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An efficient method for the synthesis of novel derivatives 4-{5-[4-(4-amino-5-mercapto-4*H*-[1,2,4]triazol-3-yl)-phenyl]-3-trifluoromethyl-pyrazol-1-yl}-benzenesulfonamide and their anti-inflammatory potential

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ABSTRACT

The present work describe the synthesis of a novel series of celecoxib derivatives (**6a-m**) and they were evaluated as Carbonic Anhydrase (CA, EC 4.2.1.1) inhibitors against the human (h) isoforms hCA I, II, IV and IX which are involved in a variety of diseases such as glaucoma, retinitis pigmentosa, epilepsy and tumors etc. These compounds showed interesting inhibitory activity for these isoforms, with several low nanomolar derivatives identified against all these enzymes. The *in-vivo* anti-inflammatory activity of the synthesized compounds were evaluated using Celecoxib as reference standard by paw Oedema model on albino Wistar. Most of the compounds showed higher *in-vivo* anti-inflammatory activity compared to Celecoxib.

1. Introduction

The heterocyclic ring systems exists in abundance in natural products and act as functional cores in most of synthetic and natural drugs [1]. Among these, five membered ring system with different nitrogen atoms such as azoles, triazoles and pyrazoles exhibit their characteristic medicinal and biological properties independently in their respective compounds [2–4]. The triazole ring systems finding a broad range of applications such as antiphytopathogenic, anticonvulsant and antitubercular agents [3,5–7]. On the other hand, pyrazole derivatives are used as analgesics, anticancer and antimicrobial agents [8–10]. When both of these moieties are combined in the same structure, the newly synthesized compounds are biologically active and behave aggressively against highly resistant bacterial species [11–13] such as triazole derivatives containing mercapto moiety and pyrazole with substituted amino groups at vicinal position showing antiviral [14], antifungal [15] and antibacterial activities cleaving DNA and confirm the antiproliferative activity [16]. Keeping in view the biological and medicinal properties associated to compounds containing 1,2,4-triazoles and pyrazoles moiety, we synthetized a novel series of dual inhibitors of cyclooxygenase (COX) and Carbonic Anhydrase (CA, EC 4.2.1.1) enzymes in order to discover and development new compounds without the toxic side effects associated with the currently used non-steroidal anti-inflammatory drugs (NSAIDs) [17,18]. Indeed, the abnormal expressions of the hCAs I, III, IV and the overexpression of CA IX and XII isoforms are involved in several inflammation processes as well as their related pain symptoms in Rheumatoid arthritis or juvenile idiopathic arthritis diseases [19–21]. In continuation to our efforts on the studies of novel multitarget and polyfunctional drugs [22,23], the results of this study revealed that most the synthesized compounds possess greater anti-inflammatory potential than the reference drug Celecoxib.

2. Results and discussion

2.1. Chemistry

The synthetic route to prepare the intermediate 4-[5-(4-hydrazinocarbonyl-phenyl)-3-trifluoromethyl-pyrazol-1-yl]-benzenesulfonamide (**4**) comprises of oxidation of methyl group of Celecoxib (**1**) by using aqueous alkaline $KMnO_4$ followed by esterification with methanol

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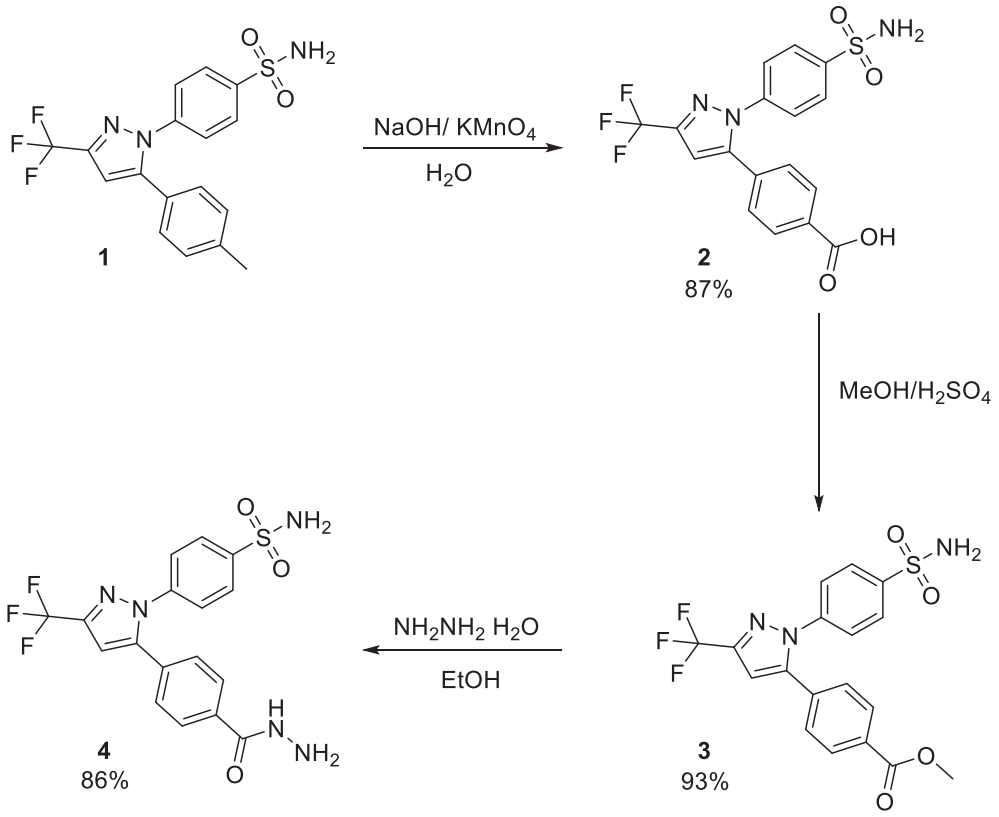


Scheme 1. Synthetic route to afford 4-[5-(4-hydrazinocarbonyl-phenyl)-3-trifluoromethyl-pyrazol-1-yl]-benzenesulfonamide (**4**). [24]

(Scheme 1). The resulting derivative **3** was refluxed with hydrazine hydrate in ethanol to afford compound **4.**

In order to synthetize derivatives of Celecoxib with triazole moiety, compound **4** was reacted with CS_2 and successively with hydrazine to afford derivative **5** (Scheme 2). Finally, compound **5** was refluxed with different aromatic aldehydes to obtain compounds **6a-m**. The characterization of the synthesized compounds was done by using spectroscopic techniques (FT-IR, ^{1}HNMR, ^{13}CNMR and mass spectrometry) along with their elemental analyses and was found in accordance with the calculated values.

