Med Chem Res (2015) 24:4191–4206
DOI 10.1007/s00044-015-1467-9

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

# Synthesis, molecular docking and biological evaluation of novel bis-pyrazole derivatives for analgesic, anti-inflammatory and antimicrobial activities

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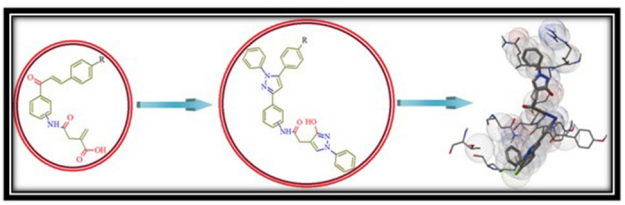
Received: 21 April 2015 / Accepted: 4 September 2015 / Published online: 11 September 2015


**Abstract** A new series of bis-pyrazoles were synthesized by Michael addition of hydrazine to chalcones. The starting-material-substituted acetophenones required for the synthesis of chalcones were prepared from itaconic anhydride. The newly synthesized compounds were characterized by IR, $^{1}$H-NMR, $^{13}$C-NMR, mass spectral and analytical data. All the synthesized compounds were evaluated for in vivo analgesic, anti-inflammatory and in vitro antimicrobial activities. Among the tested compounds, **5a**, **5b** and **5d** showed potential anti-inflammatory and analgesic activities. Further anti-inflammatory results were supported by *in silico* docking study, in which tested bis-pyrazoles were found to be more selective toward COX-2 (PDB ID: 1CX2) rather than COX-1 (PDB ID: 1CQE). The $LD_{50}$ values for these products **5**(**a**–**l**) showed a high safety margin with a dose level >2000 mg/kg. Among all synthesized compounds, *N*-[4-(5-(4-bromophenyl)-1-phenyl-1*H*-pyrazol-3-yl)phenyl-2-(3-hydroxy-1-phenyl-1*H*-pyrazol-4-yl)] acetamide (**5b**) emerged as most potent molecule with anti-inflammatory, analgesic and antimicrobial properties.

*Graphical Abstract*



**Keywords** Bis-pyrazoles · Analgesic activity · Anti-inflammatory activity · Antimicrobial activity · Molecular docking study

## Introduction

The development of an effective therapeutic agent for management of inflammation has undergone continual evolution leading to the emergence of more efficacious class of drugs. Since the discovery of aspirin, many efforts have been devoted to the development of nonsteroidal anti-inflammatory drugs (NSAIDs). Their mechanism of action involves inhibition of the enzyme cyclooxygenase (COX), which catalyzes the rate-limiting step in the formation of prostanoids, prostaglandins (PGs) and thromboxane A2 (TxA2) (Vane, 1971). It is predicted that COX-2 enzyme is responsible for some aspects of pain and inflammation in arthritis, while constitutive COX-1 enzyme appears to be

**Electronic supplementary material** The online version of this article (doi:10.1007/s00044-015-1467-9) contains supplementary material, which is available to authorized users.

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responsible for most of gastroprotective prostaglandin synthesis in the stomach and duodenum. It is also suggested that COX-2 inhibitors are not only promising agents for treatment of pain and inflammation, but also used for prevention of cancer (Marnett and Kalgutkar, 1998). Thus, search for novel anti-inflammatory drugs with minimal gastrointestinal side effects and high safety margin is still warranted.

Pyrazole derivatives have a long history of application in agrochemicals and pharmaceutical industries as herbicides and active pharmaceuticals. The recent success of pyrazole-derived COX-2 inhibitors further highlights the importance of pyrazole as biodynamic ring system. Many useful clinical agents, such as difenamizole (Kameyama and Nabeshima, 1978; Kameyama *et al.*, 1978), celecoxib (Penning *et al.*, 1997) and tepoxalin (Anderson *et al.*, 1990) do possess pyrazole ring system. The prevalence of pyrazole cores in biologically active molecules has stimulated the need for elegant and efficient ways to make these heterocyclic leads. Microbial infections often produce pain and inflammation. Analgesic and anti-inflammatory drugs are prescribed simultaneously in normal practice under chemotherapy. The compound possessing all three activities is not common. It has been reported that certain pyrazole derivatives possess analgesic, anti-inflammatory (Amir and Kumar, 2005; Zelenin *et al.*, 1999; Adnan *et al.*, 2005), antimicrobial (Susant *et al.*, 2007; Akihiko *et al.*, 2005; Goda *et al.*, 2003), antitumor, antioxidant, antiviral, antiparasitic, antitubercular and insecticidal activities (Amir *et al.*, 2008; Hes *et al.*, 1978; Grosscurt *et al.*, 1979). In addition, pyrazoles have also played a crucial part in the development of theory in heterocyclic chemistry and are also used extensively in organic synthesis (Klimova *et al.*, 1999).

Motivated by these findings, coupled with our ongoing research on pyrazoles and other heterocyclic compounds (Nayak *et al.*, 2014a, b, c, d, e), it was decided to synthesize, some novel series of bis-pyrazole derivatives with a potential for acting synergistically as analgesic, anti-inflammatory and antimicrobial agents with minimal gastrointestinal side effects and high safety margin.

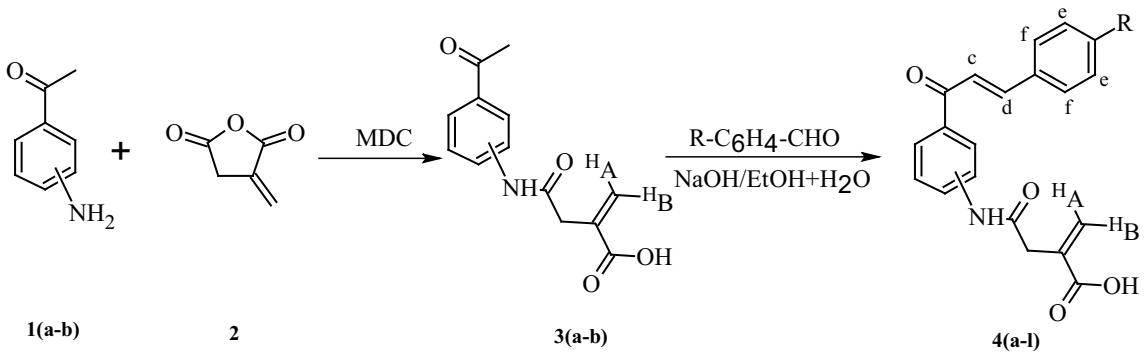
## Results and discussion

### Chemistry

A new series of bis-pyrazole derivatives were prepared starting form itaconic anhydride (ITA) and 3/4-aminoacetophenone. The resultant oxobutanoic acids **3**(**a**–**b**) (Scheme 1) were treated with 4-substituted aryl aldehydes to yield chalcone derivatives **4**(**a**–**l**). These intermediate chalcones were converted into bis-pyrazole derivatives by condensing with phenyl hydrazine according to the reaction sequence depicted in Scheme 2. The formation of bis-pyrazole products and other intermediates was confirmed by spectral and elemental analysis.

