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ORIGINAL PAPER

Synthesis and pharmacological evaluation of pyrazole derivatives containing sulfonamide moiety

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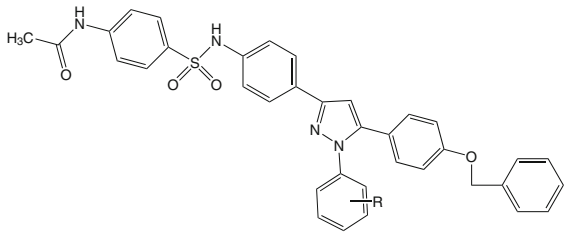
Abstract A new series of *N*-[4-[*N*-[4-[5-[4-(benzyloxy)-phenyl]-1-(substituted phenyl)-1*H*-pyrazol-3-yl]phenyl] sulfamoyl]phenyl]acetamide derivatives were synthesized and elucidated by spectral data. All the compounds were subjected to in vitro evaluation for anti-inflammatory (BSA anti-denaturation assay), antioxidant (DPPH radical scavenging assay) and in vivo screening for anti-inflammatory (carrageenan induced rat paw edema inhibition) activities. Selected active compounds were evaluated for ulcerogenic, lipid peroxidation, and LPS induced TNF-α production inhibition potential. The most active compound in the series showed an in vivo anti-inflammatory efficacy of 83.1 % when compared to diclofenac sodium (81.6 %). Evaluation of ulcer index and biochemical estimation for oxidative stress also revealed that this compound was safe on gastric mucosa and did not induce oxidative stress in tissues. When further tested for LPS induced TNF-α production inhibition in mice, it showed a better inhibition (ID_{50} = 6.23 mg/kg) when compared to standard inhibitor, SB 203580 (ID_{50} = 28.40 mg/kg). The p38α MAP kinase docking score of this active compound was also found to be better than that of SB 203580.

Graphical abstract



Keywords Pyrazole · TNF-α inhibition · Docking · Anti-inflammatory · Ulcerogenicity

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are therapeutically important class of compounds widely used in the treatment of various inflammatory diseases particularly arthritic pain [1]. Chronic NSAID therapy effectively reduces the symptoms of many painful arthritic syndromes, but invites adverse gastrointestinal (GI) complications ranging from stomach irritation to life threatening GI ulceration and bleeding [2]. NSAIDs exert their anti-inflammatory effect mainly through inhibition of cyclooxygenases (COXs), key enzymes in the prostaglandin (PG) biosynthesis from arachidonic acid. There are at least two COX isoforms, COX-1 and COX-2. Constitutive COX-1 is responsible for providing cytoprotection in GI tract, whereas inducible COX-2 mediates inflammation [3]. Traditional NSAIDs such as aspirin, diclofenac, ibuprofen, and naproxen are non-selective; however, they show greater selectivity for COX-1 than COX-2 [4]. Consequently, their long-term clinical use is associated

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with GI toxicity [5]. Thus the discovery of COX-2 provided the rationale for the development of drugs devoid of GI toxicity while retaining clinical efficacy as anti-inflammatory agents. But the recent report showed that selective COX-2 inhibitors could lead to adverse cardiovascular side effects [6]. Therefore development of novel compounds having anti-inflammatory activity with an improved safety profile is still a necessity.

p38 mitogen activated protein (MAP) kinase is a member of the intracellular family of MAP kinases implicated in the phosphorylation cascade leading to the release of tumour necrosis factor-alpha (TNF-α) and other cytokines [7]. TNF-α is a multifunctional cytokine which participates in the pathogenesis of various diseases including autoimmune, inflammatory, and neurodegenerative diseases. TNF-α also mediates many of the inflammatory processes in rheumatoid arthritis (RA) including immune cell activation, proliferation, apoptosis and regulation of leukocyte movement [8, 9]. Therefore TNF-α has emerged as an attractive target to reduce the inflammatory conditions.

Pyrazoles and their derivatives are widely known for their excellent effectiveness as analgesics and antipyretics. Antipyrin was the first pyrazoline derivative used in the management of pain and inflammation. Phenylbutazone and its derivative, oxyphenbutazone, were potent anti-inflammatory agents. The marketed selective COX-2 inhibitor (celecoxib) having a pyrazole moiety also showed high anti-inflammatory activity with reduced GI toxicity [10]. Besides these, many pyrazole derivatives are also reported in literature having potent anti-inflammatory activity [11–15]. Furthermore, sulfonamide derivatives are well known pharmaceutical agents since this group has been a part of most of the drug structures due to stability and tolerance in human beings [16]. This group is also the key constituent of COX inhibitors such as celecoxib [17] and valdecoxib [18].

Encouraged by these observations and in continuation of our research program on the synthesis of 5-membered heterocyclic compounds as anti-inflammatory agents [19–22], it was attempted in the present study to synthesize and evaluate anti-inflammatory activity and TNF-α inhibitory properties of some new pyrazole derivatives bearing sulfonamide pharmacophore. The most potent derivative was also docked into the active site of p38 MAPK so as to explore the interaction mechanism between the inhibitor and the receptor.

Results and discussion

Chemistry

4-(Benzyloxy)benzaldehyde (**1**) was prepared by treating 4-hydroxybenzaldehyde and benzyl chloride in the presence of anhydrous K_2CO_3 in dimethyl formamide (DMF) at room temperature. Treatment of **1** with 4-aminoacetophenone in presence of aqueous NaOH in methanol at room temperature yielded 1-(4-aminophenyl)-3-[4-(benzyloxy)phenyl]prop-2-en-1-one (**2**). This chalcone was then refluxed with different aryl hydrazines in ethanol to give 4-[5-[4-(benzyloxy)phenyl]-1-(substituted phenyl)-1*H*-pyrazol-3-yl]anilines **3a**–**3o**. Synthesis of target compounds **4a**–**4o** was achieved by stirring pyrazoles **3a**–**3o** with *p*-acetamidobenzenesulfonyl chloride in the presence of tetrahydrofuran (THF) and pyridine. Synthesis of these compounds is outlined in Scheme 1.

^{1}H NMR spectra of titled compounds **4a**–**4o** showed a singlet for CH_3CO and CONH protons at $\delta = 2.06$–3.01 and at 8.32–8.90 ppm, respectively. The singlets of SO_2NH and OCH_2 protons were observed at $\delta = 10.18$–10.31 and 5.15–5.22 ppm, respectively. In the ^{13}C NMR spectra of compounds **4a**–**4o**, CH_3 and OCH_2 carbon peaks were observed at $\delta = 39.49$–23.78 and 72.26–70.24 ppm, respectively. Furthermore, CONH carbon peaks were observed downfield at $\delta = 172.47$–169.92 ppm. Mass spectra of all the compounds showed a molecular ion peak M^+ at an *m/z* corresponding to their molecular formula.