2.2. Carbonic anhydrase inhibition

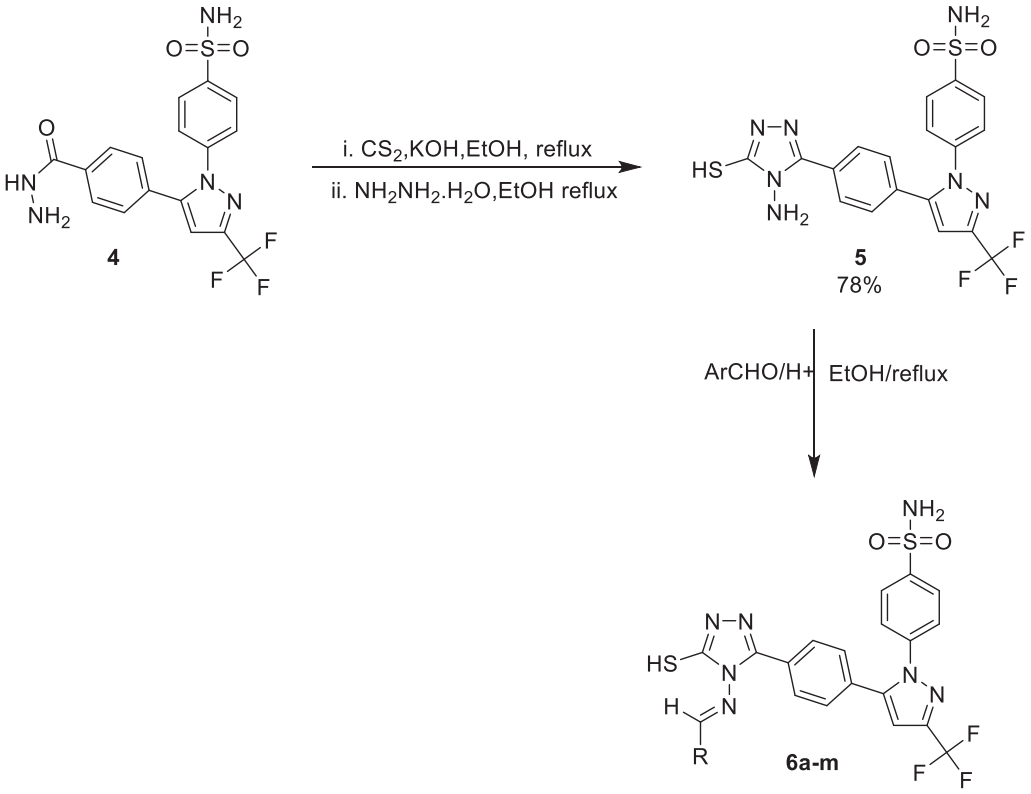
All compounds **1–5** and **6a-m** were tested in *vitro* for their inhibitory activity against the physiologically relevant human CA isoforms I, II, IV and IX by means of the stopped-flow carbon dioxide hydration assay [24] and their activities were compared to the standard Carbonic Anhydrase Inhibitor (CAI) acetazolamide (**AAZ**) (Table 1).

In this work, a novel series of celecoxib derivatives has been synthesized and investigated for their interaction with the four hCA, here considered, after a period of 15 min of incubation of the enzyme and inhibitor solutions [26–31]. The following structure activity relationship (SAR) may be concluded regarding the inhibition data of Table 1:

i) The dominant cytosolic isoform, hCA I, was not inhibited by celecoxib (**1**). On the other hand, further modification on aromatic ring system showed to be crucial for the activity. The addition of a carboxylic moiety (**2**) increased considerably the potency (K_i 35 nM), instead, ester (**3**) or hydrazine (**4**) derivatives decrease their efficacy (K_i 3592 and 172.2 nM, respectively). Further modification of the celecoxib scaffold (**6a-m**), except compound **6d**, decreased the efficacy to micromolar range (K_i 1456–8375 nM).
ii) The second dominant cytosolic isoform, hCA II, was effectively inhibited by compounds **1–4** in medium nanomolar range (K_i 21.0–59.0 nM). This time, further moieties on celecoxib scaffold (**6a-m**) played a fundamental role for the inhibition activity. Derivatives **6c** and **6d** with two chlorine atoms; derivative **6f** with nitro moiety in *para* position and heteroaromatic scaffold such as furfuryl moiety (**6k**) showed the best activity with K_is spanning from 55.4 to 84.6 nM. On the other hand, compounds **6b, g, h** and **6l** showed a medium potency (K_is 511.8–647.2 nM). Finally, the remaining compounds (**6a, e, m**) showed an inhibition constant in the micromolar range (K_is 2662–8083 nM).
iii) The membrane-bound isoform hCA IV was weakly inhibited by compounds **1–5** and the more bulky scaffold of celecoxib derivatives **6a-m** did not show any inhibition activity for most compounds.
iv) Cancer associated isoform hCA IX was inhibited by most of synthesized derivatives in nanomolar range except for **6a** and **6l** exhibited K_is value in low micromolar range. Although, derivatives of celecoxib series (**6a-m**) substituted with different aromatic scaffolds were appeared as effective hCA IX inhibitors, it was noticed that three compounds (**6c**, **6d** and **6k**) are high potency inhibitors for hCA IX (K_is 24.4, 22.8 and 24.4 nM, respectively). Thus, it seems that two chlorine atoms (**6c** and **6d**) or heteroaromatic ring such as furan (**6k**) bestowed some effective hCA IX inhibitors, while only one chlorine atom (**6a**) or nitro moiety (**6l**) failed to produce such action (K_is 1848 and 1266 nM, respectively).

