, 1H, Ar), 7.45 (d, *J* = 8.5 Hz, 2H, Ar), 7.88 (d, *J* = 8.9 Hz, 2H, Ar), 7.98 (s, 1H, Ar). IR (Nujol) 3209, 1642, 1584 cm^{-1}. m/z 428 (m+H)$^+$. Anal. calcd. for $C_{18}H_{16}F_3N_3O_4S$ (427.40) C, 50.58; H, 3.77; N, 9.83. Found C, 50.64; H, 3.76; N, 9.80.

*4.1.2.15. 3-(1-Acetyl-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl sulfamate (**9f**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **7f**. Yield 27%. M.p. 94-95 °C. ^{1}H NMR (DMSO-d_6) 2.29 (s, 3H, CH_3), 3.12 (dd, *J* = 14.0, 3.0 Hz, 1H, CH), 3.74 (dd, *J* = 14.5, 3.5 Hz, 1H, CH), 3.88 (s, 2H, NH_2), 5.54 (dd, *J* = 13.5, 3.0 Hz, 1H, CH), 6.87 (d, *J* = 7.5 Hz, 1H, Ar), 7.15 (d, *J* = 7.9 Hz, 1H, Ar), 7.22 (m, 1H, Ar), 7.39 (d, *J* = 8.5 Hz, 2H, Ar), 7.55 (d, *J* = 8.0 Hz, 2H, Ar), 8.06 (s, 1H, Ar). IR (Nujol) 3264, 1607 cm^{-1}. m/z 378 (m+H)$^+$. Anal. calcd. for $C_{17}H_{16}FN_3O_4S$ (377.39) C, 54.10; H, 4.27; N, 11.13. Found C, 54.04; H, 4.28; N, 11.17.

*4.1.2.16. 3-(1-Acetyl-5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl sulfamate (**9g**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **7g**. Yield 54%. M.p. 121-123 °C. ^{1}H NMR (DMSO-d_6) 2.35 (s, 3H, CH_3), 2.96 (dd, *J* = 14.0, 3.0 Hz, 1H, CH), 3.93 (dd, *J* = 14.5, 3.5 Hz, 1H, CH), 4.88 (s, 2H, NH_2), 5.77 (dd, *J* = 13.5, 3.0 Hz, 1H, CH), 6.87 (d, *J* = 7.5 Hz, 1H, Ar), 7.08 (d, *J* = 7.0 Hz, 1H, Ar), 7.22 (m, 1H, Ar), 7.38 (d, *J* = 7.5 Hz, 2H, Ar), 7.72 (d, *J* = 8.0 Hz, 2H, Ar), 8.08 (s, 1H, Ar). IR (Nujol) 3237, 1642, 1574 cm^{-1}. m/z 394 (m+H)$^+$. Anal. calcd. for $C_{17}H_{16}ClN_3O_4S$ (393.84) C, 51.84; H, 4.09; N, 10.67. Found C, 51.79; H, 4.11; N, 10.69.

*4.1.2.17. 3-(1-Acetyl-5-(3-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl sulfamate (**9h**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **7h**. Yield 44%. M.p. 96-98 °C. ^{1}H NMR (DMSO-d_6) 2.34 (s, 3H, CH_3), 3.14 (dd, *J* = 3.0, 14.0 Hz, 1H, CH), 3.64 (dd, *J* = 14.5, 3.5 Hz, 1H, CH), 3.82 (s, 2H, NH_2), 5.58 (dd, *J* = 13.5, 3.0 Hz, 1H, CH), 7.16 (d, *J* = 7.5 Hz, 1H, Ar), 7.27 (d, *J* = 7.0 Hz, 1H, Ar), 7.39 (s, 1H, Ar), 7.55 (d, *J* = 7.5 Hz, 2H, Ar), 7.72 (d, *J* = 8.0 Hz, 2H, Ar), 8.05 (s, 1H, Ar). IR (Nujol) 3262, 1614, 1574 cm^{-1}. m/z 394 (m+H)$^+$. Anal. calcd. for $C_{17}H_{16}ClN_3O_4S$ (393.84) C, 51.84; H, 4.09; N, 10.67. Found C, 51.88; H, 4.08; N, 10.71.