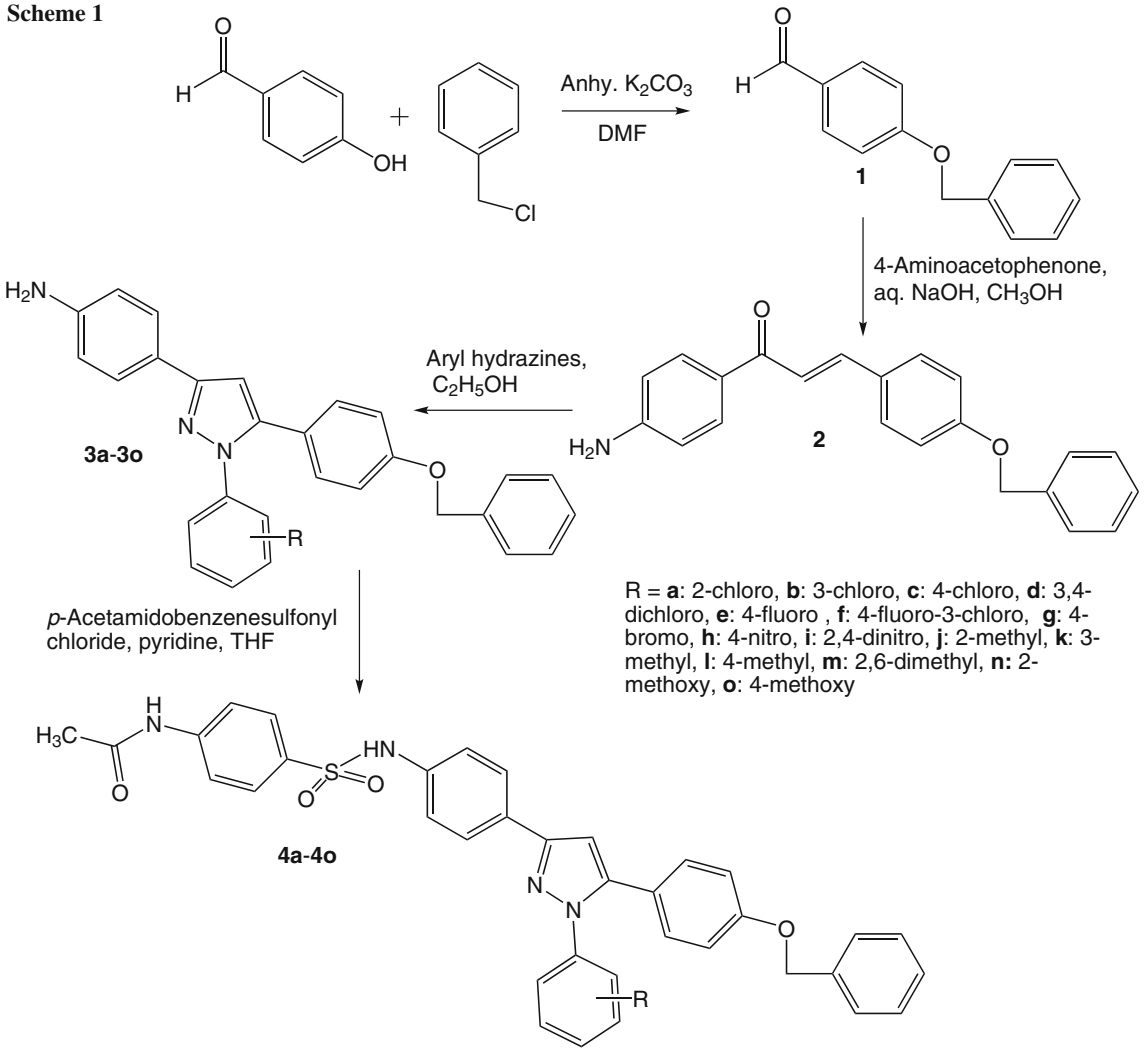
In vitro anti-inflammatory activity

The synthesized compounds **4a**–**4o** were screened for anti-inflammatory activity by inhibition of albumin denaturation technique. Compound **4a** showed the maximum anti-inflammatory activity of 89 % when compared to the standard diclofenac sodium (83.6 %). Compounds **4c** (87.5 %) and **4b** (84.5 %) also exhibited higher anti-inflammatory activity in comparison to diclofenac sodium. Other compounds were found to have anti-inflammatory activity in the range of 62.9–82.2 % (Table 1). From the results it can be stated that synthesized analogs are capable of controlling the production of auto antigens due to prevention of denaturation of proteins and the findings suggested that these compounds may show good in vivo anti-inflammatory activity.

Antioxidant activity

The free radical scavenging activity of the compounds **4a**–**4o** was evaluated by DPPH colorimetric method at 1 mM concentration. The degree of change in absorbance with respect to control was calculated as antioxidant potential. Compounds **4a** (76.0 %), **4c** (74.2 %), and **4b** (74 %) showed antioxidant activity within the same range in comparison to the standard, butylated hydroxyl anisole (BHA) (73 %). All the other compounds also exhibited good antioxidant activity which is in the range of 71–40.4 % (Table 1). The study revealed that electron

Scheme 1



withdrawing groups increased the antioxidant potential which may be due to intensification of positive charge associated with negative inductive effect of these groups. The positive charge intensification may lead to free radical quenching. Moreover, electron withdrawing groups are themselves good free radical quenchers. On the other hand, substitution with electron releasing groups was found to decrease the radical scavenging potential possibly due to their positive inductive effect.

In vivo anti-inflammatory activity

The anti-inflammatory activity of the synthesized compounds **4a**–**4o** was evaluated by carrageenan induced paw edema method of Winter et al. The compounds were tested at equimolar oral dose relative to 10 mg/kg diclofenac sodium. The tested compounds showed anti-inflammatory activity ranging from 52.3 to 83.1 %, whereas standard drug diclofenac sodium showed 81.7 %. Compound **4a** having the 2-chlorophenyl group attached to position 1 of the pyrazole ring emerged as the most promising compound of the series showing anti-inflammatory activity (83.1 %) slightly higher than diclofenac sodium, whereas compound **4c** and **4b** having 4-chloro- and 3-chlorophenyl groups showed anti-inflammatory activity 82.1 and 80.5 % respectively which is within the range of standard drug diclofenac sodium. When the chlorophenyl group was replaced by a 4-fluorophenyl (**4e**), 4-bromophenyl (**4g**), or 4-nitrophenyl (**4h**) group, the activity was found to be decreased (74.0, 72.6, and 72.1 %, respectively). A further decrease in activity was observed for compounds **4d** (71.4 %) and **4f** (70.7 %) which possessed two electron withdrawing groups i.e. 3,4-dichlorophenyl and 4-fluoro-3-chlorophenyl, respectively. Compounds **4i**–**4o** showed moderate to low activity ranging from 52.3 to 66.1 % which possessed -2,4-dinitro, $-CH_3$, and $-OCH_3$ groups attached to position 1 of the pyrazole ring (Table 2).

Table 1 In vitro anti-inflammatory activity (BSA denaturation inhibition assay) and % DPPH activity

Compounds	BSA denaturation inhibition assay[a]		% DPPH activity (at 1 mM)[b]	
	Mean absorbance	% Inhibition of denaturation ± SD	Mean absorbance	% Antioxidant activity ± SD
4a	0.353	89.0 ± 2.53	0.197	76.0 ± 0.64
4b	0.345	84.5 ± 2.83	0.214	74.0 ± 0.56
4c	0.351	87.5 ± 1.88	0.207	74.8 ± 0.49
4d	0.333	78.1 ± 1.85	0.282	65.7 ± 0.93
4e	0.341	82.2 ± 4.32	0.239	71.0 ± 0.8
4f	0.325	73.8 ± 3.51	0.286	65.3 ± 0.98
4g	0.337	80.0 ± 2.53	0.253	69.2 ± 0.56
4h	0.334	78.8 ± 2.16	0.26	68.3 ± 0.49
4i	0.311	66.1 ± 0.82	0.372	54.7 ± 0.51
4j	0.307	64.4 ± 0.31	0.381	53.6 ± 0.81
4k	0.315	68.3 ± 2.64	0.331	59.7 ± 0.73
4l	0.319	70.8 ± 4.85	0.314	61.8 ± 0.61
4m	0.305	63.3 ± 2.74	0.394	52.1 ± 0.98
4n	0.305	62.9 ± 4.97	0.49	40.4 ± 0.61
4o	0.318	70.1 ± 3.25	0.326	60.3 ± 0.73
Standard	0.343	83.6 ± 2.03	0.222	73.0 ± 1.27

SD standard deviation (average of three determinations)

[a] Diclofenac sodium

[b] Butylated hydroxyl anisole

Ulcerogenicity

Compounds **4a**–**4c**, **4e**, and **4g** exhibiting significant anti-inflammatory activity were further screened for their ulcerogenic potential. It was observed that the tested compounds exhibited a better GI safety profile, severity index ranging from 0.42 ± 0.38 to 0.92 ± 0.66, whereas standard drug diclofenac sodium showed a high severity index of 1.83 ± 0.25. Most potent compounds **4a** and **4c** showing high anti-inflammatory activity showed a reduction in severity index (0.42 ± 0.38 and 0.67 ± 0.52, respectively) in comparison to standard drug (Table 3). From the results obtained it may be suggested that due to decrease in the ulcerogenic activity the compounds may cause less gastric ulceration and disruption of gastric epithelial cells.

Lipid peroxidation

The compounds screened for ulcerogenic activity were also analyzed for lipid peroxidation. Lipid peroxidation is measured as nmol of MDA per 100 mg of gastric mucosa tissue. The tested compounds **4a**–**4c**, **4e**, and **4g** exhibited significant reduction in lipid peroxidation (in the range of 4.69 ± 0.87–6.12 ± 0.50). Most active anti-inflammatory compounds **4a** and **4c** showed reduction in lipid peroxidation (4.69 ± 0.87 and 5.26 ± 1.50, respectively). The standard drug, diclofenac sodium showed the maximum lipid peroxidation (6.53 ± 0.22) whereas the control group displayed a lipid peroxidation of 3.23 ± 0.10 (Table 3). Thus this study showed that the synthesized compounds have inhibited the induction of gastric mucosal lesions and the results further suggested that their protective action might be related to the inhibition of lipid peroxidation in the gastric mucosa.

LPS induced TNF-α production inhibition

Subsequently we evaluated the inhibitory activity of the derivatives **4a**–**4c**, **4e**, and **4g** in the LPS induced TNF-α production in mice. Compound **4a** showed the most effective activity (ID_{50} = 6.23 mg/kg), which was more than the standard compound, SB 203580 (ID_{50} = 28.40 - mg/kg). Analogs **4c** and **4b** also demonstrated good TNF-α inhibitory efficacy with ID_{50} values of 10.96 and 10.83 mg/kg, respectively. Compounds **4e** (52.8 %) and **4g** (52.8 %) showed activity more or less similar to that of the standard inhibitor, SB 203580 which showed 52.1 % inhibition (Table 3).