2.3. Anti-inflammatory studies

The synthesized compounds (**6a-m**) were evaluated for in-*vivo* anti-inflammatory activity using single dose carrageenan-induced paw oedema method in Albino Wistar [32]. The results clearly indicate that most of the derivatives have significantly greater inhibition compared with reference drug Celecoxib as reported in Fig. 1.



Compd.	-R	Compd.	-R	Compd.	-R
6a	4-Chlorophenyl	**6f**	4-Nitrophenyl	**6k**	Furfuryl
6b	2,3-Dichorophenyl	**6g**	Phenyl	**6l**	2-Nitrophenyl
6c	2,4-Dichorophenyl	**6h**	2-Chlorophenyl	**6m**	4-*N,N*-dimethylaminophenyl
6d	3,4-Dichorophenyl	**6i**	4-Methoxyphenyl		
6e	4-Bromophenyl	**6j**	2-Hydroxyphenyl		

Scheme 2. Synthetic route to afford derivatives of 4-{5-[4-(4-amino-5-mercapto-4*H*-[1,2,4]triazol-3-yl)-phenyl]-3-trifluoromethyl-pyrazol-1-yl}-benzenesulfonamides **6a-m.**

Table 1
Inhibition data of human CA isoforms I, II, IV and IX with compounds **1–5, 6a-m** and **AAZ** by a stopped flow CO_2 hydrase assay. [25]

K_I (nM)*				
Cmp	hCA I	hCAII	hCA IV	hCA IX
1	> 10,000	21.0	880.0	16.0
2	35.7	59.0	1920	1453
3	3592	56.1	6918	71.5
4	172.2	26.4	5147	157.8
5	610.9	681.6	7674	71.4
6a	4640	3493	> 10,000	1848
6b	2628	641.4	6623	115.9
6c	3787	55.4	> 10,000	24.4
6d	515.5	82.4	> 10,000	22.8
6e	> 10,000	8083	> 10,000	714.5
6f	8375	67.3	7385	120.7
6g	1456	511.8	4202	180.9
6h	2529	597.9	> 10,000	125.3
6i	1761	2602	> 10,000	214.2
6j	5944	7173	> 10,000	504.1
6k	4321	84.6	> 10,000	24.4
6l	5656	647.2	5673	1266
6m	4785	5077	> 10,000	65.5
AAZ	250	12.1	74.0	25.8

* Mean from 3 different assays, by a stopped flow technique (errors were in the range of ± 5–10% of the reported values).

The different substituents on triazole moiety played a crucial role for percentage inhibition of anti-inflammatory effect at the interval of 1hr. In particular, derivatives with chlorine atom (**6a**, **6c**, **6d** and **6h**) and nitro derivative **6f** showed an enhanced of the anti-inflammatory potency. On the other hand, bromine atom on compound **6e**, phenyl (**6g**) and 4-*N,N*-Dimethylaminophenyl (**6m**) moieties decreased their anti-inflammatory effects. At interval of 5hr, most of the compounds showed great anti-inflammatory potency than the reference drug Celecoxib. Only derivatives **6a** and **6e** resulted less efficacious.

3. Conclusions

The Celecoxib derivatives **6a-m** were synthesized and evaluated for their carbonic anhydrase inhibition and anti-inflammatory activity. These compounds were tested on different CA isoforms such as hCA I, II, IV, and hCA IX which are involved in a variety of diseases such as glaucoma, retinitis pigmentosa, epilepsy and tumors. The results showed that compounds **6a-m** increased the inhibition activity against the cytosolic isoform hCA I than Celecoxib and the different moieties modulate significantly the potency against hCA II going to nanomolar range such as for compounds **6c**, **6f** and **6k** to micromolar range for compounds **6e**, **6i** and **6m**. Moreover, most compounds here reported showed anti-inflammatory activity.

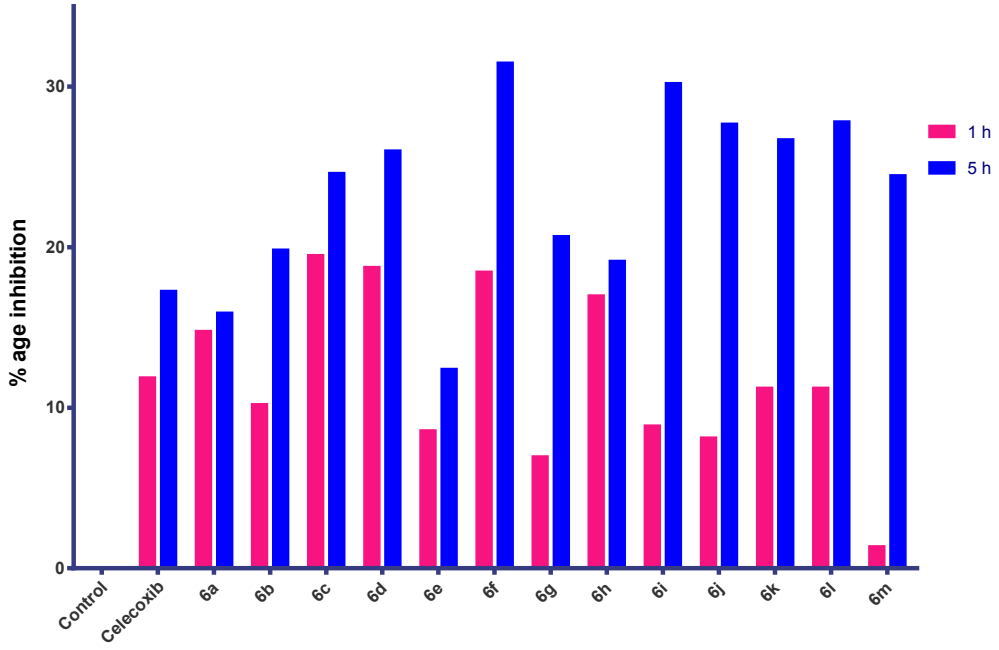


Fig. 1. Percentage inhibition of anti-inflammatory effect of derivatives **6a-m** against carrageenan induced rat paw oedema model in rats (Albino Wistar).