*4.1.2.18. 3-(1-Acetyl-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl sulfamate (**9i**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **7i**. Yield 72%. M.p. 108-110 °C. ^{1}H NMR (DMSO-d_6) 2.29 (s, 3H, CH_3), 2.95 (dd, *J* = 14.0, 3.0, Hz, 1H, CH), 3.80 (dd, *J* = 14.5, 3.5 Hz, 1H, CH), 3.91 (s, 2H, NH_2), 5.54 (dd, *J* = 13.5, 3.0 Hz, 1H, CH), 6.88 (d, *J* = 7.5 Hz, 1H, Ar), 7.16 (d, *J* = 7.0 Hz, 1H, Ar), 7.39 (m, 1H, Ar), 7.55 (d, *J* = 7.5 Hz, 2H, Ar), 7.73 (d, *J* = 8.0 Hz, 2H, Ar), 8.06 (s, 1H, Ar). IR (Nujol) 3265, 1615 cm^{-1}. m/z 394 (m+H)$^+$. Anal. calcd. for $C_{17}H_{16}ClN_3O_4S$ (393.84) C, 51.84; H, 4.09; N, 10.67. Found C, 51.79; H, 4.08; N, 10.70.

*4.1.2.19. 3-(1-Acetyl-5-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl sulfamate (**9j**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **7j**. Yield 22%. M.p. 105-107 °C. ^{1}H NMR (DMSO-d_6) 2.33 (s, 3H, CH_3), 3.14 (dd, *J* = 14.0, 4.0, Hz, 1H, CH), 3.88 (dd, *J* = 13.5, 3.5 Hz, 1H, CH), 4.01 (s, 2H, NH_2), 5.78 (dd, *J* = 13.5, 3.0 Hz, 1H, CH), 6.87 (d, *J* = 7.5 Hz, 1H, Ar), 7.11 (d, *J* = 7.0 Hz, 1H, Ar), 7.38 (m, 2H, Ar), 7.67 (m, 2H, Ar), 8.05 (s, 1H, Ar). IR (Nujol) 3220, 1640, 1584 cm^{-1}. m/z 428 (m+H)$^+$. Anal. calcd. for $C_{17}H_{15}Cl_2N_3O_4S$ (428.29) C, 47.67; H, 3.53; N, 9.81. Found C, 47.71; H, 3.52; N, 9.78.

*4.1.2.20. 3-(1-Acetyl-5-(2,5-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl sulfamate (**9k**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **7k**. Yield 88%. M.p. 160-161 °C. ^{1}H NMR (DMSO-d_6) 2.37 (s, 3H, CH_3), 3.18 (dd, *J* = 14.0, 4.0 Hz, 1H, CH), 3.97 (dd, *J* = 13.5, 3.5 Hz, 1H, CH), 4.57 (s, 2H, NH_2), 5.77 (dd, *J* = 13.5, 3.0 Hz, 1H, CH), 7.11 (d, *J* = 7.5 Hz, 1H, Ar), 7.55 (d, *J* = 7.0 Hz, 1H, Ar), 7.69 (m, 2H, Ar), 7.73 (m, 2H, Ar), 8.06 (s, 1H, Ar). IR (Nujol) 3190, 1647, 1572 cm^{-1}. m/z 428 (m+H)$^+$. Anal. calcd. for $C_{17}H_{15}Cl_2N_3O_4S$ (428.29) C, 47.67; H, 3.53; N, 9.81. Found C, 47.62; H, 3.54; N, 9.77.