Molecular docking study

The glide score value of SB 203580 was found to be −9.163, while that of compound **4a** was −10.058, indicating the strong interaction of the molecule with the enzyme (Table 2). The best binding mode of SB 203580 (p38α

Table 2 Anti-inflammatory activity and XP docking glide score of compounds **4a**–**4o** and standards

Compounds	Increase in paw edema/cm^3 ± SEM	% Inhibition	Activity relative to diclofenac	Glide score[d]
4a	0.29 ± 0.08[a]	83.1	102.1	−10.058
4b	0.33 ± 0.14[c]	80.5	98.9	−10.107
4c	0.30 ± 0.06[c]	82.1	100.5	−9.678
4d	0.48 ± 0.06[a]	71.4	87.7	−11.324
4e	0.44 ± 0.17[a]	74.0	90.9	−10.083
4f	0.50 ± 0.15[a]	70.7	86.8	−10.581
4g	0.46 ± 0.13[a]	72.6	89.2	−8.706
4h	0.47 ± 0.12[b]	72.1	88.6	−8.240
4i	0.74 ± 0.17[b]	56.4	69.3	−10.211
4j	0.78 ± 0.24[a]	54.1	66.4	−11.192
4k	0.66 ± 0.11[c]	60.7	74.5	−11.124
4l	0.57 ± 0.23[a]	66.1	81.2	−9.320
4m	0.79 ± 0.10[a]	53.3	65.4	−10.202
4n	0.81 ± 0.27[a]	52.3	64.2	−10.636
4o	0.58 ± 0.13[a]	65.5	80.5	−6.038
Control	1.69 ± 0.04[a]	–	–	–
Diclofenac sodium	0.31 ± 0.01[a]	81.7	100	−4.875
SB 203580	–	–	–	−9.163

Anti-inflammatory activity of the compounds were compared with respect to control. Data were analyzed by unpaired student's *t* test for $n = 6$. All data are significantly different from control

[a] $p < 0.05$

[b] $p < 0.01$

[c] $p < 0.001$

[d] Glide score denotes *g* score obtained for docking with p38α MAP kinase (PDB ID: 3D83)

Table 3 Ulcerogenic, lipid peroxidation, and LPS induced TNF-α production inhibition potential

Compounds	Mean severity index ± SEM	Lipid peroxidation nmol MDA/100 mg tissue	% TNF-α inhibition ± SEM	ID_{50}/mg/kg
4a	0.42 ± 0.38[b]	4.69 ± 0.87[b]	64.1 ± 1.24[a]	6.23
4b	0.83 ± 0.26[b]	5.89 ± 0.44[a]	56.4 ± 1.80[b]	10.83
4c	0.67 ± 0.52[a]	5.26 ± 1.50[a]	57.3 ± 0.66[a]	10.96
4e	0.83 ± 0.41[b]	5.69 ± 0.67[a]	52.8 ± 1.00[b]	–
4g	0.92 ± 0.66[a]	6.12 ± 0.50[a]	52.8 ± 0.85[b]	–
Control	0.00 ± 0.00	3.23 ± 0.10[a]	–	–
Diclofenac sodium	1.83 ± 0.25[a]	6.53 ± 0.22[a]	–	–
SB203580	–	–	52.1 ± 1.96[b]	28.40

Relative to standard and data were analyzed by unpaired student's *t* test for $n = 6$

[a] $p < 0.01$

[b] $p < 0.001$

prototype inhibitor) and compound **4a** in the active site of p38α MAP kinase are presented in Fig. 1a, b, respectively. The docked pose of compound **4a** showed a hydrogen bond interaction with Met 109 (O···HN, 2.13 Å) and Thr 106 (O···HN, 2.40 Å) (Fig. 2). Interestingly, compound **4a** also forms a π–π interaction between a phenyl ring and Phe 169. Standard SB 203580 was found to show two hydrogen bond interactions with Glu 71 and Asp 168.

Conclusion

We have reported here a simple synthesis of a series of new pyrazole derivatives bearing a sulfonamide pharmacophore. The compounds having chloro, fluoro, and bromo groups in position 1 of the pyrazole ring have exhibited very good anti-inflammatory activity in comparison to diclofenac sodium along with good antioxidant potential.

Compound **4a** showing high anti-inflammatory activity was found to be safe on gastric mucosa with least ability to induce oxidative stress in tissues. The LPS induced TNF-α production inhibition for compound **4a** was found to be superior in comparison to that of SB 203580 in mice. Furthermore, molecular docking study of compound **4a** revealed favorable orientation within the active binding site of p38α MAP kinase with a comparatively higher docking score than SB 203580. Therefore, compound **4a** represent a fruitful matrix for development of anti-inflammatory agents that would deserve further investigation and derivatization.

Experimental

All reagents and solvents were of laboratory grade and were procured from Merck (Darmstadt, Germany) and S.D. Fine chemicals (Delhi, India). Melting points were determined in open capillaries using Digital Auto Melting Point Apparatus (Labtronics). Purity of the compounds was ascertained by TLC using precoated aluminium TLC plates (Merck) visualized in a UV/Visible chamber. Infrared spectra were recorded on Perkin-Elmer 1720 FTIR spectrometer. ^{1}H NMR spectra (400 MHz) and ^{13}C NMR spectra (100 MHz, with complete proton decoupling) were recorded on a Bruker Avance-400 instrument. Chemical shifts were reported in ppm downfield from tetramethylsilane (TMS) as the internal standard. Mass spectra were recorded on Jeol SX-102/DA-6000 spectrometer. Elemental analyses (C, H and N) were undertaken with a CHNS Vario EL III (Elementar Analysen systeme GmbH, Germany) and the results are within ±0.4 % of theoretical values.

4-(Benzyloxy)benzaldehyde(**1**)

Equimolar quantities of 4-hydroxybenzaldehyde and benzyl chloride (5 mmol each) were stirred at room temperature for 8 h in the presence of anhydrous potassium carbonate (7.5 mmol) in 10 cm of DMF. The solution was then poured into ice cold water and the precipitate thus obtained was filtered, dri^{3}ed and crystallized from ethanol. Yield: 92 %; m.p.: 74–75 °C (Ref. [23] 65–66 °C).

1-(4-Aminophenyl)-3-[4-(benzyloxy)phenyl]prop-2-en-1-one (**2**)

4-(Benzyloxy)benzaldehyde (**1**, 5 mmol) and 4-aminoacetophenone (5 mmol) in the presence of 10 cm^{3} aqueous sodium hydroxide (4 %) and 25 cm^{3} methanol was stirred at room temperature for 4h. Yellow crystalline precipitate was obtained which was filtered, washed with water, and dried. Yield 89 %, m.p.: 182–183 °C; IR (KBr): $\bar{\nu}$ = 3487, 3209 (NH), 3021 (CH), 1665 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 5.09 (s, 2H, OCH_2), 5.72 (s, 2H, NH_2, D_2O exchangeable), 7.05 (d, J = 7.6 Hz, 2H, ArH), 7.42 (d, J = 7.6 Hz, 2H, ArH), 7.43–7.64 (m, 5H, ArH), 7.67 (d, J = 8.4 Hz, 2H, ArH), 7.72 (d, J = 15.2 Hz, 1H, CO–CH=CH), 7.91 (d, J = 15.2 Hz, 1H, CO–CH=C*H*), 7.97 (d, J = 8.4 Hz, 2H, ArH) ppm; ^{13}C NMR ($CDCl_3$): δ = 70.1 (OCH_2), 113.9 (CH=CH), 115.3 (CH=CH), 119.9, 127.5, 128.2, 128.3, 128.6, 128.7, 130.0, 131.0, 136.5, 142.9, 151.2, 160.5 (C=O) ppm.

General method for the synthesis of 4-[5-[4-(benzyloxy)phenyl]-1-(substituted phenyl)-1H-pyrazol-3-yl]anilines ***3a–3o***

Amixture of 1-(4-aminophenyl)-3-[4-(benzyloxy)phenyl] prop-2-en-1-one (**2**, 5 mmol) and different aryl hydrazines (7.5 mmol) in 25 cm^{3} of ethanol was refluxed at 60 °C for 3–7 h. The solution was cooled at room temperature and poured into ice cold water. The precipitate thus obtained was filtered, washed with water, dried, and recrystallized from ethanol.

4-[5-[4-(Benzyloxy)phenyl]-1-(2-chlorophenyl)-1H-pyrazol-3-yl]aniline (**3a**, $C_{28}H_{22}ClN_3O$)

Yield 88 %; m.p.: 126–127 °C; IR (KBr): $\bar{\nu}$ = 3522, 3351 (NH), 3035 (CH), 1596 (C=N), 736 (C–Cl) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 5.12 (s, 2H, OCH_2), 5.17 (s, 2H, NH_2,

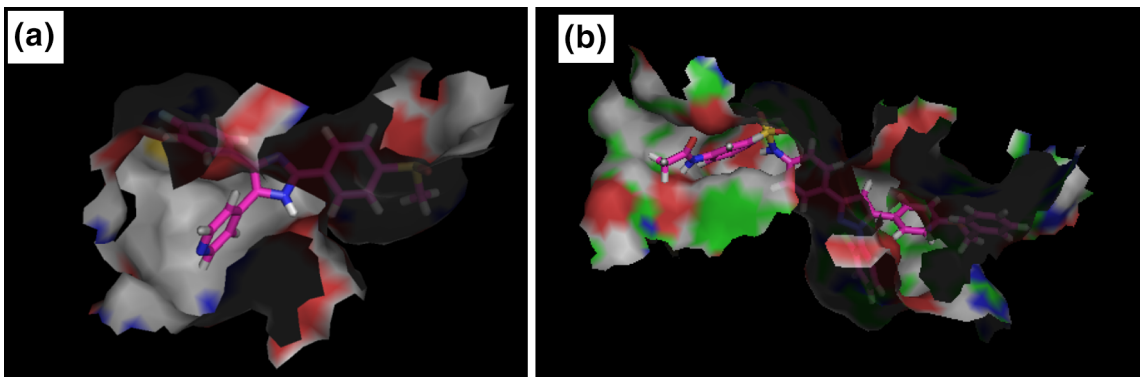


Fig. 1 A suggested binding mode of (**a**) SB 203580 and (**b**) compound **4a** into p38α MAP kinase (PDB code: 3D83)