4. Experimental part

4.1. General

The chemicals used in this study were obtained from scharlau, E. Merck and Fluka and were used without further purification. ^{1}H NMR spectra were recorded in DMSO-d_6 on a Bruker DPX-400 instrument at 500 MHz and ^{13}C NMR spectra at 100 MHz. Chemical shifts are reported in ppm referenced to the residual solvent signal. FT-IR spectra were recorded on a Thermo Nicolet IR 200 spectrometer. Mass spectra were recorded on Agilent 5973 N instrument equipped with direct probe EI mode. Melting points were recorded on a Gallenkamp melting point apparatus and are uncorrected.

4.1.1. Synthesis of 4-{1-[4-(aminosulfonyl)phenyl]-3-(trifluoromethyl)-1H-pyrazol-5-yl}benzoic acid (2)

A mixture of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzenesulfonamide (**1**) (0.5 g, 1.31 mmol), sodium hydroxide (0.056 g, 1.31 mmol) and water (100 ml) was stirred at ambient temperature for 20 min followed by addition of potassium permanganate (0.036 g, 2.25 mmol). After completion of the reaction (as indicated by TLC), reaction mixture was filtered off at pump to remove the precipitated manganese dioxide followed by decolorization of the filtrate with sodium metabisulfite solution. Filtrate was acidified to get the precipitated 4-{1-[4-(aminosulfonyl)phenyl]-3-(trifluoromethyl)-1*H*-pyrazol-5-yl}benzoic acid (**2**), which was filtered, washed with distilled water and dried. The precipitates were crystallized from ethanol. White powder; M.p. 246 °C. IR (KBr) cm^{-1}: 2972, 1679, 1090 ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.27 (s, 3H, C*H*$_3$), 7.41 (s, 1H, Ar*H*), 7.49–7.53 (m, 6H, Ar*H*), 7.87(d, J = 8.1 Hz, 2H, N*H*$_2$), 12.81 (s, 1H, O*H*) Anal. Calculated for $C_{17}H_{12}F_3N_3O_4S$: C, 49.64, H, 2.94, N, 10.22; Found: C, 49.6, H, 2.97, N, 10.25; MS m/z [M + H]$^+$: 412.08.

4.1.2. Synthesis of methyl 4-{1-[4-(aminosulfonyl)phenyl]-3-(trifluoromethyl)-1H-pyrazol-5-yl}benzoate (3)

A mixture of 4-{1-[4-(aminosulfonyl)phenyl]-3-(trifluoromethyl)-1*H*-pyrazol-5-yl}benzoic acid (**2**) (5 g; 121.5 mmol), methanol (25 ml) and conc. sulfuric acid (4 drops) was refluxed in microwave oven at 300 MHz for a period of ninety minutes till the completion of reaction (as indicated by TLC). After removing the solvent under vacuum, resultant solids were partitioned with chloroform – water mixture followed by separation of organic layer, which was dried to get the compound **3**.

White powder; M.p. 180 °C. IR (KBr) cm^{-1}: 2972, 1790, 1030, ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.81 (s, 3H, OC*H*$_3$), 7.33 (s, 1H, Ar*H*), 7.43–7.51 (m, 8H, Ar*H*), 7.84(d, J = 8.2 Hz, 2H, N*H*$_2$), Anal. Calculated for $C_{18}H_{14}F_3N_3O_4S$: C, 50.82, H, 3.32, N, 9.88; Found: C, 50.78, H, 3.35, N, 9.84; MS m/z [M + H]$^+$: 426.17.

4.1.3. Synthesis of 4-[5-(4-hydrazinocarbonyl-phenyl)-3-trifluoromethyl-pyrazol-1-yl]-benzenesulfonamide (4)

A mixture of methyl 4-{1-[4-(aminosulfonyl)phenyl]-3-(trifluoromethyl)-1*H*-pyrazol-5-yl}benzoate (**4**) (5 g, 11.75 mmol), hydrazine hydrate (80%; 5 ml) and ethanol (30 ml) was refluxed in a microwave oven till completion of the reaction (as indicated by TLC). Excess of ethanol and hydrazine was removed under vacuum and the crude product was stirred with dilute HCl (at pH ~ 5) for 30 min to obtain white precipitates which were filtered, washed with distilled water and dried in oven.

White powder M.p. 209–210 °C; IR (KBr) cm^{-1}:3272, 2970, 1396, ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 4.48 (s, 2H, N*H*$_2$), 7.25 (s, 1H, Ar*H*), 7.47–7.71 (m, 8H, Ar*H*), 7.75–7.83 (d, J = 8.2 Hz, 2H, N*H*$_2$), 10.01 (s, 1H, N*H*) Anal. Calculated for $C_{17}H_{14}F_3N_5O_3S$: C, 48.00, H, 3.32, N, 16.46; Found: C, 48.05, H, 3.35, N, 16.49; MS m/z [M + H]$^+$: 426.03.