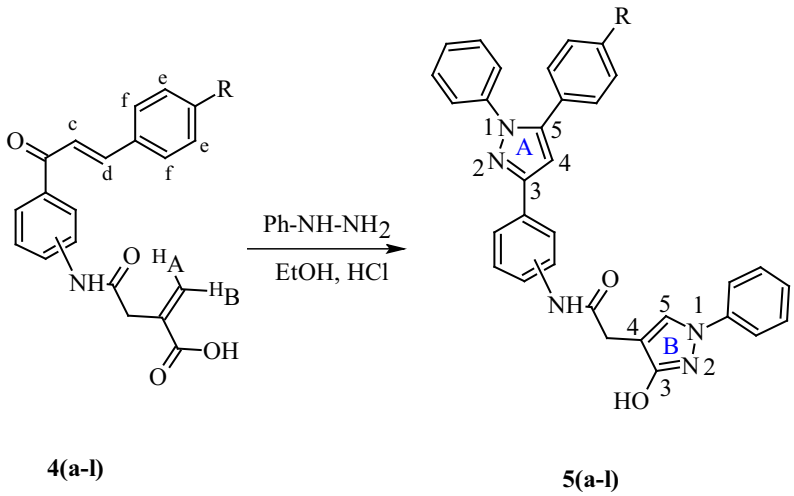
In the first step, the intermediate γ-oxobutanoic acids (**3a**, **3b**) required for the synthesis of chalcones were prepared by the reaction of nucleophilic reagents with ITA. This formation of the products was confirmed by single-crystal XRD data (Fig. 1) (Narayana *et al.*, 2014; Nayak *et al.*, 2014a, b, c, d, e). During single-crystal X-ray diffraction study of compounds **3a** (CCDC No. 1005968), **3b** (CCDC No. 949575), a twist between the mean planes of methylidene group and amide group to an extent of approximately 100° was found, which confirmed *E* (*trans*) form of the oxobutanoic acids (**3a**, **3b**) (Nayak *et al.*, 2014c).

The Claisen–Schmidt condensation of oxobutanoic acids (**3a, 3b**) with differently substituted aldehydes yielded intermediate chalcones. The IR spectra of intermediate chalcones exhibited stretching bands in the region of 3275–3345, 3059–3136, 1678–1688 and 1652–1665 $cm^{-1}$ representing NH, OH, amide C=O and acid C=O functional




**Scheme 1** Synthetic pathways to chalcone derivatives **4**(**a**–**l**)

**Scheme 2** Synthetic pathways to bis-pyrazole derivatives **5(a–l)**



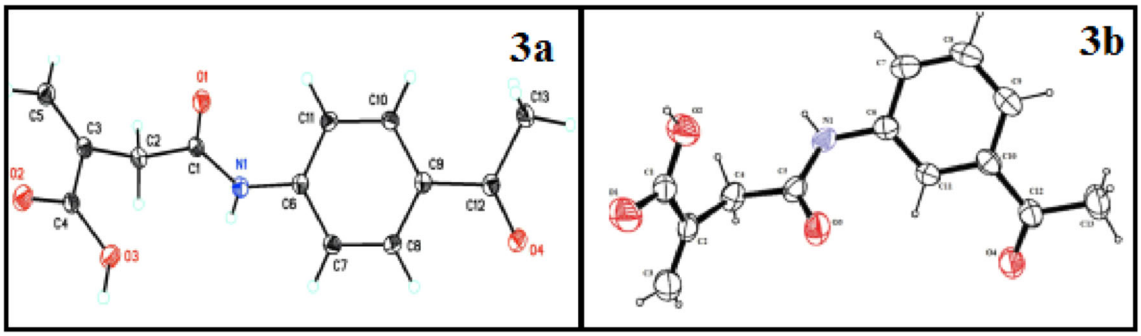




**Fig. 1** ORTEP drawing of the molecules, showing the labeling scheme with 30 % (**3a**) and 40 % (**3b**) probability displacement *ellipsoids*

groups, respectively. These data suggested the formation of intermediate chalcones. The $^{1}$H-NMR spectrum of representative chalcone **4k** displayed two doublets at $\delta$ 7.48 ppm and $\delta$ 7.79 ppm due to $H_c$ and $H_d$ protons. The coupling constant ($J$) for $H_c$ and $H_d$ was 15.6 Hz characteristic of (*E*)-isomer of the chalcone. Similarly, two more doublets appeared at $\delta$ 5.57 ppm and $\delta$ 6.50 ppm due to $H_A$ and $H_B$ of methylidene protons of oxobutanoic acid group. The coupling constant ($J$) for $H_A$ and $H_B$ was found to be 1.6 Hz characteristic of geminal coupling of methylidene group. The signals for aromatic protons appeared as multiplets in the range of $\delta$ 6.95–7.96 ppm. The two singlets observed at $\delta$ 10.50 ppm and $\delta$ 11.55 ppm confirmed the presence of NH and OH protons. Further formation of chalcone products **4**(**a**–**i**) was confirmed by mass spectral data and elemental analysis.

The formation of bis-pyrazole derivatives **5**(**a**–**l**) was explained via formation of hydrazones followed by cyclization and dehydration process on the chalcone end, while at the other end, phenyl hydrazine condenses with oxobutanoic acid followed by the cyclization and dehydration. In the IR spectra of **5**(**a**–**l**), stretching band observed around 1670–1709 cm$^{-1}$ was due to the stretching vibration of amide carbonyl group, suggesting that acid group of oxobutanoic acid was also involved in the reaction with phenyl hydrazine to form an additional pyrazole ring. This suggested the formation of bis-pyrazole products. The $^{1}$H-NMR spectrum of representative bis-pyrazole **5a** exhibited two singlets at $\delta$ 6.58 ppm and $\delta$ 8.15 ppm, representing the protons attached to C-4 of pyrazole ring A and C-5 of pyrazole ring B, respectively. Similarly, a singlet observed at $\delta$ 5.43 ppm could be due to OH proton of pyrazolyl alcohol. The upfield shift of this signal suggested that enol form predominated over keto form. A singlet found at $\delta$ 10.10 ppm was attributed to NH protons. Absence of a broad singlet at $\delta$ 12.56 due to carboxylic OH of the precursor **4a** confirmed the formation of **5a**. Further signals for methylidene protons were also absent in the bis-pyrazole spectrum, which confirmed the formation of product **5a**. In $^{13}$C-NMR spectrum, peaks observed at $\delta$ 157.64, 149.56, 147.45, 122.67, 119.43 and 104.49 ppm were attributed to the number of pyrazole carbons (pyrazole A C-3, pyrazole A C-5, pyrazole B C-3, pyrazole B C-5, pyrazole B C-4 and pyrazole A C-4). Further

formation of bis-pyrazole products **5(a–i)** was confirmed by mass spectral studies and elemental analysis.