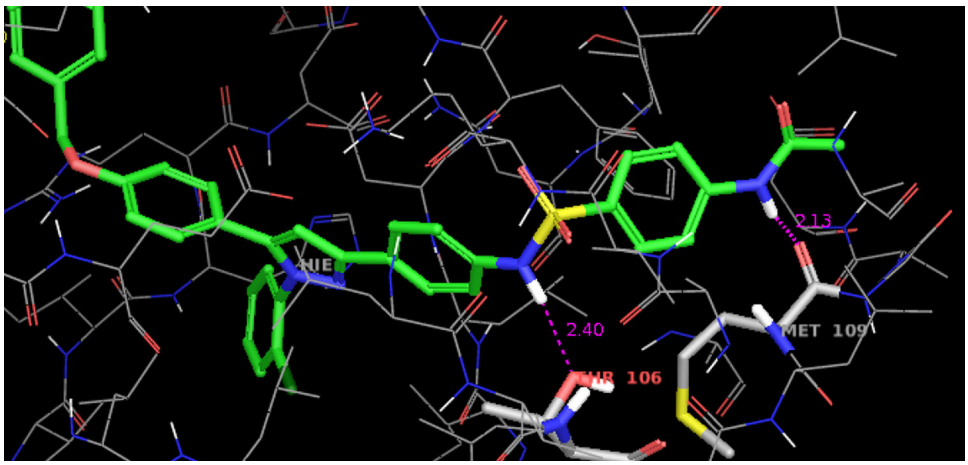


Fig. 2 Docked pose of compound **4a** in the binding site of p38α MAP kinase showing hydrogen bond interaction

D_2O exchangeable), 6.65 (s, 1H, pyrazole H-4), 7.05–7.82 (m, 17H, Ar–H) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 68.9$ (OCH_2), 115.6 (pyrazole C_4), 118.7, 120.1, 128.2, 128.9, 129.1, 129.3, 130.0, 130.2, 131.1, 131.8, 132.0, 132.2, 132.4, 132.8, 132.9, 136.2, 143.6, 144.0, 160.5 (pyrazole C_5), 163.4 (pyrazole C_3) ppm.

4-[5-[4-(Benzyloxy)phenyl]-1-(3-chlorophenyl)-1H-pyrazol-3-yl]aniline (**3b**, $C_{28}H_{22}ClN_3O$)
Yield 89 %; m.p.: 120 °C; IR (KBr): $\bar{\nu} = 3520$, 3340 (NH), 3035 (CH), 1592 (C=N), 741 (C–Cl) cm^{-1}; ^1H NMR (DMSO-d_6): $\delta = 5.16$ (s, 2H, OCH_2), 5.19 (s, 2H, NH_2, D_2O exchangeable), 6.62 (s, 1H, pyrazole H-4), 7.02–7.81 (m, 17H, Ar–H) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 68.3$ (OCH_2), 115.9 (pyrazole C_4), 118.3, 120.8, 128.2, 128.8, 129.1, 129.2, 129.8, 130.4, 131.1, 131.7, 132.1, 132.3, 132.4, 132.7, 132.8, 136.4, 143.5, 144.2, 160.4 (pyrazole C_5), 163.3 (pyrazole C_3) ppm.

4-[5-[4-(Benzyloxy)phenyl]-1-(4-chlorophenyl)-1H-pyrazol-3-yl]aniline (**3c**, $C_{28}H_{22}ClN_3O$)
Yield 80 %; m.p.: 163 °C; IR (KBr): $\bar{\nu} = 3496$, 3348 (NH), 3029 (CH), 1599 (C=N), 749 (C–Cl) cm^{-1}; ^1H NMR (DMSO-d_6): $\delta = 5.15$ (s, 2H, OCH_2), 5.19 (s, 2H, NH_2, D_2O exchangeable), 6.62 (s, 1H, pyrazole H-4), 7.09–7.83 (m, 17H, Ar–H) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 68.9$ (OCH_2), 115.3 (pyrazole C_4), 118.2, 120.4, 128.3, 128.8, 129.1, 129.3, 129.8, 130.2, 131.3, 131.8, 132.1, 132.3, 132.5, 136.2, 143.5, 144.2, 160.6 (pyrazole C_5), 163.7 (pyrazole C_3) ppm.

4-[5-[4-(Benzyloxy)phenyl]-1-(3,4-dichlorophenyl)-1H-pyrazol-3-yl]aniline (**3d**, $C_{28}H_{21}Cl_2N_3O$)
Yield 89 %; m.p.: 127–129 °C; IR (KBr): $\bar{\nu} = 3570$, 3345 (NH), 3037 (CH), 1596 (C=N), 742 (C–Cl) cm^{-1}; ^1H NMR (DMSO-d_6): $\delta = 5.13$ (s, 2H, OCH_2), 5.22 (s, 2H, NH_2, D_2O exchangeable), 6.71 (s, 1H, pyrazole H-4), 7.15–7.82 (m, 16H, Ar–H) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 68.6$ (OCH_2), 115.7 (pyrazole C_4), 118.3, 120.5, 128.4, 128.9, 129.1, 129.3, 130.1, 130.3, 131.2, 131.6, 132.0, 132.1, 132.4, 132.8, 132.9, 136.1, 143.6, 144.1, 160.5 (pyrazole C_5), 163.5 (pyrazole C_3) ppm.

4-[5-[4-(Benzyloxy)phenyl]-1-(4-fluorophenyl)-1H-pyrazol-3-yl]aniline (**3e**, $C_{28}H_{22}FN_3O$)
Yield 85 %; m.p.: 132–133 °C; IR (KBr): $\bar{\nu} = 3565$, 3350 (NH), 3032 (CH), 1585 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6): $\delta = 5.18$ (s, 2H, OCH_2), 5.22 (s, 2H, NH_2, D_2O exchangeable), 6.66 (s, 1H, pyrazole H-4), 7.05–7.82 (m, 17H, Ar–H) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 68.5$ (OCH_2), 115.7 (pyrazole C_4), 118.4, 120.2, 128.2, 128.8, 129.2, 129.4, 130.0, 130.3, 131.2, 131.7, 132.2, 132.4, 132.8, 136.3, 143.5, 144.2, 160.4 (pyrazole C_5), 163.3 (pyrazole C_3) ppm.

4-[5-[4-(Benzyloxy)phenyl]-1-(3-chloro-4-fluorophenyl)-1H-pyrazol-3-yl]aniline (**3f**, $C_{28}H_{21}ClFN_3O$)
Yield 88 %; m.p.: 145–146 °C; IR (KBr): $\bar{\nu} = 3560$, 3354 (NH), 3026 (CH), 1591 (C=N), 729 (C–Cl) cm^{-1}; ^1H NMR (DMSO-d_6): $\delta = 5.16$ (s, 2H, OCH_2), 5.22 (s, 2H, NH_2, D_2O exchangeable), 6.70 (s, 1H, pyrazole H-4), 7.06–7.84 (m, 16H, Ar–H) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 68.3$ (OCH_2), 115.4 (pyrazole C_4), 118.6, 120.2, 128.4, 128.9, 129.2, 129.3, 130.1, 130.3, 131.1, 131.7, 132.0, 132.1, 132.4, 132.7, 132.9, 136.4, 143.5, 144.2, 161.5 (pyrazole C_5), 163.6 (pyrazole C_3) ppm.

4-[5-[4-(Benzyloxy)phenyl]-1-(4-bromophenyl)-1H-pyrazol-3-yl]aniline (**3g**, $C_{28}H_{22}BrN_3O$)
Yield 87 %; m.p.: 132–133 °C; IR (KBr): $\bar{\nu} = 3520$, 3368 (NH), 3033 (CH), 1585 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6): $\delta = 5.16$ (s, 2H, OCH_2), 5.17 (s, 2H, NH_2, D_2O exchangeable), 6.65 (s, 1H, pyrazole H-4), 7.05–7.82 (m, 17H, Ar–H) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 68.7$ (OCH_2), 115.8 (pyrazole C_4), 118.6, 120.8, 128.3, 128.8, 129.1, 129.2, 129.7, 130.2, 131.3, 131.8, 132.1, 132.3,

132.8, 136.2, 143.5, 144.2, 160.3 (pyrazole C_5), 163.7 (pyrazole C_3) ppm.

4-[5-[4-(Benzyloxy)phenyl]-1-(4-nitrophenyl)-1H-pyrazol-3-yl]aniline (**3h**, $C_{28}H_{22}N_4O_3$)
Yield 88 %; m.p.: 182–183 °C; IR (KBr): $\bar{\nu}$ = 3560, 3341 (NH), 3039 (CH), 1597 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 5.09 (s, 2H, OCH_2), 5.17 (s, 2H, NH_2, D_2O exchangeable), 6.69 (s, 1H, pyrazole H-4), 7.07–7.81 (m, 17H, Ar–H) ppm; ^{13}C NMR (DMSO-d_6): δ = 68.6 (OCH_2), 115.6 (pyrazole C_4), 118.4, 120.6, 128.3, 128.8, 129.2, 129.3, 129.8, 130.2, 131.1, 131.6, 132.2, 132.5, 132.9, 136.1, 143.6, 144.1, 161.2 (pyrazole C_5), 163.6 (pyrazole C_3) ppm.