4.1.4. Synthesis of 4-{5-[4-(4-amino-5-mercapto-4H-[1,2,4]triazol-3-yl)-phenyl]-3-trifluoromethyl-pyrazol-1-yl}-benzenesulfonamide (5)

A mixture of 4-[5-(4-hydrazinocarbonyl-phenyl)-3-trifluoromethyl-pyrazol-1-yl]-benzenesulfonamide (**4**) (4.0 g, 9.4 mmol) and potassium hydroxide (1.06 g, 18.81 mmol) was stirred in ethanol (30 ml) for 30 min. Then added to it CS_2 (5 ml) and refluxed for 3hrs. The progress of the reaction was monitored by TLC. The excess of ethanol and CS_2 was removed under reduced pressure to obtain a light yellow solid. This crude product was extracted with Et_2O to remove any unreacted hydrazide. After drying the product, hydrazine hydrate (5 ml) was added to it and refluxed the reaction mixture for 3hrs. After completion of the reaction (as indicated by TLC), the excess of hydrazine was removed by distillation under vacuum. The reaction mixture was treated with dilute H_3PO_4 at pH (5–6) and stirred for 30 min. The precipitates obtained were dried and recrystallized from ethanol. White powder; Yield 78%;

M.p.161 °C. IR (KBr) cm^{-1}: 3274.5 (N—H of sulfonamide), 2542 (S—H), 1332 (S═O), 764 (C—S); ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 5.77 (s, 2H, NH_2), 7.31 (s, 1H, Pyrazol), 7.42 (s, 2H, NH_2), 7.48–7.67 (m, 4H, Ar*H*), 7.84–7.96 (m, 2H, Ar*H*), 8.02–8.10 (m, 2H, Ar*H*), 14.01 (s, 1H, N*H*) ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 106.97, 120.18, 122.31, 126.12, 126.19, 126.45, 126.91, 126.96, 127.37, 128.92, 129.96, 140.95, 142.18, 142.48, 144.24, 144.38, 148.70, 167.26; Anal. Calculated for $C_{18}H_{14}F_3N_7O_2S_2$: C, 44.90, H, 2.93, N, 20.36; Found: C, 44.94, H, 2.89, N, 20.31; MS *m/z* $[M + H]^+$: 481.84.

4.1.5. General procedure for the synthesis of 4-[5-(4-hydrazinocarbonyl-phenyl)-3-trifluoromethyl-pyrazol-1-yl]-benzenesulfonamides (6a-m)

A mixture of 4-{5-[4-(4-amino-5-mercapto-4*H*-[1,2,4]triazol-3-yl)-phenyl]-3-trifluoromethyl-pyrazol-1-yl}-benzenesulfonamide (**5**) (0.2 g, 0.41 mmol), corresponding aldehyde (0.41 mmol), *o*-phosphoric acid (2drps) and ethanol (30 ml) was refluxed till the completion of reaction. The contents were cooled to 5 °C in an ice bath, filtered and the resultant solids were washed with cold ethanol to get the pure product.

4.1.6. 4-[5-(4-{4-[(4-Chloro-benzylidene)-amino]-5-mercapto-4H-[1,2,4] triazol-3-yl}-phenyl)-3-trifluoromethyl-pyrazol-1-yl]-benzenesulfonamide (6a)

White powder; m.p.222 °C. IR (KBr) cm^{-1}: 3267 (N—H of sulfonamide), 2730 (S—H), 1583.4 (HC = N), 1354.9 (S═O), 743.8 (C—S) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 7.35 (s, 1H, Pyrazol) 7.43 (s, 2H, NH_2), 7.46–7.66 (m, 6H, Ar*H*), 7.86–7.97 (m, 6H, Ar*H*), 8.02–8.10 (m, 2H, Ar*H*), 8.78 (s, 1H, *H*C = N), 14.35 (s, 1H, S*H*) ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 106.99, 120.16, 122.29, 126.06, 126.15, 126.19, 126.95, 128.55, 129.02, 129.09, 129.22, 129.47, 130.03, 130.19, 130.39, 130.76, 137.66, 140.89, 142.20, 142.50, 144.23, 147.86, 160.62, 162.47, 165.33; Anal. Calculated for $C_{25}H_{17}ClF_3N_7O_2S_2$: C, 49.7, H, 2.84, N, 16.23; Found: C, 49.75, H, 2.80, N, 16.27; MS *m/z* $[M + H]^+$ 604.20, $[M + H + 2]^+$ 606.21.

*4.1.7. **4**-[5-(4-{4-[(2,3-Dichloro-benzylidene)-amino]-5-mercapto-4H-[1,2,4]triazol-3-yl}-phenyl)-3-trifluoromethyl-pyrazol-1-yl]-benzenesulfonamide (6b)*

Light yellow powder; m.p.245 °C. IR (KBr) cm^{-1}: 3300 (N—H of sulfonamide), 2740 (S—H), 1596 (HC = N), 1342 (S═O), 748 (C—S) cm^{-1}; ^{1}HNMR (DMSO-d_6, 500 MHz) δ: 7.35 (s, 1H, Pyrazol), 7.42 (s, 2H, NH_2), 7.48–7.59 (m, 6H, Ar*H*, NH_2), 7.63–7.89 (m, 5H, Ar*H*), 8.85 (s, 1H, *H*C = N), 14.40 (s, 1H, N*H*) ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 107.01, 120.15, 122.29, 125.47, 125.95, 125.99, 126.15, 126.22, 126.93, 128.22, 128.94, 128.97, 129.11, 130.19, 132.14, 132.91, 133.09, 134.09, 140.90, 142.20, 142.50, 144.23, 148.39, 159.67, 162.29; Anal. Calculated for $C_{25}H_{16}Cl_2F_3N_7O_2S_2$: C, 47.03, H, 2.53, N, 15.36; Found: C, 47.03, H, 2.53, N, 15.36; MS *m/z* $[M + H]^+$ 638.02, $[M + H]^+$640.01, $[M + H]^+$642.19