## Pharmacology

### *Analgesic activity*

Abdominal constriction induced by acetic acid is used to screen the peripheral analgesic effect (nonsteroidal type of analgesic action) (Vogel and Vogel, 2002a, b) mediated by local peritoneal receptors (Gene *et al.*, 1998). The results of analgesic activity as shown in Table 1 indicated that para amido bis-pyrazoles **5(a–f)** showed better activity in comparison with the meta amido bis-pyrazoles 5(**g–i**). Among the para amido bis-pyrazoles, halogen-substituted compounds **5a** (66.66 %), **5b** (66.66 %) and **5c** (64.58 %) showed good percentage of inhibition in comparison with that of the standard drug aspirin (68.75 %). Other compounds exhibited comparably less activity. The enhanced activity of these compounds might be due to the presence of halogen-substituted aryl moieties and para amido pyrazole functional group.

### *Anti-inflammatory activity*

The anti-inflammatory activity of the newly synthesized compounds **5(a–i)** was evaluated by carrageenan-induced paw edema model in rats using indomethacin as a reference drug. Results were expressed as mean ± SD and given in Table 2. Difference between control and treatment groups was evaluated for statistical significance using one-way ANOVA followed by Dunnett's *t* test. Compound **5b** (68.87 %), *N*-[4-(5-(4-bromophenyl)-1-phenyl-1*H*-pyrazol-3-yl)phenyl-2-(3-hydroxy-1-phenyl-1*H*-pyrazol-4-yl) acetamide] conferred maximum anti-inflammatory activity followed by **5a** (68.42 %) bearing fluoro substitution. Other compounds **5(c–i)** showed moderate-to-less activity at the dosage of 10 mg/kg p.o. Interestingly, it was also observed that para amido bis-pyrazoles **5(a–f)** showed better activity in comparison with the meta amido bis-pyrazoles **5(g–i)** similar to that of analgesic activity.

In general, para amido bis-pyrazoles [*N*-(4-(5-(4-substituted-phenyl)-1-phenyl-1*H*-pyrazol-3-yl)phenyl-2-(3-hydroxy-1-phenyl-1*H*-pyrazol-4-yl)acetamide)] **5(a–f)** showed good analgesic and anti-inflammatory activity. Among these, halogen-substituted compounds **5(a–c)** exhibited better activities than nonhalogenated compounds **5(d–f)**.

### *Acute toxicity*

There was no incidence of mortality among groups of animals which were fed with compounds **5(a–i)**, and they did not exhibit any toxicity or behavioral changes at a dose level of 2000 mg/kg. The compounds showed a high safety margin when screened at dose of 2000 mg/kg, p.o., and lethal dose ($LD_{50}$) values were found to be >2000 mg/kg.

### *Molecular docking study*

The synthesized bis-pyrazole derivatives **5(a–i)** were screened for *in silico* molecular docking with COX-1 (PDB

**Table 1** Analgesic activity compounds **5(a–l)** by acetic acid-induced writhing test

| Compounds | *R* | Dose (mg/kg) | Number of writhings | % inhibition |
|---|---|---|---|---|
| Control | – | 0.5 | 48.00 ± 0.90 | – |
| Aspirin | – | 10 | 15.00 ± 0.72* | 68.75 |
| **5a** | F | 25 | 16.00 ± 0.68* | 66.66 |
| **5b** | Br | 25 | 16.00 ± 0.79* | 66.66 |
| **5c** | Cl | 25 | 17.00 ± 0.59* | 64.58 |
| **5d** | $CH_3$ | 25 | 19.00 ± 0.81* | 60.41 |
| **5e** | $OCH_3$ | 25 | 19.00 ± 0.81* | 60.41 |
| **5f** | $CH(CH_3)_2$ | 25 | 18.00 ± 0.72* | 62.50 |
| **5g** | F | 25 | 19.00 ± 0.78* | 60.41 |
| **5h** | Br | 25 | 19.00 ± 0.67* | 60.41 |
| **5i** | Cl | 25 | 20.00 ± 0.79* | 58.33 |
| **5j** | $CH_3$ | 25 | 22.00 ± 0.63* | 54.16 |
| **5k** | $OCH_3$ | 25 | 21.00 ± 0.62* | 56.25 |
| **5l** | $CH(CH_3)_2$ | 25 | 20.00 ± 0.75* | 58.33 |

* The mean difference is significant at the 0.001 level, when compared to the control group

**Table 2** Anti-inflammatory activity compounds **5(a–l)** by carrageenan-induced paw edema model

| Compounds | *R* | Dose (mg/kg) | % decrease in paw volume at 3 h | | | | % decrease in paw volume at 3 h |
|---|---|---|---|---|---|---|---|
| | | | 0 h | 1 h | 2 h | 3 h | |
| Control | – | 0.1 | 0.073 ± 0.005 | 0.113 ± 0.005 | 0.160 ± 0.006 | 0.221 ± 0.008 | – |
| Indomethacin | – | 10 | 0.025 ± 0.009 | 0.031 ± 0.007* | 0.033 ± 0.005* | 0.043 ± 0.005* | 80.63 |
| **5a** | F | 10 | 0.048 ± 0.005 | 0.056 ± 0.006 | 0.065 ± 0.004 | 0.070 ± 0.005* | 68.42 |
| **5b** | Br | 10 | 0.042 ± 0.003 | 0.051 ± 0.006 | 0.060 ± 0.002 | 0.069 ± 0.004* | 68.87 |
| **5c** | Cl | 10 | 0.040 ± 0.005 | 0.050 ± 0.004 | 0.061 ± 0.005 | 0.072 ± 0.005* | 67.52 |
| **5d** | $CH_3$ | 10 | 0.044 ± 0.005 | 0.052 ± 0.006 | 0.066 ± 0.007 | 0.073 ± 0.006* | 67.07 |
| **5e** | $OCH_3$ | 10 | 0.048 ± 0.005 | 0.058 ± 0.006 | 0.067 ± 0.004 | 0.079 ± 0.008* | 64.36 |
| **5f** | $CH(CH_3)_2$ | 10 | 0.051 ± 0.004 | 0.065 ± 0.006 | 0.072 ± 0.005 | 0.073 ± 0.006* | 67.07 |
| **5g** | F | 10 | 0.050 ± 0.005 | 0.058 ± 0.006 | 0.069 ± 0.004 | 0.078 ± 0.005* | 64.70 |
| **5h** | Br | 10 | 0.450 ± 0.006 | 0.059 ± 0.005 | 0.068 ± 0.003 | 0.077 ± 0.005* | 65.51 |
| **5i** | Cl | 10 | 0.050 ± 0.006 | 0.058 ± 0.004 | 0.068 ± 0.005 | 0.080 ± 0.007* | 63.80 |
| **5j** | $CH_3$ | 10 | 0.046 ± 0.005 | 0.059 ± 0.006 | 0.071 ± 0.008 | 0.085 ± 0.006* | 61.53 |
| **5k** | $OCH_3$ | 10 | 0.050 ± 0.004 | 0.059 ± 0.005 | 0.069 ± 0.007 | 0.083 ± 0.006* | 62.44 |
| **5l** | $CH(CH_3)_2$ | 10 | 0.047 ± 0.005 | 0.058 ± 0.004 | 0.067 ± 0.006 | 0.081 ± 0.007* | 63.34 |