4-[5-[4-(Benzyloxy)phenyl]-1-(2,4-dinitrophenyl)-1H-pyrazol-3-yl]aniline (**3i**, $C_{28}H_{21}N_5O_5$)
Yield 89 %; m.p.: 148–149 °C; IR (KBr): $\bar{\nu}$ = 3525, 3352 (NH), 3085 (CH), 1584 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 5.11 (s, 2H, OCH_2), 5.17 (s, 2H, NH_2, D_2O exchangeable), 6.68 (s, 1H, pyrazole H-4), 7.10–7.86 (m, 16H, Ar–H) ppm; ^{13}C NMR (DMSO-d_6): δ = 68.8 (OCH_2), 115.9 (pyrazole C_4), 118.4, 120.8, 128.4, 128.8, 129.1, 129.4, 130.0, 130.4, 131.2, 131.8, 132.0, 132.1, 132.5, 132.7, 132.9, 136.3, 143.6, 144.0, 160.2 (pyrazole C_5), 163.8 (pyrazole C_3) ppm.

4-[5-[4-(Benzyloxy)phenyl]-1-(2-methylphenyl)-1H-pyrazol-3-yl]aniline (**3j**, $C_{29}H_{25}N_3O$)
Yield 86 %; m.p.: 134–135 °C; IR (KBr): $\bar{\nu}$ = 3562, 3347 (NH), 3021 (CH), 1585 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 2.18 (s, 3H, CH_3), 5.10 (s, 2H, OCH_2), 5.27 (s, 2H, NH_2, D_2O exchangeable), 6.61 (s, 1H, pyrazole H-4), 7.06–7.82 (m, 17H, Ar–H) ppm; ^{13}C NMR (DMSO-d_6): δ = 16.3 (CH_3), 68.5 (OCH_2), 115.8 (pyrazole C_4), 118.5, 120.7, 128.3, 128.8, 129.2, 129.3, 129.8, 130.1, 131.1, 131.7, 132.1, 132.3, 132.6, 132.8, 132.9, 136.2, 143.7, 144.0, 160.4 (pyrazole C_5), 163.9 (pyrazole C_3) ppm.

4-[5-[4-(Benzyloxy)phenyl]-1-(3-methylphenyl)-1H-pyrazol-3-yl]aniline (**3k**, $C_{29}H_{25}N_3O$)
Yield 84 %; m.p.: 200–202 °C; IR (KBr): $\bar{\nu}$ = 3560, 3355 (NH), 3037 (CH), 1593 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 2.29 (s, 3H, CH_3), 5.11 (s, 2H, OCH_2), 5.20 (s, 2H, NH_2, D_2O exchangeable), 6.75 (s, 1H, pyrazole H-4), 7.05–7.85 (m, 17H, Ar–H) ppm; ^{13}C NMR (DMSO-d_6): δ = 16.1 (CH_3), 68.4 (OCH_2), 115.9 (pyrazole C_4), 118.2, 120.7, 128.2, 128.9, 129.0, 129.4, 130.0, 130.2, 131.1, 131.8, 132.0, 132.2, 132.4, 132.7, 132.8, 136.2, 143.7, 144.1, 161.5 (pyrazole C_5), 163.6 (pyrazole C_3) ppm.

4-[5-[4-(Benzyloxy)phenyl]-1-(4-methylphenyl)-1H-pyrazol-3-yl]aniline (**3l**, $C_{29}H_{25}N_3O$)
Yield 87 %; m.p.: 178–179 °C; IR (KBr): $\bar{\nu}$ = 3520, 3370 (NH), 3032 (CH), 1590 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 2.32 (s, 3H, CH_3), 5.22 (s, 2H, OCH_2), 5.25 (s, 2H, NH_2, D_2O exchangeable), 6.65 (s, 1H, pyrazole H-4), 7.05–7.82 (m, 17H, Ar–H) ppm; ^{13}C NMR (DMSO-d_6): δ = 16.1 (CH_3), 68.8 (OCH_2), 115.6 (pyrazole C_4), 118.5, 120.8, 128.4, 128.9, 129.0, 129.5, 130.1, 130.3, 131.2, 131.7, 132.3, 132.7, 132.9, 136.1, 143.7, 144.2, 160.5 (pyrazole C_5), 163.7 (pyrazole C_3) ppm.

4-[5-[4-(Benzyloxy)phenyl]-1-(2,6-dimethylphenyl)-1H-pyrazol-3-yl]aniline (**3m**, $C_{30}H_{27}N_3O$)
Yield 87 %; m.p.: 132–133 °C; IR (KBr): $\bar{\nu}$ = 3525, 3328 (NH), 3024 (CH), 1590 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 2.10 (s, 6H, 2 CH_3), 5.12 (s, 2H, OCH_2), 5.17 (s, 2H, NH_2, D_2O exchangeable), 6.68 (s, 1H, pyrazole H-4), 7.01–7.88 (m, 16H, Ar–H) ppm; ^{13}C NMR (DMSO-d_6): δ = 15.6 (2 CH_3), 68.8 (OCH_2), 115.6 (pyrazole C_4), 118.3, 120.5, 128.2, 128.8, 129.1, 129.5, 130.1, 130.4, 131.2, 131.8, 132.1, 132.3, 132.6, 132.8, 132.9, 136.3, 143.6, 144.1, 161.7 (pyrazole C_5), 163.5 (pyrazole C_3) ppm.

4-[5-[4-(Benzyloxy)phenyl]-1-(2-methoxyphenyl)-1H-pyrazol-3-yl]aniline (**3n**, $C_{29}H_{25}N_3O$)
Yield 88 %; m.p.: 158–160 °C; IR (KBr): $\bar{\nu}$ = 3520, 3344 (NH), 3019 (CH), 1592 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 3.52 (s, 3H, OCH_3), 5.18 (s, 2H, OCH_2), 5.27 (s, 2H, NH_2, D_2O exchangeable), 6.71 (s, 1H, pyrazole H-4), 7.09–7.82 (m, 17H, Ar–H) ppm; ^{13}C NMR (DMSO-d_6): δ = 53.2 (OCH_3), 68.5 (OCH_2), 115.8 (pyrazole C_4), 118.6, 120.4, 128.3, 128.9, 129.1, 129.3, 129.8, 130.2, 131.1, 131.6, 132.0, 132.2, 132.4, 132.7, 132.8, 136.3, 143.6, 144.1, 160.6 (pyrazole C_5), 163.8 (pyrazole C_3) ppm.

4-[5-[4-(Benzyloxy)phenyl]-1-(4-methoxyphenyl)-1H-pyrazol-3-yl]aniline (**3o**, $C_{29}H_{25}N_3O$)
Yield 88 %; m.p.: 184–185 °C; IR (KBr): $\bar{\nu}$ = 3525, 3353 (NH), 3028 (CH), 1575 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 3.71 (s, 3H, OCH_3), 5.21 (s, 2H, OCH_2), 5.25 (s, 2H, NH_2, D_2O exchangeable), 6.68 (s, 1H, pyrazole H-4), 7.05–7.84 (m, 17H, Ar–H) ppm; ^{13}C NMR (DMSO-d_6): δ = 53.2 (OCH_3), 68.4 (OCH_2), 115.7 (pyrazole C_4), 118.2, 120.4, 128.2, 128.8, 129.2, 129.4, 129.7, 130.1, 131.3, 131.6, 132.2, 132.8, 132.8, 136.3, 143.5, 144.0, 161.7 (pyrazole C_5), 163.9 (pyrazole C_3) ppm.

General method for the synthesis of N-[4-[N-[4-[5-[4-(benzyloxy)phenyl]-1-(substituted phenyl)-1H-pyrazol-3-yl]phenyl]sulfamoyl]phenyl]acetamide ***4a–4o***
Equimolar quantities of **3a–3o** and *p*-acetamidobenzenesulfonyl chloride (5 mmol each) were stirred at room temperature for 6–9 h in the presence of pyridine (5 mmol) in 30 cm^3 of THF. The mixture was then poured into crushed ice and the precipitate obtained was filtered, dried, and crystallized from ethanol.

N-[4-[N-[4-[5-[4-(Benzyloxy)phenyl]-1-(2-chlorophenyl)-1H-pyrazol-3-yl]phenyl]sulfamoyl]phenyl]acetamide (**4a**, $C_{36}H_{29}ClN_4O_4S$)
Yield 78 %; m.p.: 193–194 °C; IR (KBr): $\bar{\nu}$ = 3332, 3321 (NH), 3031 (CH), 1640 (C=O), 1610 (C=N), 1184 (SO_2), 743 (C–Cl) cm^{-1}; ^1H NMR (DMSO-d_6): δ = 2.76 (s, 3H, CH_3), 5.15 (s, 2H, OCH_2), 7.05 (s, 1H, pyrazole H-4), 7.07–8.38 (m, 21H, Ar–H), 8.40 (s, 1H, CONH, D_2O exchangeable), 10.26 (s, 1H, SO_2NH, D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6): δ = 29.8 (CH_3), 71.4 (OCH_2), 105.3 (pyrazole C_4), 113.7, 114.0, 114.8, 115.3, 118.7, 119.8, 121.2, 122.3, 123.5, 125.1, 128.4, 128.6, 128.7, 128.9, 130.1, 130.4, 134.7, 136.4, 136.9, 138.2, 144.6, 151.5, 159.0 (pyrazole C_5), 160.8 (pyrazole C_3), 171.8 (CONH) ppm; MS: *m/z* = 650 ($[M + 1]^+$), 651 ($[M + 2]^+$).