4.1.8. 4-[5-(4-{4-[(2,4-Dichloro-benzylidene)-amino]-5-mercapto-4H-[1,2,4]triazol-3-yl}-phenyl)-3-trifluoromethyl-pyrazol-1-yl]-benzenesulfonamide (6c)

Light yellow powder; m.p.236 °C. IR (KBr) cm^{-1}: 3287.2 (N—H of sulfonamide), 2730 (S—H), 1586 (HC = N), 1347.4 (S═O), 752 (C—S) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 7.36 (s, 1H, Pyrazol), 7.46 (s, 2H, NH_2), 7.48–7.54 (m, 3H, Ar*H*), 7.55–7.71 (m, 4H, Ar*H*), 7.76–7.82 (m, 3H, Ar*H*), 8.63 (s, 1H, *H*C = N), 14.40 (s, 1H, S*H*),^{13}C NMR (DMSO-d_6, 100 MHz) δ: 106.99, 120.15, 122.29, 126.01, 126.16, 126.94, 128.47, 128.75, 128.77, 128.9, 129.0, 129.11, 129.9, 130.17, 132.24, 136.09, 138.04, 140.89, 142.20, 142.50, 144.24, 144.2, 148.35, 159.07, 162.26; Anal. Calculated for $C_{25}H_{16}Cl_2F_3N_7O_2S_2$: C, 47.03, H, 2.53, N, 15.36; Found: C47.07, H, 2.49, N, 15.31; MS *m/z* $[M + H]^+$ 638.07, $[M + H + 2]^+$ 640.01, $[M + H + 4]^+$ 642.19.

4.1.9. 4-[5-(4-{4-[(3,4-Dichloro-benzylidene)-amino]-5-mercapto-4H-[1,2,4]triazol-3-yl}-phenyl)-3-trifluoromethyl-pyrazol-1-yl]-benzenesulfonamide (6d)

Light yellow powder; m.p.232 °C. IR (KBr) cm^{-1}: 3280 (N—H of sulfonamide), 2738 (S—H), 1598 (HC = N), 1338 (S═O), 743 (C—S); ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 7.28 (s, 1H, Pyrazol), 7.45 (s, 2H, NH_2), 7.46–7.64 (m, 7H, Ar*H*), 7.88–8.01 (m, 4H, Ar*H*), 9.21 (s, 1H *H*C = N), 14.23 (s, 1H, S*H*), ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 107.01, 109.72, 111.69, 120.16, 122.30, 123.34, 124.24, 124.35, 126.12, 126.18, 126.26, 126.94, 128.37, 129.22, 130.12, 140.91, 142.19, 142.49, 144.20, 144.27, 147.58, 149.18, 153.03, 162.54, 166.92; Anal. Calculated for $C_{25}H_{16}Cl_2F_3N_7O_2S_2$: C, 47.03, H, 2.53, N, 15.36; Found: C, 47.07, H, 2.58, N, 15.38; MS *m/z* $[M + H]^+$: 638.91, $[M + H + 2]^+$ 640.90, $[M + H + 2]^+$ 642.93.

4.1.10. 4-[5-(4-{4-[(4-Bromo-benzylidene)-amino]-5-mercapto-4H-[1,2,4]triazol-3-yl}-phenyl)-3-trifluoromethyl-pyrazol-1-yl]-benzenesulfonamide (6e)

Off white powder; m.p. 235 °C. IR (KBr) cm^{-1}: 3291 (N—H of sulfonamide), 2740 (S—H), 1587.8 (HC = N), 1361.0 (S═O), 760 (C—S); ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 7.35 (s, 1H, Pyrazol), 7.41 (s, 2H, NH_2), 7.46–7.66 (m, 6H, Ar*H*), 7.89–7.95 (m, 6H, Ar*H*), 9.32 (s, 1H, *H*C = N), 14.35 (s, 1H, S*H*) ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 106.98. 120.14, 122.28, 126.05, 126.14, 126.68, 126.93, 128.53, 129.20, 130.18, 130.50, 131.09, 132.39, 131.61, 131.89, 135.52, 140.87, 142.18, 142.48, 144.22, 147.85, 153.76, 155.34, 162.46, 165.43; Anal. Calculated for $C_{25}H_{17}BrF_3N_7O_2S_2$: C, 46.30, H, 2.69, N, 15.16; Found: C, 46.34, H, 2.69, N, 15.16; MS *m/z* $[M + H]^+$ 649.74, $[M + H + 2]^+$ 651.75.

4.1.11. 4-[5-(4-{5-Mercapto-4-[(4-nitro-benzylidene)-amino]-4H-[1,2,4] triazol-3-yl}-phenyl) −3-trifluoromethyl-pyrazol-1-yl]-benzenesulfonamide (6f)

Yellow powder; m.p.206 °C. IR (KBr) cm^{-1}: 3130 (N—H of sulfonamide), 2926 (S—H), 1583.4 (HC = N), 1347.4 (S═O), 750 (C—S) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 7.35 (s, 1H, Pyrazol), 7.42–7.57 (m, 4H, Ar*H*), 7.56 (s, 2H, NH_2), 7.63–7.90 (m, 4H, Ar*H*), 8.04–8.17 (m, 2H, Ar*H*), 8.34–8.40 (m, 2H, Ar*H*), 9.41 (s, 1H, *H*C = N), 14.35 (s, 1H, S*H*) ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 106.97, 117.81, 119.93, 120.14, 122.28, 124.32, 125.92, 126.15, 126.93, 128.68, 129.22, 129.80, 130.25, 137.73, 140.34, 140.87, 140.92, 142.19, 142.49, 144.21, 144.2, 148.10, 149.67, 162.44; Anal. Calculated for $C_{25}H_{17}F_3N_8O_4S_2$: C, 48.86, H, 2.79, N, 18.19; Found: C, 48.82, H, 2.79, N, 18.19; MS *m/z* $[M + H]^+$: 615.07