* The mean difference is significant at the 0.001 level, when compared to the control group

ID: 1CQE) and COX-2 (PDB ID: 1CX2) enzymes to test the putative interaction. Automated docking was used to determine the orientation of inhibitors bound in the active site of COX-1 and COX-2 enzymes. A Lamarckian genetic algorithm method, implemented in the program AutoDock 4.2, was employed.

Docking results between the screened bis-pyrazole derivatives **5(a–i)** and selected receptors, i.e., the cyclooxygenase-1 (PDB ID: 1CQE) and cyclooxygenase-2 (PDB ID: 1CX2) enzymes from mouse, are tabulated in Table 3. The structure of receptor, ligand and ligand–receptor complex is shown in Figs. 2, 3. Among bis-pyrazoles **5(a–i)**, meta amido bis-pyrazoles **5(g–i)** could not be docked against COX-1 and COX-2 enzymes as they were showing some mathematical error. This might be due to the size or geometry of the molecules resisting the docking. From the docking study results (Table 3), it could be concluded that ligand–receptor fitting of all screened compounds was best with highest binding energy for COX-2 than for COX-1 enzyme. The compound **5b** showed better fitting with minimum total energy value −11.92 kcal/mol followed by **5a** (energy value −11.21 kcal/mol), **5f** and **5c** (energy value −10.33 kcal/mol) with COX-2 enzyme. The compound **5b** showed binding energy with total energy value −3.04 kcal/mol with COX-1 enzyme followed by **5c** (energy value −2.41 kcal/mol) and **5a** (energy value −2.36 kcal/mol). These *in silico* study results are complementing with the other biological experimental results and support the overall outcome of the study. The selective binding of tested molecules to COX-2 in preference to COX-1 is an encouraging result in favor of anti-inflammatory activity.

**Table 3** Docking study results of screened active compounds with COX-1 and COX-2 enzymes

| Compounds | Binding energy (BE) (kcal/mol) | | Ligand efficiency (LE) | | Inhibition constant (IC) | | Electrostatic energy (EE) | | H-bond | |
|---|---|---|---|---|---|---|---|---|---|---|
| | COX-1 | COX-2 | COX-1 | COX-2 | COX-1 (mM) | COX-2 (nM) | COX-1 | COX-2 | COX-1 | COX-2 |
| **5a** | −2.36 | −11.21 | −0.06 | −0.28 | 6.8 | 6.11 | −0.02 | −0.32 | 0 | 2 |
| **5b** | −3.04 | −11.92 | −0.08 | −0.29 | 5.9 | 5.81 | −0.03 | −0.35 | 1 | 2 |
| **5c** | −2.41 | −10.33 | −0.06 | −0.26 | 6.3 | 26.97 | −0.02 | −0.20 | 0 | 2 |
| **5d** | −2.19 | −10.27 | −0.05 | −0.25 | 7.1 | 27.01 | −0.01 | −0.12 | 0 | 2 |
| **5e** | −2.04 | −9.31 | −0.03 | −0.17 | 7.9 | 33.41 | 0.02 | −0.08 | 0 | 1 |
| **5f** | −2.13 | −10.46 | −0.04 | −0.26 | 7.3 | 26.72 | 0.01 | −0.18 | 0 | 1 |

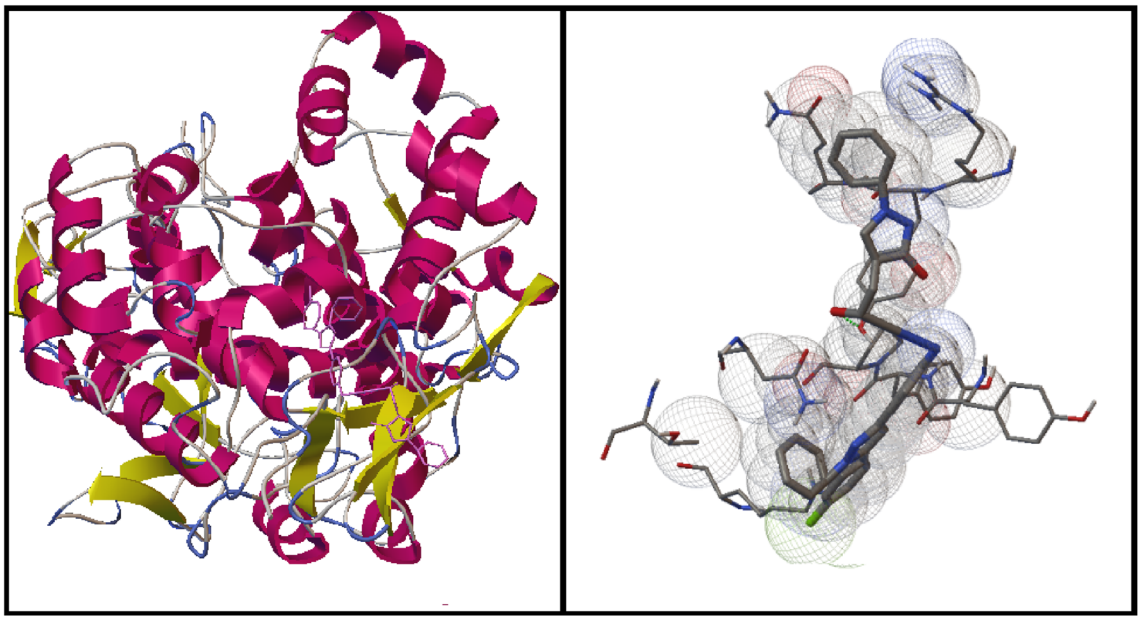


**Fig. 2** Compound **5b** in the active pocket of COX-1 (PDB ID: 1CQE) and interaction with COX-1 residues in an active pocket