N-[4-[N-[4-[5-[4-(Benzyloxy)phenyl]-1-(3-chlorophenyl)-1H-pyrazol-3-yl]phenyl]sulfamoyl]phenyl]acetamide (**4b**, $C_{36}H_{29}ClN_4O_4S$)
Yield 71 %; m.p.: 185–186 °C; IR (KBr): $\bar{\nu}$ = 3328, 3217 (NH), 3037 (CH), 1630 (C=O), 1612 (C=N), 1191 (SO_2), 792 (C–Cl) cm^{-1}; ^1H NMR (DMSO-d_6): δ = 2.89 (s, 3H, CH_3), 5.18 (s, 2H, OCH_2), 7.13 (s, 1H, pyrazole H-4), 7.29–8.14 (m, 21H, Ar–H), 8.42 (s, 1H, CONH, D_2O exchangeable), 10.18 (s, 1H, SO_2NH, D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6): δ = 29.3 (CH_3), 71.6 (OCH_2), 105.2 (pyrazole C_4), 113.6, 114.2, 114.6, 115.1, 118.6, 118.9, 120.8, 122.5, 123.7, 126.7, 127.3, 128.2, 128.5, 128.8, 130.1, 130.4, 133.4, 136.7, 137.5, 138.7, 142.3, 152.1, 158.8 (pyrazole C_5), 159.6 (pyrazole C_3), 170.2 (CONH) ppm; MS: *m/z* = 650 ($[M + 1]^+$), 651 ($[M + 2]^+$).

N-[4-[N-[4-[5-[4-(Benzyloxy)phenyl]-1-(4-chlorophenyl)-1H-pyrazol-3-yl]phenyl]sulfamoyl]phenyl]acetamide (**4c**, $C_{36}H_{29}ClN_4O_4S$)
Yield 70 %; m.p.: 183–184 °C; IR (KBr): $\bar{\nu}$ = 3337, 3220 (NH), 3031 (CH), 1645 (C=O), 1614 (C=N), 1190 (SO_2), 790 (C–Cl) cm^{-1}; ^1H NMR (DMSO-d_6): δ = 2.78 (s, 3H, CH_3), 5.19 (s, 2H, OCH_2), 7.05 (s, 1H, pyrazole H-4), 7.11–7.83 (m, 21H, Ar–H), 8.90 (s, 1H, CONH, D_2O exchangeable), 10.22 (s, 1H, SO_2NH, D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6): δ = 32.1 (CH_3), 71.9 (OCH_2), 105.1 (pyrazole C_4), 113.4, 114.1, 114.8, 115.5, 116.7, 119.5, 120.5, 122.3, 123.5, 125.7, 127.7, 128.7, 128.9, 129.6, 130.3, 130.8, 133.8, 136.1, 137.4, 139.0, 142.5 (pyrazole C_5), 160.7 (pyrazole C_3), 171.8 (CONH) ppm; MS: *m/z* = 650 ($[M + 1]^+$), 651 ($[M + 2]^+$).

N-[4-[N-[4-[5-[4-(Benzyloxy)phenyl]-1-(3,4-dichlorophenyl)-1H-pyrazol-3-yl]phenyl]sulfamoyl]phenyl]acetamide (**4d**, $C_{36}H_{28}Cl_2N_4O_4S$)
Yield 77 %; m.p.: 220–222 °C; IR (KBr): $\bar{\nu}$ = 3342, 3219 (NH), 3028 (CH), 1665 (C=O), 1609 (C=N), 1187 (SO_2), 788 (C–Cl) cm^{-1}; ^1H NMR (DMSO-d_6): δ = 2.73 (s, 3H, CH_3), 5.21 (s, 2H, OCH_2), 7.08 (s, 1H, pyrazole H-4), 7.23–8.10 (m, 20H, Ar–H), 8.45 (s, 1H, CONH, D_2O exchangeable), 10.30 (s, 1H, SO_2NH, D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6): δ = 31.5 (CH_3), 71.3 (OCH_2), 107.3 (pyrazole C_4), 113.2, 114.1, 114.6, 115.3, 115.7, 118.7, 119.6, 120.1, 123.8, 125.9, 127.5, 128.0, 128.5, 129.4, 130.8, 131.9, 133.6, 134.1, 136.2, 137.3, 139.7, 142.4, 152.3 (pyrazole C_5), 160.0 (pyrazole C_3), 171.8 (CONH) ppm; MS: *m/z* = 684 ($[M + 1]^+$), 685($[M + 2]^+$).

N-[4-[N-[4-[5-[4-(Benzyloxy)phenyl]-1-(4-fluorophenyl)-1H-pyrazol-3-yl]phenyl]sulfamoyl]phenyl]acetamide (**4e**, $C_{36}H_{29}FN_4O_4S$)
Yield 77 %; m.p.: 226–227 °C; IR (KBr): $\bar{\nu}$ = 3340, 3221 (NH), 3025 (CH), 1669 (C=O), 1613 (C=N), 1192 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ = 2.92 (s, 3H, CH_3), 5.21 (s, 2H, OCH_2), 7.06 (s, 1H, pyrazole H-4), 7.11–7.86 (m, 21H, Ar–H), 8.51 (s, 1H, CONH, D_2O exchangeable), 10.24 (s, 1H, SO_2NH, D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6): δ = 31.5 (CH_3), 72.1 (OCH_2), 105.1 (pyrazole C_4), 113.9, 114.3, 114.9, 115.5, 119.8, 120.4, 123.2, 125.5, 127.7, 128.1, 128.9, 129.1, 130.2, 131.6, 133.4, 134.2, 135.5, 137.8, 139.2, 140.1, 152.6 (pyrazole C_5), 160.7 (pyrazole C_3), 171.4 (CONH) ppm; MS: *m/z* = 633 ($[M + 1]^+$).

N-[4-[N-[4-[5-[4-(Benzyloxy)phenyl]-1-(4-fluoro-3-chlorophenyl)-1H-pyrazol-3-yl]phenyl]sulfamoyl]phenyl]acetamide (**4f**, $C_{36}H_{28}ClFN_4O_4S$)
Yield 74 %; m.p.: 224–225 °C; IR (KBr): $\bar{\nu}$ = 3343, 3218 (NH), 3015 (CH), 1642 (C=O), 1614 (C=N), 1187 (SO_2), 782 (C–Cl) cm^{-1}; ^1H NMR (DMSO-d_6): δ = 2.74 (s, 3H, CH_3), 5.20 (s, 2H, OCH_2), 7.03 (s, 1H, pyrazole H-4), 7.14–7.89 (m, 20H, Ar–H), 8.83 (s, 1H, CONH, D_2O exchangeable), 10.27 (s, 1H, SO_2NH, D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6): δ = 28.5 (CH_3), 71.6 (OCH_2), 105.4 (pyrazole C_4), 113.1, 113.4, 113.5, 114.0, 114.3, 115.8, 119.0, 120.6, 121.2, 125.7, 127.2, 128.9, 129.5, 129.8, 130.6, 131.1, 133.3, 134.5, 135.8, 137.2, 139.2, 140.5, 152.6 (pyrazole C_5), 160.2 (pyrazole C_3), 172.4 (CONH) ppm; MS: *m/z* = 668 ($[M + 1]^+$), 669 ($[M + 2]^+$).

N-[4-[N-[4-[5-[4-(Benzyloxy)phenyl]-1-(4-bromophenyl)-1H-pyrazol-3-yl]phenyl]sulfamoyl]phenyl]acetamide (**4g**, $C_{36}H_{29}BrN_4O_4S$)
Yield 72 %; m.p.: 220–222 °C; IR (KBr): $\bar{\nu}$ = 3345, 3320 (NH), 3018 (CH), 1645 (C=O), 1608 (C=N), 1183 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ = 2.76 (s, 3H, CH_3), 5.21 (s, 2H, OCH_2), 7.06 (s, 1H, pyrazole H-4), 7.11–7.89 (m, 21H, Ar–H), 8.72 (s, 1H, CONH, D_2O exchangeable), 10.19 (s, 1H, SO_2NH, D_2O exchangeable) ppm; ^{13}C NMR

(DMSO-d_6): $\delta = 32.6$ (CH_3), 72.1 (OCH_2), 105.3 (pyrazole C_4), 113.7, 114.3, 114.8, 115.5, 119.6, 120.2, 121.2, 125.7, 127.1, 128.5, 129.0, 129.4, 130.3, 131.2, 133.9, 134.3, 135.5, 137.3, 138.6, 142.5, 152.5 (pyrazole C_5), 160.8 (pyrazole C_3), 172.6 (CONH) ppm; MS: $m/z = 694$ ($[M + 1]^+$).