*4.1.2.21. 3-(1-Acetyl-5-(2,6-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl sulfamate (**9l**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **7l**. Yield 41%. M.p. 129-130 °C. ^{1}H NMR (DMSO-d_6) 2.25 (s, 3H, CH_3), 3.22 (dd, *J* = 14.0, 4.0 Hz, 1H, CH), 3.72 (dd, *J* = 13.5, 3.5 Hz, 1H, CH), 3.88 (s, 2H, NH_2), 6.07 (dd, *J* = 13.5, 3.0 Hz, 1H, CH), 6.14 (d, *J* = 7.5 Hz, 1H, Ar), 6.89 (d, *J* = 7.0 Hz, 1H, Ar), 7.28 (m, 1H, Ar), 7.33 (d, *J* = 7.5 Hz, 1H, Ar), 7.71 (d, *J* = 8.0 Hz, 1H, Ar), 8.09 (s, 1H, Ar). IR (Nujol) 3230, 1642, 1576 cm^{-1}. m/z 428 (m+H)$^+$. Anal. calcd. for $C_{17}H_{15}Cl_2N_3O_4S$ (428.29) C, 47.67; H, 3.53; N, 9.81. Found C, 47.72; H, 3.54; N, 9.78.

*4.1.2.22. 3-(1-Acetyl-5-(naphthalen-1-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl sulfamate (**9m**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **7m**. Yield 79%. M.p. 118-120 °C. ^{1}H NMR (DMSO-d_6) 2.42 (s, 3H, CH_3), 2.96 (dd, *J* = 14.0, 4.0 Hz, 1H, CH), 3.04 (s, 2H, NH_2), 4.12 (dd, *J* = 13.5, 4.0 Hz Hz, 1H, CH), 6.27 (dd, *J* = 13.5, 3.5 Hz, 1H, CH), 6.87 (d, *J* = 8.0 Hz, 2H, Ar), 7.22 (d, *J* = 8.5 Hz, 2H, Ar), 7.45 (m, 3H, Ar), 7.64 (m, 2H, Ar), 7.87 (m, 2H, Ar), 8.16 (s, 1H, Ar). IR (Nujol) 3213, 1640 cm^{-1}. m/z 410 (m+H)$^+$. Anal. calcd. for $C_{21}H_{19}N_3O_4S$ (409.46) C, 61.60; H, 4.68; N, 10.26. Found C, 61.67; H, 4.69; N, 10.23.

*4.1.2.23. 4-(1-Acetyl-3-phenyl-4,5-dihydro-1H-pyrazol-5-yl)phenyl sulfamate (**17a**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **15a**. Yield 19%. M.p. 124-125 °C. ^{1}H NMR (DMSO-d_6) 2.29 (s, 3H, CH_3), 3.13 (dd, *J* = 14.0, 4.0 Hz, 1H, CH), 3.87 (dd, *J* = 13.5, 4.0 Hz, 1H, CH), 4.62 (s, 2H, NH_2), 5.61 (dd, *J* = 13.5, 3.5 Hz, 1H, CH), 7.07 (d, *J* = 8.0 Hz, 2H, Ar), 7.23 (d, *J* = 8.5 Hz, 2H, Ar), 7.48 (m, 3H, Ar), 7.98 (m, 2H, Ar). IR (Nujol) 3196, 1596 cm^{-1}. m/z 360 (m+H)$^+$. Anal. calcd. for $C_{17}H_{17}N_3O_4S$ (359.40) C, 56.81; H, 4.77; N, 11.69. Found C, 56.76; H, 4.76; N, 11.73.