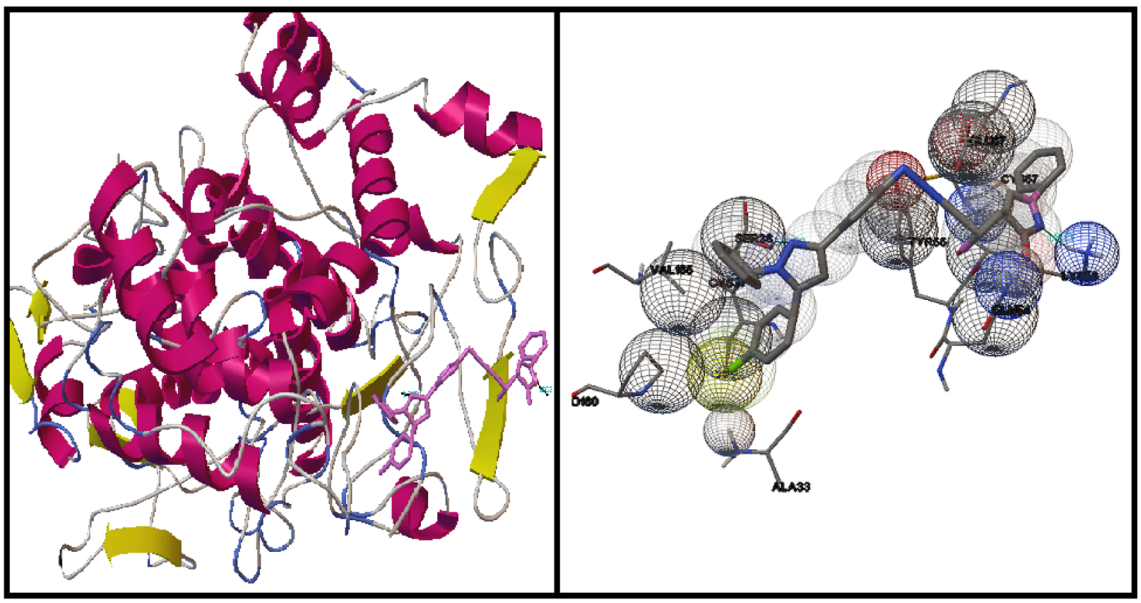



**Fig. 3** Compound **5b** in the active pocket of COX-2 (PDB ID: 1CX2) and interaction with COX-2 residues in an active pocket

*Antimicrobial activity*

The results are depicted in Table 4. The results revealed that the synthesized compounds displayed variable inhibitory effects on the growth of the tested gram-positive (*Staphylococcus aureus* NCIM 2079) and gram-negative (*Escherichia coli* NCIM 2931) bacterial strains. In general, most of the tested compounds exhibited moderate activity against both the gram-positive and gram-negative bacteria. It could also be noticed that zone of inhibition by the compounds containing halogen substituents **5a**, **5b**, **5c**, **5g**, **5h** and **5i** was better than that of nonhalogenated derivatives.

The minimum inhibitory concentration (MIC) values of compounds were tested to measure antimicrobial activity of compounds (**5a–i**) (Table 5). The MIC values for the compounds **5a**, **5b** and **5g** were found to be 125 μg/mL against both the tested strains. MIC values for other

**Table 4** Results of antimicrobial activity of the compounds **5(a–l)**

| Compounds | *R* | Zone of inhibition (mm) | |
|---|---|---|---|
| | | Gram-positive *S. aureus* | Gram-negative *E. coli* |
| **5a** | F | 10.00 ± 0.57 | 12.00 ± 0.00 |
| **5b** | Br | 11.00 ± 0.28 | 11.00 ± 0.57 |
| **5c** | Cl | 09.00 ± 0.28 | 11.00 ± 0.00 |
| **5d** | $CH_3$ | 09.00 ± 0.57 | 12.00 ± 0.28 |
| **5e** | $OCH_3$ | 09.00 ± 0.00 | 11.00 ± 0.00 |
| **5f** | $CH(CH_3)_2$ | 08.00 ± 0.57 | 09.00 ± 0.28 |
| **5g** | F | 11.00 ± 0.00 | 11.00 ± 0.28 |
| **5h** | Br | 11.00 ± 0.28 | 10.00 ± 0.57 |
| **5i** | Cl | 10.00 ± 0.57 | 11.00 ± 0.28 |
| **5j** | $CH_3$ | 09.00 ± 0.57 | 11.00 ± 0.00 |
| **5k** | $OCH_3$ | 07.00 ± 0.28 | 09.00 ± 0.57 |
| **5l** | $CH(CH_3)_2$ | 09.00 ± 0.28 | 08.00 ± 0.28 |
| Streptomycin (10 μg/mL) | | 19.00 ± 0.28 | 21.00 ± 0.57 |

**Table 5** MIC values of compounds **5(a–l)**

| Compounds | *R* | Minimum inhibitory concentration (MIC) (μg/mL) | |
|---|---|---|---|
| | | *S. aureus* | *E. coli* |
| **5a** | F | 125 | 125 |
| **5b** | Br | 125 | 125 |
| **5c** | Cl | 250 | 250 |
| **5d** | $CH_3$ | 250 | 125 |
| **5e** | $OCH_3$ | 250 | 250 |
| **5f** | $CH(CH_3)_2$ | 250 | 250 |
| **5g** | F | 125 | 125 |
| **5h** | Br | 125 | 250 |
| **5i** | Cl | 250 | 125 |
| **5j** | $CH_3$ | >400 | 125 |
| **5k** | $OCH_3$ | >400 | 250 |
| **5l** | $CH(CH_3)_2$ | >400 | >400 |
| Streptomycin | | <12.5 | <12.5 |

compounds were found to be in the range from 250 to >400 μg/mL, revealing their lower activity against *S. aureus* and *E. coli* bacterial strains.