N-[4-[N-[4-[5-[4-(Benzyloxy)phenyl]-1-(4-nitrophenyl)-1H-pyrazol-3-yl]phenyl]sulfamoyl]phenyl]acetamide (**4h**, $C_{36}H_{29}N_5O_6S$)
Yield 71 %; m.p.: 230–232 °C; IR (KBr): $\bar{\nu} = 3338$, 3221 (NH), 3014 (CH), 1642 (C=O), 1610 (C=N), 1190 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): $\delta = 2.94$ (s, 3H, CH_3), 5.22 (s, 2H, OCH_2), 7.04 (s, 1H, pyrazole H-4), 7.16–7.83 (m, 21H, Ar–H), 8.87 (s, 1H, CONH, D_2O exchangeable), 10.29 (s, 1H, SO_2NH, D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 32.3$ (CH_3), 71.8 (OCH_2), 109.2 (pyrazole C_4), 113.6, 114.2, 114.8, 115.1, 119.4, 120.8, 124.7, 125.1, 127.8, 128.3, 129.4, 129.9, 130.1, 133.2, 133.6, 134.2, 135.7, 137.8, 138.9, 142.2, 155.0 (pyrazole C_5), 160.4 (pyrazole C_3), 171.8 (CONH) ppm; MS: $m/z = 660$ ($[M + 1]^+$).

N-[4-[N-[4-[5-[4-(Benzyloxy)phenyl]-1-(2,4-dinitrophenyl)-1H-pyrazol-3-yl]phenyl]sulfamoyl]phenyl]acetamide (**4i**, $C_{36}H_{28}N_6O_8S$)
Yield 71 %; m.p.: 256–257 °C; IR (KBr): $\bar{\nu} = 3342$, 3220 (NH), 3017 (CH), 1647 (C=O), 1607 (C=N), 1188 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): $\delta = 2.76$ (s, 3H, CH_3), 5.19 (s, 2H, OCH_2), 7.08 (s, 1H, pyrazole H-4), 7.14–7.89 (m, 20H, Ar–H), 8.76 (s, 1H, CONH, D_2O exchangeable), 10.22 (s, 1H, SO_2NH, D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 31.3$ (CH_3), 72.8 (OCH_2), 105.7 (pyrazole C_4), 112.1, 113.8, 114.2, 114.7, 115.4, 118.7, 119.6, 120.2, 124.3, 125.7, 127.2, 128.1, 129.3, 129.7, 130.5, 133.6, 134.1, 135.2, 135.6, 137.1, 138.2, 141.1, 155.8 (pyrazole C_5), 160.7 (pyrazole C_3), 171.2 (CONH) ppm; MS: $m/z = 705$ ($[M + 1]^+$).

N-[4-[N-[4-[5-[4-(Benzyloxy)phenyl]-1-(2-methylphenyl)-1H-pyrazol-3-yl]phenyl]sulfamoyl]phenyl]acetamide (**4j**, $C_{37}H_{32}N_4O_4S$)
Yield 72 %; m.p.: 150–152 °C; IR (KBr): $\bar{\nu} = 3343$, 3323 (NH), 3019 (CH), 1642 (C=O), 1616 (C=N), 1191 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): $\delta = 2.28$ (s, 3H, CH_3), 2.98 (s, 3H, CH_3), 5.17 (s, 2H, OCH_2), 7.05 (s, 1H, pyrazole H-4), 7.18–7.82 (m, 21H, Ar–H), 8.77 (s, 1H, CONH, D_2O exchangeable), 10.29 (s, 1H, SO_2NH, D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 18.2$ (CH_3), 32.3 (CH_3), 71.8 (OCH_2), 105.7 (pyrazole C_4), 112.4, 113.2, 113.6, 114.1, 114.5, 115.6, 119.2, 120.4, 124.8, 126.9, 127.8, 128.4, 129.3, 129.8, 130.1, 134.6, 134.9, 135.1, 135.8, 138.3, 138.5, 141.6, 155.2 (pyrazole C_5), 160.3 (pyrazole C_3), 169.9 (CONH) ppm; MS: $m/z = 629$ ($[M + 1]^+$).

N-[4-[N-[4-[5-[4-(Benzyloxy)phenyl]-1-(3-methylphenyl)-1H-pyrazol-3-yl]phenyl]sulfamoyl]phenyl]acetamide (**4k**, $C_{37}H_{32}N_4O_4S$)
Yield 73 %; m.p.: 155–156 °C; IR (KBr): $\bar{\nu} = 3337$, 3225 (NH), 3021 (CH), 1640 (C=O), 1611 (C=N), 1189 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): $\delta = 2.17$ (s, 3H, CH_3), 3.12 (s, 3H, CH_3), 5.20 (s, 2H, OCH_2), 7.07 (s, 1H, pyrazole H-4), 7.12–7.89 (m, 21H, Ar–H), 8.71 (s, 1H, CONH, D_2O exchangeable), 10.31 (s, 1H, SO_2NH, D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 17.1$ (CH_3), 32.9 (CH_3), 71.5 (OCH_2), 109.3 (pyrazole C_4), 112.3, 114.0, 114.1, 114.4, 115.3, 115.8, 119.1, 122.0, 124.4, 126.7, 127.6, 128.2, 129.1, 129.6, 130.4, 133.5, 134.6, 135.2, 135.8, 138.2, 138.9, 140.6, 155.7 (pyrazole C_5), 160.7 (pyrazole C_3), 171.3 (CONH) ppm; MS: $m/z = 629$ ($[M + 1]^+$).

N-[4-[N-[4-[5-[4-(Benzyloxy)phenyl]-1-(4-methylphenyl)-1H-pyrazol-3-yl]phenyl]sulfamoyl]phenyl]acetamide (**4l**, $C_{37}H_{32}N_4O_4S$)
Yield 73 %; m.p.: 159–160 °C; IR (KBr): $\bar{\nu} = 3341$, 3220 (NH), 3014 (CH), 1637 (C=O), 1614 (C=N), 1190 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): $\delta = 2.04$ (s, 3H, CH_3), 2.73 (s, 3H, CH_3), 5.18 (s, 2H, OCH_2), 7.03 (s, 1H, pyrazole H-4), 7.15–7.87 (m, 21H, Ar–H), 8.73 (s, 1H, CONH, D_2O exchangeable), 10.20 (s, 1H, SO_2NH, D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 17.4$ (CH_3), 32.9 (CH_3), 71.1 (OCH_2), 110.3 (pyrazole C_4), 112.9, 113.8, 114.7, 115.2, 119.6, 122.8, 124.1, 126.3, 127.5, 128.2, 129.0, 129.8, 130.2, 133.9, 134.5, 134.7, 135.2, 138.6, 138.9, 140.1, 155.3 (pyrazole C_5), 160.1 (pyrazole C_3), 172.3 (CONH) ppm; MS: $m/z = 629$ ($[M + 1]^+$).

N-[4-[N-[4-[5-[4-(Benzyloxy)phenyl]-1-(2,6-dimethylphenyl)-1H-pyrazol-3-yl]phenyl]sulfamoyl]phenyl]acetamide (**4m**, $C_{38}H_{34}N_4O_4S$)
Yield 70 %; m.p.: 262–264 °C; IR (KBr): $\bar{\nu} = 3339$, 3221 (NH), 3013 (CH), 1642 (C=O), 1612 (C=N), 1186 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): $\delta = 2.18$ (s, 6H, 2 CH_3), 3.01 (s, 3H, CH_3), 5.19 (s, 2H, OCH_2), 7.08 (s, 1H, pyrazole H-4), 7.13–7.83 (m, 20H, Ar–H), 8.33 (s, 1H, CONH, D_2O exchangeable), 10.18 (s, 1H, SO_2NH, D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 17.7$ (2 CH_3), 32.6 (CH_3), 71.3 (OCH_2), 110.3 (pyrazole C_4), 113.2, 114.4, 114.9, 115.4, 119.7, 122.7, 124.3, 127.3, 127.6, 128.8, 129.1, 129.5, 130.9, 133.9, 134.7, 134.9, 135.0, 138.8, 138.9, 140.3, 155.5 (pyrazole C_5), 160.7 (pyrazole C_3), 171.3 (CONH) ppm; MS: $m/z = 643$ ($[M + 1]^+$).

N-[4-[N-[4-[5-[4-(Benzyloxy)phenyl]-1-(2-methoxyphenyl)-1H-pyrazol-3-yl]phenyl]sulfamoyl]phenyl]acetamide (**4n**, $C_{37}H_{32}N_4O_5S$)
Yield 74 %; m.p.: 285–286 °C; IR (KBr): $\bar{\nu} = 3342$, 3217 (NH), 3017 (CH), 1653 (C=O), 1610 (C=N), 1191 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): $\delta = 2.89$ (s, 3H, CH_3), 3.71 (s,

3H, OCH_3), 5.21 (s, 2H, OCH_2), 7.06 (s, 1H, pyrazole H-4), 7.14–7.89 (m, 21H, Ar–H), 8.32 (s, 1H, CONH, D_2O exchangeable), 10.26 (s, 1H, SO_2NH, D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 32.0$ (CH_3), 56.1 (OCH_3), 71.3 (OCH_2), 110.1 (pyrazole C_4), 113.4, 113.7, 114.2, 114.9, 115.4, 119.7, 122.5, 124.0, 127.4, 127.6, 128.1, 129.5, 129.8, 130.4, 133.1, 133.3, 134.6, 134.9, 135.8, 138.3, 138.5, 140.2, 155.0 (pyrazole C_5), 160.1 (pyrazole C_3), 171.6 (CONH) ppm; MS: $m/z = 647$ ($[M + 1]^+$).