*4.1.2.24. 4-(1-acetyl-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-5-yl)phenyl sulfamate (**17b**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **15b**. Yield 47%. M.p. 126-127 °C. ^{1}H NMR (DMSO-d_6) 2.27 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 3.14 (dd, *J* = 14.0, 3.5 Hz, 1H, CH), 3.87 (dd, *J* =13.5, 3.5 Hz, 1H, CH), 4.23 (s, 2H, NH_2), 5.57 (dd, *J* = 13.5, 3.0 Hz, 1H, CH), 6.69 (d, *J* = 8.0 Hz, 2H, Ar), 6.98 (d, *J* = 8.0 Hz, 2H, Ar), 7.37 (d, *J* = 7.5 Hz, 2H, Ar), 7.90 (d, *J* = 7.0 Hz, 2H, Ar). IR (Nujol) 3346, 1637, 1607 cm^{-1}. m/z 374 (m+H)$^+$. Anal. calcd. for $C_{18}H_{19}N_3O_4S$ (373.43) C, 57.89; H, 5.13; N, 11.25. Found C, 57.96; H, 5.11; N, 11.22.

*4.1.2.25. 4-(1-Acetyl-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenyl sulfamate (**17c**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **15c**. Yield 30%. M.p. 177-178 °C. ^{1}H NMR (DMSO-d_6) 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, NH2). IR (Nujol) 3321, 3167, 1637, 1568 cm^{-1}. m/z 390 (M + H)$^+$. Anal. calcd. for $C_{18}H_{19}N_3O_5S$ (389.10) C, 55.52; H, 4.92; N, 10.79. Found C, 55.58; H, 4.90; N, 10.76.

*4.1.2.26. 4-(1-Acetyl-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenyl sulfamate (**17d**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **15d**. Yield 60%. M.p. 134-135 °C. ^{1}H NMR (DMSO-d_6) 2.31 (s, 3H, CH_3), 2.95 (s, 2H, NH_2), 3.13 (dd, *J* = 13.0, 3.0 Hz, 1H, CH), 3.86 (dd, *J* = 14.0 , 4.0 Hz, 1H, CH), 5.57 (dd, *J* = 13.0, 3.5 Hz, 1H, CH), 6.72 (d, *J* = 8.5 Hz, 2H, Ar), 7.19 (d, *J* = 8.0 Hz, 2H, Ar), 8.13 (d, *J* = 7.0 Hz, 2H, Ar), 8.33 (d, J = 7.5 Hz, 2H, Ar). IR (Nujol) 3203, 1643, 1605 cm^{-1}. m/z 405 (M + H)$^+$. Anal. calcd. for $C_{17}H_{16}N_4O_6S$ (404.40) C, 50.49, H, 3.99, N, 13.85. Found C, 50.53, H, 4.01; N 13.81.

*4.1.2.27. 4-(1-Acetyl-3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenyl sulfamate (**17f**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **15f**. Yield 61%. M.p. 118-120 °C. ^{1}H NMR (DMSO-d_6) 2.27 (s, 3H, CH_3), 3.14 (dd, *J* = 14.0, 3.5 Hz, 1H, CH), 3.83 (dd, *J* = 13.5, 3.5 Hz, 1H, CH), 4.47 (s, 2H, NH_2), 5.57 (dd, *J* = 13.5, 3.0 Hz, 1H, CH), 6.71 (d, *J* = 8.0 Hz, 2H, Ar), 7.09 (d, *J* = 8.0 Hz, 2H, Ar), 7.54 (d, J = 7.5 Hz, 2H, Ar), 7.93 (d, J = 8.0 Hz, 2H, Ar). IR (Nujol) 3187, 1640, 1592 cm^{-1}. m/z 394 (m+H)$^+$. Anal. calcd. for $C_{17}H_{16}ClN_3O_4S$ (393.84) C, 51.84; H, 4.09; N, 9.00. Found C, 51.79; H, 4.08; N, 9.04.