## Experimental protocol

### *Chemistry*

The melting points were measured in open capillary tubes and were not corrected. Thin-layer chromatography was performed on Merck silica gel 60 F254. The IR spectra were recorded on a Shimadzu-FTIR infrared spectrometer in KBr ($\nu_{max}$ in $cm^{-1}$). The $^1$H-NMR (400 MHz) spectra were recorded on a BRUKER AV400 NMR spectrometer, with 5-mm PABBO BB-1H TUBES. The $^{13}$C-NMR (100 MHz) spectra were recorded for approximately 0.03 M solutions in DMSO at 100 MHz with TMS as the internal standard. LCMS was completed using the Agilent 1200 series LC and Micromass ZQ spectrometer. Elemental analysis was carried out with the VARIO EL-III (Elementar Analysensysteme, GmBH). Crystallographic data were collected on a Agilent Eos Gemini diffractometer with a CuKα radiation ($\lambda = 1.54184$ Å) for compound **3a** and Bruker SMART APEXII CCD area-detector diffractometer with a graphite-monochromated $MoK_\alpha$ radiation ($\lambda = 0.71073$ Å) for compound **3b** equipped with an X'Calibur CCD area-detector diffractometer. The geometry of the molecule was calculated using the PLATON, PARST and OLEX2 software. The structure was solved by direct method and refined by least squares using the SHELX97, SHELXL2012 software package. CCDC 1005968 and 949575 contain the supplementary crystallographic data for **3a** and **3b**. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: 44 1223 336033: email: deposit@ccdc.cam.ac.uk).

### *Synthesis of 4-[(3/4-acetylphenyl)amino]-2-methylidene-4-oxobutanoic acid (**3a–b**)*

*4-[(4-Acetylphenyl)amino]-2-methylidene-4-oxobutanoic acid (**3a**)* ITA (**2**) (0.112 g, 1 mmol) was dissolved in acetone (30 mL), and 4-amino acetophenone (0.135 g,

1 mmol) was added in small portions under stirring at room temperature over time span of 30 min. The mixture became yellow slurry. Stirring was continued for 1.5 h, after which the solution was filtered. The solid obtained was washed with acetone and dried. The crude product was further purified by recrystallization method, and single crystals were grown from methanol by the slow evaporation. The product was obtained as a light yellow solid with a yield of 84 %, m.p. 188–190 °C; IR (KBr): $\nu_{max}$ ($cm^{-1}$), 3282 (OH), 3055 (NH), 2900 (Ar–H), 2634 (aliphatic C-H) 1685 (amide C=O), 1650 (acid C=O), 1591 (C=C). $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta$ ppm, 2.53 (3H, s, $CH_3$), 3.35 (2H, s, O=C-$CH_2$), 5.75 (1H, d, $J_{AB} = 1.6$ Hz, $H_A$), 6.18 (1H, d, $J_{AB} = 1.6$ Hz, $H_B$), 7.69–7.93 (4H, m, Ar–H), 10.37 (1H, s, NH), 12.57 (1H, s, OH). LCMS (*m/z*): 248 ($M^+ + 1$). Calcd. for $C_{13}H_{13}NO_4$: C, 63.15; H, 5.30; N, 5.67; Found: C, 63.13; H, 5.32; N, 5.65 %. XRD data: triclinic, $P\ \bar{1}$, $a = 5.0164(5)$ Å, $b = 5.2908(4)$ Å, $c = 21.8464(18)$ Å, $V = 575.67(8)$ Å$^3$, $Z = 2$.

*4-[(3-Acetylphenyl)amino]-2-methylidene-4-oxobutanoic acid (**3b**)* ITA (**2**) (0.112 g, 1 mmol) was dissolved in acetone (30 mL), and 3-amino acetophenone (0.135 g, 1 mmol) was added in small portions under stirring at room temperature over time span of 30 min. The mixture became yellow slurry. Stirring was continued for 1.5 h, after which the solution was filtered. The obtained solid was washed with acetone and dried. The crude product was further purified by recrystallization method, and single crystals were grown from methanol by the slow evaporation method. The product was obtained as a light yellow solid with 80 % yield. m.p. 180–182 °C; IR (KBr): $\nu_{max}$ ($cm^{-1}$), 3282 (OH), 3055 (NH), 2900 (Ar–H), 2634 (aliphatic C-H) 1685 (amide C=O), 1591 (C=C). $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta$ ppm, 2.55 (3H, s, –$CH_3$), 3.35(2H, s, O=C-$CH_2$), 5.75 (1H, d, $J_{AB} = 1.6$ Hz, $H_A$), 6.18 (1H, d, $J_{AB} = 1.6$ Hz, $H_B$), 7.43–8.17 (4H, m, Ar–H), 10.23 (1H, s, NH), 12.55 (1H, s, OH). LCMS (*m/z*): 248 ($M^+ + 1$). Calcd. for $C_{13}H_{13}NO_4$: C, 63.15; H, 5.30; N, 5.67; Found: C, 63.13; H, 5.32; N, 5.65 %. XRD data: triclinic, P-1, $a = 4.9485$ (3) Å, $b = 5.3614$ (6) Å, $c = 22.457$ (2) Å, V = 592.77(9) Å$^3$, $Z = 2$.

*Synthesis of chalcones (**4a–l**)*

*4-({4-[3-(4-Fluorophenyl)prop-2-enoyl]phenyl}amino)-2-methylidene-4-oxobutanoic acid (**4a**)* To a mixture of 4-[(4-acetylphenyl)amino]-2-methylidene-4-oxobutanoic acid (**3a**) (0.1 mol) and 4-substituted benzaldehyde (0.1 mol) in 30 mL ethanol, 10 mL of 10 % sodium hydroxide solution was added and stirred at room temperature for 3 h. The precipitate formed was collected by filtration and recrystallized in ethanol. The product was obtained as a yellow solid with 68 % yield. m.p. 170–172 °C; IR (KBr): $\nu_{max}$ ($cm^{-1}$), 3340 (OH), 3103 (NH), 2922 (Ar–H), 2706 (aliphatic C-H) 1678 (amide C=O), 1662 (acid C=O), 1517 (C=C). $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta$ ppm, 3.41 (2H, s, O=C-$CH_2$), 5.97 (1H, d, $J_{AB} = 1.6$ Hz, $H_A$), 6.50 (1H, d, $J_{AB} = 1.6$ Hz, $H_B$), 7.26 (2H, dt, $J = 2.0$ Hz, 5.2 Hz, Ar–$H_e$), 7.65 (1H, d, $J = 16.4$ Hz, $H_c$), 7.92–8.30 (7H, m, Ar–H), 10.36 (1H, s, NH),12.56 (1H, s, OH). LCMS (*m/z*): 354 ($M^+ + 1$). Calcd. for $C_{20}H_{16}FNO_4$: C, 67.98; H, 4.56; N, 3.96; Found: C, 67.96; H, 4.58; N, 3.94 %.