N-[4-[N-[4-[5-[4-(Benzyloxy)phenyl]-1-(4-methoxyphenyl)-1H-pyrazol-3-yl]phenyl]sulfamoyl]phenyl]acetamide (**4o**, $C_{37}H_{32}N_4O_5S$)
Yield 74 %; m.p.: 286–287 °C; IR (KBr): $\bar{\nu} = 3337$, 3215 (NH), 3010 (CH), 1653 (C=O), 1615 (C=N), 1186 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): $\delta = 2.72$ (s, 3H, CH_3), 3.76 (s, 3H, OCH_3), 5.21 (s, 2H, OCH_2), 7.08 (s, 1H, pyrazole H-4), 7.14–7.92 (m, 21H, Ar–H), 8.34 (s, 1H, CONH, D_2O exchangeable), 10.20 (s, 1H, SO_2NH, D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 32.6$ (CH_3), 57.2 (OCH_3), 71.6 (OCH_2), 110.7 (pyrazole C_4), 113.2, 113.2, 114.6, 115.1, 119.7, 120.7, 122.6, 124.5, 127.3, 127.4, 128.9, 129.0, 129.6, 130.1, 134.5, 134.6, 135.8, 138.3, 138.5, 140.8, 153.7 (pyrazole C_5), 160.6 (pyrazole C_3), 172.4 (CONH) ppm; MS: $m/z = 647$ ($[M + 1]^+$).

Anti-inflammatory screening

The synthesized compounds were screened for anti-inflammatory activity by the inhibition of albumin denaturation technique [24]. Diclofenac sodium (standard drug) and test compounds were dissolved in minimum amount of DMF and diluted with phosphate buffer saline (pH 7.4) in such a way that concentration of DMF in all solutions was less than 2.5 %. Test solutions (1 cm^3, 100 μg/cm^3) were mixed with 1 cm^3 of 1 % albumin solution in phosphate buffer saline and incubated at 27 ± 1 °C in an incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60 ± 1 °C in a water bath for 10 min. After cooling, the turbidity was measured at 660 nm with UV–Vis spectrophotometer. Percentage inhibition of denaturation was then calculated from control where no drug was added. Each experiment was done in triplicate and average taken. The percentage of inhibition was calculated using the formula:

$$\% \text{ inhibition of denaturation} = [(V_t/V_c) - 1] \times 100$$

where V_t = mean absorption of test compound and V_c = mean absorption of control.

Antioxidant assay

All the compounds were evaluated in vitro for their 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging potential as described earlier [25]. Stock solutions of different compounds (1 mM) were mixed with DPPH methanol solution (0.5 cm^3, 0.3 mM) in 3 cm^3 of total reaction mixture and allowed to react at room temperature. After 30 min, absorbance was measured at 520 nm and converted to % antioxidant activity. For a comparative study BHA was used as the standard. The percentage inhibition activity was calculated by using the formula:

$$\% \text{ activity} = [1 - (A_t/A_c)] \times 100$$

where A_t = absorbance of test compound and A_c absorbance of control compound.

In vivo studies

Twelve-week-old adult male/female Wistar rats (150–200 g) and Balb/c mice (20–30 g) used in the present study were housed and kept in accordance with the Hamdard University Animal Care Unit, which applies the guidelines and rules laid down by Committee for the purpose of control and supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Animals were housed in a ventilated room at 25 ± 2 °C under a 12 h light/dark cycle in large spacious polypropylene cages. Animals were allowed to acclimatize for 1 week before the study and had free access to standard laboratory feed and water ad libitum.

Anti-inflammatory activity

The synthesized compounds were evaluated for their anti-inflammatory activity using carrageenan induced rat hind paw edema method [26]. The animals were randomly allocated into groups of six animals each and fasted for 24 h before the experiment with free access to water. Control group received only 0.5 % CMC solution. Standard, diclofenac sodium was administered orally at a dose of 10 mg/kg. The test compounds were administered orally at an equimolar oral dose relative to 10 mg/kg diclofenac sodium. 0.1 cm^3 of 1 % carrageenan solution in saline was injected subcutaneously into the sub plantar region of the right hind paw of each rat, 1 h after the administration of the test compounds and standard drug. The right hind paw volume was measured before and after 3 and 4 h of carrageenan treatment by means of a plethysmometer. The percent edema inhibition was calculated from the mean effect in the control and treated animals according to the following equation:

$$\% \text{ edema inhibition} = (V_c - V_t/V_c) \times 100$$

where V_t represents the mean increase in paw volume in rats treated with test compounds and V_c represents the mean increase in paw volume in control group of rats. For

the ulcerogenic activity the same group of rats which were used for anti-inflammatory activity were used after a washout period of 15 days.

Ulcerogenic activity

The ulcerogenic effect of five active compounds and diclofenac sodium was evaluated by the reported method [27]. The rats were allocated into different groups consisting of six animals in each group. Ulcerogenic activity was evaluated after oral administration of the test compounds at an equimolar dose relative to 30 mg/kg diclofenac sodium. Control group received only 0.5 % carboxymethylcellulose (CMC) solution. Food but not water was stopped 24 h before administration of the test compounds. After the drug treatment, the rats were fed with normal diet for 17 h and then sacrificed. The stomach was removed and opened along the greater curvature, washed with distilled water, and cleaned gently by dipping in normal saline. The mucosal damage was examined by means of a magnifying glass. For each stomach the mucosal damage was assessed according to the following scoring system: 0.5: redness, 1.0: spot ulcers, 1.5: hemorrhagic streaks, 2.0: ulcers >3 but $\leq$5, 3.0: ulcers >5. The mean score of each treated group minus the mean score of control group was regarded as severity index of gastric mucosal damage.

Lipid peroxidation

Lipid peroxidation in the gastric mucosa was determined according to the method of Ohkawa et al. [28] method. After screening the stomach for ulcers the gastric mucosa of glandular portion was scrapped with the help of two glass slides. Gastric mucosa was weighed (100 mg) and homogenized in mortar and pestle with 1.8 cm^3 ice-cold 1.15 % KCl solution. The homogenate supplemented with 0.2 cm^3 of 8.1 % sodium dodecyl sulfate (SDS), 1.5 cm^3 of acetate buffer, and 1.5 cm^3 of 0.8 % thiobarbituric acid. The mixture was then incubated at 95 °C for 60 min on boiling water bath. The reaction mixture was kept at room temperature for some time and then extracted with a mixture of *n*-butanol: pyridine (15:1, v/v; 5 cm^3) by shaking vigorously for 1 min and then keeping in ice for 2 min. The organic layer was centrifuged at 3000 rpm for 10 min. Organic layer was separated out and absorbance measured at 532 nm on UV spectrophotometer. Results were expressed as nmol MDA/100 mg tissue using extinction coefficient 1.56×10^5 cm^{-1} M^{-1}. Concentration = (absorbance $\times$ volume $\times$ 10^9)/(1.56×10^8).

Determination of the inhibition of LPS-induced TNF-α production

TNF-α production inhibition in mice (Balb/c) weighing 20–30 g was carried out by the already reported procedure [29, 30]. Mice were deprived of food overnight, but not water. The next day, these mice were orally administered with test compounds at an equimolar oral dose relative to 30 mg/kg SB 203580 inhibitor suspended in 0.5 % CMC aqueous solution at 10 cm^3/kg. The groups of normal and control mice were administered only with 0.5 % CMC aqueous solution at 10 cm^3/kg. Thirty minutes later, LPS solution dissolved in physiological saline was intravenously injected at 0.45 mg/10 cm^3/kg except for the group of normal mice, which were injected with physiological saline at 10 cm^3/kg. One hour later, blood samples were taken from the mice from the vena cava inferior into a syringe containing heparin sodium. After centrifugation of the blood samples at 13,230*g* for 3 min at 4 °C, plasma samples were taken immediately and frozen at −20 °C until measurement of the concentration of TNF-α. The concentrations of TNF-α in the plasma samples were measured with commercially available ELISA kit. The percent inhibition of TNF-α production was obtained by the following equation: Percent inhibition = [1 − (concentration of TNF-α in plasma sample of mice administrated test compounds − mean concentration of TNF-α in the plasma samples of normal mice)/(concentration of TNF-α in the plasma samples in control mice − mean concentration of TNF-α in the plasma samples of normal mice)] × 100.

Molecular docking study

For the purpose of assessment of docking of ligands to protein active sites for estimation of binding affinities of docked compounds, an advanced molecular docking programme GLIDE (Schrodinger Inc., USA) version 9.8 was used. GLIDE, the Grid-based ligand docking with energetics algorithm approximates a systematic search of positions, orientations and conformations and eliminates unwanted conformations using scoring in the enzyme pocket via a series of hierarchical filters. Finally the conformations were refined via Monte Carlo sampling of pose conformation. Glide provides three different levels of docking precisions viz. High Throughput Virtual Screening, HTVS; Standard precision, SP and Extra precision, XP. We carried out our calculations using XP docking mode as the tool is designed for better refinement in ligands. X-ray crystal structure of p38α MAP kinase was

taken from PDB entry 3D83 having resolution of 1.90 Å. For docking studies protein structures were prepared using protein preparation wizard in Maestro 9.8. Protein preparation was carried out in two steps viz. preparation and refinement. Chemical correctness was ensured and water molecules in crystal structure were deleted and hydrogen atoms were added at missing positions and bond order for crystal ligand and protein was adjusted and minimized up to 0.30 Å RMSD. Ligprep 2.2 module in maestro 9.8 build panel was used for ligand preparation which produced low energy conformations of ligand using OPLS 2005 force field [31].

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