4.1.12. 4-(5-{4-[4-(Benzylidene-amino)-5-mercapto-4H-[1,2,4]triazol-3-yl]-phenyl}-3-trifluoromethyl-pyrazol-1-yl)-benzenesulfonamide (6g)

Light yellow powder; m.p.235 °C. IR (KBr) cm^{-1}: 3347 (N—H of sulfonamide), 2750 (S—H), 1602(HC = N), 1336.3 (S═O), 752.9 (C—S); ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 5.79 (s, 2H, NH_2), 7.35 (s, 1H, Pyrazol), 7.42–7.57 (m, 4H, Ar*H*), 7.59 (s, 2H, NH_2), 7.63–7.90 (m, 4H, Ar*H*), 8.04–8.17 (m, 2H, Ar*H*), 8.34–8.40 (m, 2H, Ar*H*), 9.54 (s, 1H, *H*C = N), 14.35 (s, 1H, S*H*) ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 107.01, 122.30, 126.19, 126.45, 126.94, 126.96, 128.24, 128.51, 128.79, 129.02, 129.21, 129.96, 130.17, 131.86, 132.96, 140.90, 142.48, 144.22, 144.24, 144.38, 147.82, 148.70, 162.49, 166.90, 167.26; Anal. Calculated for $C_{25}H_{18}F_3N_7O_2S_2$: C, 52.72, H, 3.23, N, 17.24; Found: C, 52.75, H, 3.23, N, 17.24; MS *m/z* $[M + H]^+$: 570.12.

4.1.13. 4-[5-(4-{4-[(2-Chloro-benzylidene)-amino]-5-mercapto-4H-[1,2,4]triazol-3-yl}-phenyl)-3-trifluoromethyl-pyrazol-1-yl]-benzenesulfonamide (6h)

Light yellow powder; m.p.235°C. IR (KBr) cm^{-1}: 3205, 3071, 1655, 1546; ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 7.36 (s, 1H, Pyrazol), 7.57 – 7.46 (m, 6H, Ar*H*), 7.54–7.69 (m, 4H, Ar*H*), 7.86–7.98 (m, 4H, Ar*H*), 9.36 (s, 1H, *H*C = N), 14.37 (s, 1H, S*H*) ^{13}C NMR (DMSO-d_6, 100 MHz)

δ: 107.04, 117.81, 120.18, 122.32, 126.11, 126.19, 126.97, 127.69, 128.09, 128.92, 129.14, 129.68, 130.19, 130.43, 134.22, 135.36, 140.94, 142.23, 142.53, 144.26, 144.28, 148.34, 155.45, 160.51, 162.32; Anal. Calculated for $C_{25}H_{17}ClF_3N_7O_2S_2$: C, 49.71, H, 2.84, N, 16.23; Found: C, 49.74, H, 2.81, N, 16.25; MS *m/z* $[M + H]^+$ 604.07, $[M + H + 2]^+$ 6.06

4.1.14. 4-[5-(4-{5-Mercapto-4-[(4-methoxy-benzylidene)-amino]-4H-[1,2,4]triazol-3-yl}-phenyl)-3-trifluoromethyl-pyrazol-1-yl]-benzenesulfonamide (6i)

White powder; m.p.222°C. IR (KBr) cm^{-1}: 3350.9 (N—H of sulfonamide), 2760 (S—H), 1610 (HC = N), 1340.0 (S═O), 743 (C—S); ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 3.85 (s, 3H, OC*H*$_3$), 7.07–7.14 (m, 3H, Pyrazol), 7.33 (s, 2H, N*H*$_2$), 7.38–7.52 (m, 2H, Ar*H*), 7.56 – 7.64 (m, 4H, Ar*H*), 7.79–7.98 (m, 6H, Ar*H*), 9.38 (s, 1H, *H*C = N), 14.27 (s, 1H, S*H*) ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 55.66, 107.03, 114.57, 114.86, 117.81, 120.18, 122.32, 124.30, 126.20, 126.25, 126.28, 126.99, 128.46, 130.14, 129.25, 130.87, 131.89, 140.95, 144.26, 144.30, 147.70, 155.45, 162.58, 163.18, 166.97; Anal. Calculated for $C_{25}H_{18}F_3N_7O_3S_2$: C, 51.28, H, 3.10, N, 16.74; Found: C, 51.32, H, 3.14, N, 16.77; MS *m/z* $[M + H]^+$: 599.65.

4.1.15. 4-[5-(4-{4-[(2-Hydroxy-benzylidene)-amino]-5-mercapto-4H-[1,2,4]triazol-3-yl}-phenyl)-3-trifluoromethyl-pyrazol-1-yl]-benzenesulfonamide (6j)

Light yellow powder; m.p.230°C. IR (KBr) cm^{-1}: 3265.1 (N—H of sulfonamide), 2732 (S—H), 1600.9 (HC = N), 1341.8 (S═O), 758.5 (C—S); ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 4.36 (s, 1H, O*H*), 6.91–7.03 (s, 1H, Pyrazol), 7.35 (s, 2H, N*H*$_2$), 7.51–7.60 (m, 6H, Ar*H*), 7.82–7.91 (m, 6H, Ar*H*), 9.51 (s, 1H, *H*C = N), 14.25 (s, 1H, S*H*) ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 106.99, 116.72, 118.20, 119.74, 120.15, 122.28, 126.11, 126.18, 126.29, 127.19, 128.23, 128.53, 129.00, 129.14, 130.0, 134.51, 140.90, 142.17, 142.47, 144.20, 144.25, 147.82, 158.59, 162.46, 163.18; Anal. Calculated for $C_{25}H_{18}F_3N_7O_3S_2$: C, 51.28, H, 3.10, N, 16.74; Found: C, 51.32, H, 3.14, N, 16.77; MS *m/z* $[M + H]^+$: 585.40.