*4-({4-[3-(4-Bromophenyl)prop-2-enoyl]phenyl}amino)-2-methylidene-4-oxobutanoic acid (**4b**)* To a mixture of 4-[(4-acetylphenyl)amino]-2-methylidene-4-oxobutanoic acid (**3a**) (0.1 mol) and 4-substituted benzaldehyde (0.1 mol) in 30 mL ethanol, 10 mL of 10 % sodium hydroxide solution was added and stirred at room temperature for 3 h. The precipitate formed was collected by filtration and recrystallized in ethanol. The product was obtained as a yellow solid with 65 % yield. m.p. 175–177 °C; IR (KBr): $\nu_{max}$ ($cm^{-1}$), 3344 (OH), 3100 (NH), 2927 (Ar–H), 2716 (aliphatic C-H) 1687 (amide C=O), 1652 (acid C=O), 1520 (C=C). $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta$ ppm, 3.40 (s, 2H, O=C-$CH_2$), 5.93 (d, 1H, $H_A$, $J_{AB} = 1.6$ Hz), 6.45 (d, 1H, $H_B$, $J_{AB} = 1.6$ Hz), 7.27 (dt, 2H, Ar–$H_e$, $J = 2.0$ Hz, 5.2 Hz), 7.67 (d, 1H, $H_c$, $J = 16.4$ Hz), 7.40–8.30 (m, 7H, Ar–H), 10.35 (s, 1H, NH),12.54 (s, 1H, OH). LCMS (*m/z*): 415 ($M^+ + 1$). Calcd. for $C_{20}H_{16}BrNO_4$: C, 57.99; H, 3.89; N, 3.38; Found: C, 57.97; H, 3.91; N, 3.36 %.

*4-({4-[3-(4-Chlorophenyl)prop-2-enoyl]phenyl}amino)-2-methylidene-4-oxobutanoic acid (**4c**)* To a mixture of 4-[(4-acetylphenyl)amino]-2-methylidene-4-oxobutanoic acid (**3a**) (0.1 mol) and 4-substituted benzaldehyde (0.1 mol) in 30 mL ethanol, 10 mL of 10 % sodium hydroxide solution was added and stirred at room temperature for 3 h. The precipitate formed was collected by filtration and recrystallized in ethanol. The product was obtained as a yellow solid with 65 % yield. m.p. 159–16 °C. IR (KBr): $\nu_{max}$ ($cm^{-1}$), 3345 (OH), 3105 (NH), 2972 (Ar–H), 2761 (aliphatic C-H) 1684 (amide C=O), 1658 (acid C=O), 1528 (C=C). $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta$ ppm, 3.40 (s, 2H, O=C-$CH_2$), 5.95 (1H, d, $J_{AB} = 1.6$ Hz, $H_A$), 6.55 (1H, d, $J_{AB} = 1.6$ Hz, $H_B$), 7.25 (2H, dt, $J = 2.0$ Hz, 5.2 Hz, Ar–$H_e$), 7.64 (1H, d, $J = 16.4$ Hz, $H_c$), 7.91–8.32 (7H, m, Ar–H), 10.34 (1H, s, NH),12.52 (1H, s, OH). LCMS (*m/z*): 370 ($M^+ + 1$). Calcd. for $C_{20}H_{16}ClNO_4$: C, 64.96; H, 4.36; N, 3.79; Found: C, 64.94; H, 4.38; N, 3.77 %.

*2-Methylidene-4-({4-[3-(4-methylphenyl)prop-2-enoyl] phenyl}amino)-4-oxobutanoic acid (**4d**)* To a mixture of 4-[(4-acetylphenyl)amino]-2-methylidene-4-oxobutanoic acid (**3a**) (0.1 mol) and 4-substituted benzaldehyde (0.1 mol) in 30 mL ethanol, 10 mL of 10 % sodium hydroxide solution was added and stirred at room temperature for 3 h. The precipitate formed was collected by filtration and recrystallized in ethanol. The product was obtained as a yellow solid with 63 % yield. m.p. 168–170 °C. IR (KBr): $\nu_{max}$ ($cm^{-1}$), 3295 (OH), 3103 (NH), 2972 (Ar–H), 2760 (aliphatic C-H) 1683 (amide C=O), 1654 (acid C=O), 1526 (C=C). $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta$ ppm, 2.35 (3H, s, $CH_3$), 3.43 (2H, s, O=C-$CH_2$), 5.98 (1H, d, $J_{AB}$ = 1.6 Hz, $H_A$), 6.45 (1H, d, $J_{AB}$ = 1.6 Hz, $H_B$), 7.25 (2H, dt, $J$ = 2.0 Hz, 5.2 Hz, Ar–$H_e$), 7.64 (1H, d, $J$ = 16.4 Hz, $H_c$), 7.93–8.30 (7H, m, Ar–H), 10.35 (1H, s, NH),12.67 (1H, s, OH). LCMS (*m/z*): 350 ($M^+ + 1$). Calcd. for $C_{21}H_{19}NO_4$: C, 72.19; H, 5.48; N, 4.01; Found: C, 72.17; H, 5.50; N, 3.99 %.

*4-({4-[3-(4-Methoxyphenyl)prop-2-enoyl]phenyl}amino)-2-methylidene-4-oxobutanoic acid (**4e**)* To a mixture of 4-[(4-acetylphenyl)amino]-2-methylidene-4-oxobutanoic acid (**3a**) (0.1 mol) and 4-substituted benzaldehyde (0.1 mol) in 30 mL ethanol, 10 mL of 10 % sodium hydroxide solution was added and stirred at room temperature for 3 h. The precipitate formed was collected by filtration and recrystallized in ethanol. The product was obtained as a yellow solid with 60 % yield. m.p. 195–197 °C. IR (KBr): $\nu_{max}$ ($cm^{-1}$), 3340 (OH), 3103 (NH), 2922 (Ar–H), 2706 (aliphatic C-H) 1678 (amide C=O), 1662 (acid C=O), 1517 (C=C). $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta$ ppm, 3.37 (2H, s, O=C-$CH_2$), 3.36 (3H, s, $CH_3$), 5.76 (1H, d, $J_{AB}$ = 1.6 Hz, $H_A$), 6.18 (1H, d, $J_{AB}$ = 1.6 Hz, $H_B$), 7.01 (1H, d, $J$ = 15.6 Hz, $H_c$), 7.47–8.22 (9H, m, Ar–H), 10.25 (1H, s, NH), 12.72 (1H, s, OH). LCMS (*m/z*): 366 ($M^+ + 1$). Calcd. for $C_{21}H_{19}NO_5$: C, 69.03; H, 5.24; N, 3.83; Found: C, 69.01; H, 5.26; N, 3.81 %.