*4.1.2.28. 4-(1-Acetyl-3-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenyl sulfamate (**17g**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **15g**. Yield 77%. M.p. 114-115 °C. ^{1}H NMR (DMSO-d_6) 2.26 (s, 3H, CH_3), 3.17 (dd, *J* = 14.0, 4.0 Hz, 1H, CH), 3.76 (s, 2H, NH_2), 3.89 (dd, *J* = 13.5, 3.5 Hz, 1H, CH), 5.45 (dd, *J* = 13.5, 3.0 Hz, 1H, CH), 6.71 (d, *J* = 8.5 Hz, 2H, Ar), 7.02 (d, *J* = 8.0 Hz, 2H, Ar), 7.62 (d, *J* = 7.5 Hz, 1H, Ar), 7.78 (d, *J* = 8.0 Hz, 1H, Ar), 7.82 (s, 1H, Ar). IR (Nujol) 3271, 1646, 1587 cm^{-1}. m/z 428 (m+H)$^+$. Anal. calcd. for $C_{17}H_{15}Cl_2N_3O_4S$ (428.29) C, 47.67; H, 3.53; N, 9.81. Found C, 47.63; H, 3.52; N, 9.84.

*4.1.2.29. 4-(1-acetyl-3-(3,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenyl sulfamate (**17h**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **15h**. Yield 66% M.p. 123-125 °C. ^{1}H NMR (DMSO-d_6) 2.29 (s, 3H, CH_3), 3.14 (dd, *J* = 14.0, 4.5 Hz, 1H), CH, 3.81 (dd, *J* = 13.5, 3.5 Hz, 1H, CH), 4.21 (s, 2H, NH_2), 5.47 (dd, *J* = 13.5, 3.0 Hz, 1H, CH), 6.69 (d, *J* = 8.0 Hz, 2H, Ar), 7.08 (d, *J* = 8.0 Hz, 2H, Ar), 7.73 (d, *J* = 7.5 Hz, 1H, Ar), 7.81 (d, *J* = 8.0 Hz, 1H, Ar), 7.98 (s, 1H, Ar). IR (Nujol) 3250, 1645,

1594 cm^{-1}. m/z 428 $(m+H)^+$. Anal. calcd. for $C_{17}H_{15}Cl_2N_3O_4S$ (428.29) C, 47.67; H, 3.53; N, 9.81. Found C, 47.73; H, 3.54; N, 9.78.

*4.1.2.30. 3-(1-Acetyl-3-phenyl-4,5-dihydro-1H-pyrazol-5-yl)phenyl sulfamate (**18a**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **16a**. Yield 51%. M.p. 94-95 °C. ^{1}H NMR (DMSO-d_6) 2.22 (s, 3H, CH_3), 3.11 (dd, *J* = 13.0, 3.5 Hz, 1H, CH), 3.87 (dd, J =14.5, 3.5 Hz, 1H, CH), 5.01 (s, 2H, NH_2), 5.12 (dd, *J* = 14.5, 3.0 Hz, 1H, CH), 6.71 (d, *J* = 8.0 Hz, 1H, Ar), 6.83 (d, *J* = 8.5 Hz, 1H, Ar), 7.26 (m, 1H, Ar), 7.55 (m, 3H, Ar), 7.88 (m, 2H, Ar). IR (Nujol) 3200, 1598 cm^{-1}. m/z 360 $(m+H)^+$. Anal. calcd. for $C_{17}H_{17}N_3O_4S$ (359.40) C, 56.81; H, 4.77; N, 11.69. Found C, 56.86; H, 4.76; N, 11.65.

*4.1.2.31. 3-(1-Acetyl-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-5-yl)phenyl sulfamate (**18b**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **16b**. Yield 88%. M.p. 108-110 °C. ^{1}H NMR (DMSO-d_6) 2.17 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 3.17 (dd, *J* = 13.0, 3.5 Hz, 1H, CH), 3.89 (dd, *J* = 13.5, 3.5 Hz, 1H, CH), 5.21 (s, 2H, NH_2), 5.33 (dd, *J* = 14.5, 3.0 Hz, 1H, CH), 6.70 (d, *J* = 8.5 Hz, 1H, Ar), 6.88 (d, *J* = 8.5 Hz, 1H, Ar), 7.12 (m, 1H, Ar), 7.41 (d, *J* = 7.0 Hz, 2H, Ar), 7.84 (d, *J* = 7.0 Hz, 2H, Ar). IR (Nujol) 3250, 1609 cm^{-1}. m/z 374 $(m+H)^+$. Anal. calcd. for $C_{18}H_{19}N_3O_4S$ (373.43) C, 57.89; H, 5.13; N, 11.25; Found C, 57.84; H, 5.15; N, 11.29.