4.1.16. 4-[5-(4-{4-[(Furan-2-ylmethylene)-amino]-5-mercapto-4H-[1,2,4]triazol-3-yl}-phenyl)-3-trifluoromethyl-pyrazol-1-yl]-benzenesulfonamide (6k)

White powder; m.p.249°C. IR (KBr) cm^{-1}: 3304 (N—H of sulfonamide), 2730 (S—H), 1615.8 (HC = N), 1345.6 (S═O), 747.3 (C—S); ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 7.31 (s, 1H, Pyrazol), 7.31 (s, 2H, N*H*$_2$), 7.37–7.40 (m, 3H, Furan Ring), 7.46–7.56 (m, 4H, Ar*H*), 7.91–8.07 (m, 4H, Ar*H*), 9.61 (s, 1H, *H*C = N), 14.29 (s, 1H, S*H*) ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 106.98, 108.96, 113.12, 120.14, 120.60, 122.28, 126.13, 126.16, 126.94, 128.47, 129.00, 129.15, 130.14, 140.90, 142.17, 142.47, 144.22, 144.25, 147.11, 147.76, 148.27, 154.45, 162.43; Anal. Calculated for $C_{23}H_{16}F_3N_7O_3S_2$: C, 49.37, H, 2.88, N, 17.52; Found: C, 49.35, H, 2.85, N, 17.49; MS *m/z* $[M + H]^+$: 560.20

4.1.17. 4-[5-(4-{5-Mercapto-4-[(2-nitro-benzylidene)-amino]-4H-[1,2,4]triazol-3-yl}-phenyl)-3-trifluoromethyl-pyrazol-1-yl]-benzenesulfonamide (6l)

Yellow powder; m.p.237 °C. IR (KBr) cm-1: 3205, 3071, 1655, 1546; ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 7.23 (s, 1H, Pyrazol), 7.35 (s, 2H, N*H*$_2$), 7.43–7.56 (m, 8H, Ar*H*), 7.88–8.08 (m, 4H, Ar*H*), 8.48 (s, 1H, *H*C = N), 14.39 (s, 1H, S*H*) ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 107.01, 120.13, 122.27, 124.94, 125.92, 126.11, 126.17, 126.38, 126.94, 128.22, 128.87, 129.01, 129.14, 130.22, 133.18, 134.13, 140.89, 142.18, 142.49, 144.21, 148.34, 148.78, 150.10, 160.22, 162.44; Anal. Calculated for $C_{25}H_{17}F_3N_8O_4S_2$: C, 48.86, H, 2.79, N, 18.23; Found: C, 48.89, H, 2.75, N, 18.27; MS *m/z* $[M + H]^+$: 614.74

4.1.18. 4-[5-(4-{4-[(4-Dimethylamino-benzylidene)-amino]-5-mercapto-4H-[1,2,4]triazol-3-yl}-phenyl)-3-trifluoromethyl-pyrazol-1-yl]-benzenesulfonamide (6m)

Orange powder; m.p.210 °C. IR (KBr) cm^{-1}: 3205, 3071, 1655, 1546; ^{1}HNMR (DMSO-d_6, 500 MHz) δ: 3.03 (s, 3H, NC*H*$_3$), 3.07 (s, 3H, NC*H*$_3$), 6.80 (s, 1H, Pyrazol), 7.54–7.73 (m, 4H, Ar*H*), 7.80–7.97 (m, 8H, Ar*H*), 9.23 (s, 1H, *H*C = N), 14.17 (s, 1H, S*H*) ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 55.89, 56.04, 106.93, 106.94, 111.62, 118.33, 120.13, 122.27, 126.10, 126.17, 126.39, 126.92, 128.24, 129.13, 129.95, 130.56, 140.88, 142.16, 142.46, 144.18, 144.25, 145.82, 147.41, 151.92, 153.35, 162.61, 167.65; Anal. Calculated for $C_{27}H_{23}F_3N_8O_2S_2$: C, 52.93, H, 3.78, N, 18.29; Found: C, 52.96, H, 3.73, N, 18.34; MS *m/z* $[M + H]^+$: 612.89.

4.2. Carbonic anhydrase inhibition

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity. [25] Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (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 were 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 [26–31], and represent the mean from at least three different determinations. All CA isofoms were recombinant ones obtained in-house as reported earlier [26–31].

4.3. Anti-inflammatory assays

Young adult male Holtzman rats of 125 to 165 g body weight were maintained in air conditioned quarters with water and food (Rockland Mouse Diet) ad libitum. Test compounds in aqueous suspension were administered by gastric gavage in a volume of 1 ml per 100 g body weight, followed immediately by tap water to a total of 5 ml per rat. Controls received only the tap water. This treatment was given 1 h before injection of the phlogistic agent into the foot. It was found that by thus insuring uniform hydration of all rats, variability of edematous response in the paw was minimized. The phlogistic agent was carrageenin, an extract of *Chondrus* obtained from Algin Corp. of America, prepared as 1% suspension in sterile 0.9% NaCl. A volume of 0.05 ml was injected through a 26-gauge needle into the plantar tissue of the right hind paw. Immediately thereafter, the volume of the injected foot was measured. Swelling of the paw reached a peak in 3 to 5 h, then retained about the same degree of edema for several hours. For routine drug testing, increase in foot volume 3 h after phlogistic agent was adopted as a measure of effect. The paw of the unanesthetized rat was immersed in mercury exactly to an ink mark on the skin over the lateral malleolus. The mercury was contained in a glass cylinder 25 mm diameter and 60 mm deep. The mercury column was connected with a Statham pressure transducer model P23BB range 0–5 cm Hg. The output from the transducer was led, through a Statham control unit powered by a 12-volt constant battery eliminator, to a galvanometer, Leeds and Northrup model 2430, 0.005 microamp. per scale division. Galvanometer readings were calibrated in terms of ml displacement of mercury; immersion in the mercury of an object with volume of 1 ml produced a deflection of 35 scale divisions on the galvanometer.

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