*4.1.2.32. 3-(1-Acetyl-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenyl sulfamate (**18d**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **16d**. Yield 47%. M.p. 174-175 °C. ^{1}H NMR (DMSO-d_6) 2.23 (s, 3H, CH_3), 3.04 (dd, J = 14.0, 3.0 Hz, 1H, CH), 3.88 (dd, *J* = 14.5, 3.5 Hz, 1H, CH), 4.58 (s, 2H, NH_2), 5.44 (dd, *J* = 13.5, 3.0 Hz, 1H, CH), 6.79 (d, *J* = 7.5 Hz, 1H, Ar), 7.09 (d, *J* = 7.0 Hz, 1H, Ar), 7.23 (m, 1H, Ar), 7.47 (d, *J* = 8.5 Hz, 2H, Ar), 7.90 (d, *J* = 8.5 Hz, 2H, Ar). IR (Nujol) 3252, 1650, 1605 cm^{-1}. m/z 405 $(m+H)^+$. Anal. calcd. for $C_{17}H_{16}N_4O_6S$ (404.08) C, 50.49; H, 3.99; N, 13.85. Found C, 50.554; H, 4.01; N, 13.81.

*4.1.2.38. 4-(1-Acetyl-3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenyl sulfamate (**18e**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **16e**. Yield 40%. M.p. 190-191 °C. ^{1}H NMR (DMSO-d_6) 2.32 (s, 3H, CH_3), 3.18 (dd, *J* = 18.0, 4.0 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) 3325, 1638, 1608 cm^{-1}. m/z 378 $(m+H)^+$. Anal. calcd. for $C_{17}H_{16}FN_3O_4S$ (377.39) C, 54.10; H, 4.27; N, 11.13. Found C, 54.05; H, 4.28; N, 11.17.

*4.1.2.39. 3-(1-Acetyl-3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenyl sulfamate (**18f**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **16f**. Yield 69%. M.p. 93-95 °C. ^{1}H NMR (DMSO-d_6) 2.18 (s, 3H, CH_3), 3.08 (dd, J = 14.0, 3.0 Hz, 1H, CH), 4.01 (dd, *J* = 14.5, 3.5 Hz, 1H, CH), 4.69 (s, 2H, NH_2), 5.08 (dd, *J* = 13.5, 3.0 Hz, 1H, CH), 6.71 (d, J = 7.5 Hz, 1H, Ar), 6.78 (d, J = 7.0 Hz, 1H, Ar), 7.22 (m, 1H, Ar), 7.36 (d, J = 7.5 Hz, 2H, Ar), 7.68 (d, J = 8.0 Hz, 2H, Ar), 7.88 (s, 1H, Ar). IR (Nujol) 3222, 1673, 1589 cm^{-1}. m/z 394 $(m+H)^+$. Anal. calcd. for $C_{17}H_{16}ClN_3O_4S$ (393.84) C, 51.84; H, 4.09; N, 10.67. Found C, 51.80; H, 4.11; N, 10.70.

*4.1.2.40. 3-(1-Acetyl-3-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenyl sulfamate (**18g**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **16g**. Yield 77%. M.p. 124-125 °C. ^{1}H NMR (DMSO-d_6) 2.29 (s, 3H, CH_3), 3.18 (dd, *J* = 14.0, 4.0 Hz, 1H, CH), 3.96 (dd, *J* = 13.5, 3.5 Hz, 1H, CH), 4.57 (s, 2H, NH_2), 5.03 (dd, *J* = 13.5, 3.0 Hz, 1H, CH), 6.64 (d, *J* = 7.5 Hz, 1H, Ar), 6.88 (d, *J* =7.5 Hz, 1H, Ar), 7.13 (m, 1H, Ar), 7.19 (d, *J* = 7.5 Hz, 1H, Ar), 7.53 (d, *J* = 8.0 Hz, 1H, Ar), 7.77 (s, 1H, Ar). IR (Nujol) 3238, 1684, 1583 cm^{-1}. m/z 428 $(m+H)^+$. Anal. calcd. for $C_{17}H_{15}Cl_2N_3O_4S$ (428.29) C, 47.67; H, 3.53; N, 9.81. Found C, 47.71; H, 3.54; N, 9.79.

*4.1.2.41. 3-(1-Acetyl-3-(3,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenyl sulfamate(**18h**)*

Following the general procedure, the title compound was prepared starting from pyrazoline **16h**. Yield 70%. M.p. 108-110 °C. ^{1}H NMR (DMSO-d_6) 2.23 (s, 3H, CH_3), 3.22 (dd, *J* = 14.0, 4.0 Hz, 1H, CH), 4.01 (dd, *J* =13.5, 3.5 Hz, 1H, CH), 5.13 (dd, *J* = 13.5, 3.0 Hz, 1H, CH), 5.36 (s, 2H, NH_2), 6.46 (d, *J* = 7.0 Hz, 1H, Ar), 6.72 (d, *J* =7.0 Hz, 1H, Ar), 7.28 (m, 1H, Ar), 7.49 (d, *J* = 7.5 Hz, 1H, Ar), 7.75 (d, *J* = 8.0 Hz, 1H, Ar), 7.95 (s, 1H, Ar). IR (Nujol) 3241, 1679, 1584 cm^{-1}. m/z 428 $(m+H)^+$. Anal. calcd. for $C_{17}H_{15}Cl_2N_3O_4S$ (428.29) C, 47.67; H, 3.53; N, 9.81. Found C, 47.62; H, 3.52; N, 9.85.

4.2. Carbonic anhydrase inhibition

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity [50]. 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 10% DMSO aqueous solution 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 [56-58], and represent the mean from at least three different determinations. All CA isofoms were recombinant ones obtained in-house as reported earlier [59-62].

4.3. Molecular modeling

Molecular Docking studies were carried out to try to understand the binding mechanism of our compounds using rDock [63].

The four crystal structures of CAI (PDBID: 3w6h), CAII (PDBID: 4g0c), CAIX (PDBID: 3iai), CAXII (PDBID: 1jd0) were retrieved from RCSB Protein Data Bank web page (http://www.rcsb.org/). The preparation of the proteins was performed using HTMD (High Throughput MD) tool [64], in order to add hydrogens, ionize side chain of amino acids at physiological pH using propKa, deleting water molecules and co-crystallized small molecules (AAZ included).

3D ligands were prepared using an in-house python script developed using RDKit toolkit [65] and minimized using MMFF94 forcefield.

Considering the high conservation of the binding mode of the sulfamide moiety of co-crystallized compounds a tethered docking was executed [63] to enforce the partial binding modes of the sulfamates group. The 'dock_solv' rDock protocol was executed, this protocol allows a full docking search but using the desolvation scoring function, the docking grid was centered on the co-crystalized ligand and the radius settled at 5.0 Å. For each ligand, the number of possible poses was set to 10 and the one with the best score was used to evaluate its binding interactions.

Protocol validation was carried out with re-docking procedure, AAZ 3D structure was prepared and docked on the four crystals as the other ligands, then the resulting poses were compared with the crystallographic and RMSD have been calculated (Table 2).

Table 2. RMSD and SCORE of the best pose of AAZ redocked on the four CA crystals

	CAI	CAII	CAIX	CAXII
RMSD	1.54	1.62	0.76	0.67
SCORE	-21.28	-23.67	-23.90	-25.75

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Conflict of interest

The authors declare no conflict of interest.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at…….

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