*4-(4-(3-(4-Isopropylphenyl)acryloyl)phenylamino)-2-methylene-4-oxobutanoic acid (**4f**)* To a mixture of 4-[(4-acetylphenyl)amino]-2-methylidene-4-oxobutanoic acid (**3a**) (0.1 mol) and 4-substituted benzaldehyde (0.1 mol) in 30 mL ethanol, 10 mL of 10 % sodium hydroxide solution was added and stirred at room temperature for 3 h. The precipitate formed was collected by filtration and recrystallized in ethanol. The product was obtained as a yellow solid with 69 % yield. m.p. 165–167 °C. IR (KBr): $\nu_{max}$ ($cm^{-1}$), 3295 (OH), 3104 (NH), 2972 (Ar–H), 2760 (aliphatic C-H) 1683 (amide C=O), 1664 (acid C=O), 1516 (C=C). $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta$ ppm, 1.29 [6H, d, $J_{AB}$ = 6.8 Hz, $(CH_3)_2$], 3.23 (2H, s, O=C-$CH_2$), 3.34 (1H, m, CH) 5.95 (1H, d, $J_{AB}$ = 1.6 Hz, $H_A$), 6.52 (1H, d, $J_{AB}$ = 1.6 Hz, $H_B$), 7.65 (1H, d, $J$ = 16.4 Hz, $H_c$), 7.42–8.15 (9H, m, Ar–H), 10.36 (1H, s, NH),12.56 (1H, s, OH). LCMS (*m/z*): 378 ($M^+ + 1$). Calcd. for $C_{23}H_{23}NO_4$: C, 73.19; H, 6.14; N, 3.71; Found: C, 73.17; H, 6.16; N, 3.69 %.

*4-({3-[3-(4-Fluorophenyl)prop-2-enoyl]phenyl}amino)-2-methylidene-4-oxobutanoic acid (**4g**)* To a mixture of 4-[(3-acetylphenyl)amino]-2-methylidene-4-oxobutanoic acid (**3b**) (0.1 mol) and 4-substituted benzaldehyde (0.1 mol) in 30 mL ethanol, 10 mL of 10 % sodium hydroxide solution was added and stirred at room temperature for 3 h. The precipitate formed was collected by filtration and recrystallized in ethanol. The product was obtained as a yellow solid with 60 % yield. m.p. 161–163 °C. IR (KBr): $\nu_{max}$ ($cm^{-1}$), 3273 (OH), 3136 (NH), 3057 (Ar–H), 2924 (aliphatic C-H) 1685 (amide C=O), 1656 (acid C=O), 1585 (C=C). $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta$ ppm, 3.33 (2H, s, O=C-$CH_2$), 5.75 (1H, d, $J_{AB}$ = 1.6 Hz, $H_A$), 6.17 (1H, d, $J_{AB}$ = 1.6 Hz, $H_B$), 6.99–8.30 (m, 8H, Ar–H), 7.85 (1H, d, $J$ = 16.4 Hz, $H_d$), 10.36 (1H, s, NH), 12.56 (1H, s, OH). LCMS (*m/z*): 354 ($M^+ + 1$). Calcd. for $C_{20}H_{16}FNO_4$: C, 67.98; H, 4.56; N, 3.96; Found: C, 67.96; H, 4.58; N, 3.94 %.

*4-({3-[3-(4-Bromophenyl)prop-2-enoyl]phenyl}amino)-2-methylidene-4-oxobutanoic acid (**4h**)* To a mixture of 4-[(3-acetylphenyl)amino]-2-methylidene-4-oxobutanoic acid (**3b**) (0.1 mol) and 4-substituted benzaldehyde (0.1 mol) in 30 mL ethanol, 10 mL of 10 % sodium hydroxide solution was added and stirred at room temperature for 3 h. The precipitate formed was collected by filtration and recrystallized in ethanol. The product was obtained as a yellow solid with 65 % yield. m.p. 176–178 °C. IR (KBr): $\nu_{max}$ ($cm^{-1}$), 3274 (OH), 3135 (NH), 3058 (Ar–H), 2942 (aliphatic C-H) 1686 (amide C=O), 1665 (acid C=O), 1587 (C=C). $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta$ ppm, 3.31(2H, s, O=C-$CH_2$), 5.72 (1H, d, $J_{AB}$ = 1.6 Hz, $H_A$), 6.15 (1H, d, $J_{AB}$ = 1.6 Hz, $H_B$), 6.99–8.33 (8H, m, Ar–H), 7.84 (1H, d, $J$ = 16.4 Hz, $H_d$), 10.35 (1H, s, NH), 12.55 (1H, s, OH). LCMS (*m/z*): 415 ($M^+ + 1$). Calcd. for $C_{20}H_{16}BrNO_4$: C, 57.99; H, 3.89; N, 3.38; Found: C, 57.97; H, 3.91; N, 3.36 %.

*4-({3-[3-(4-Chlorophenyl)prop-2-enoyl]phenyl}amino)-2-methylidene-4-oxobutanoic acid (**4i**)* To a mixture of 4-[(3-acetylphenyl)amino]-2-methylidene-4-oxobutanoic acid (**3b**) (0.1 mol) and 4-substituted benzaldehyde (0.1 mol) in 30 mL ethanol, 10 mL of 10 % sodium hydroxide solution was added and stirred at room temperature for 3 h. The precipitate formed was collected by filtration and recrystallized in ethanol. The product was obtained as a yellow solid with 63 % yield. m.p. 172–174 °C. IR (KBr): $\nu_{max}$ ($cm^{-1}$), 3277 (OH), 3130

(NH), 3054 (Ar–H), 2945 (aliphatic C-H) 1685 (amide C=O), 1655 (acid C=O), 1585 (C=C). $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta$ ppm, 3.33 (2H, s, O=C-$CH_2$), 5.75 (1H, d, $J_{AB} = 1.6$ Hz, $H_A$), 6.17 (1H, d, $J_{AB} = 1.6$ Hz, $H_B$), 6.89–8.30 (8H, m, Ar–H), 7.85 (1H, d, $J = 16.4$ Hz, $H_d$), 10.36 (1H, s, NH), 12.56 (1H, s, OH). LCMS (*m/z*): 370 ($M^+ + 1$). Calcd. for $C_{20}H_{16}ClNO_4$: C, 64.96; H, 4.36; N, 3.79; Found: C, 64.94; H, 4.38; N, 3.77 %.

*2-Methylidene-4-({3-[3-(4-methylphenyl)prop-2-enoyl]-phenyl}amino)-4-oxobutanoic acid (**4j**)* To a mixture of 4-[(3-acetylphenyl)amino]-2-methylidene-4-oxobutanoic acid (**3b**) (0.1 mol) and 4-substituted benzaldehyde (0.1 mol) in 30 mL ethanol, 10 mL of 10 % sodium hydroxide solution was added and stirred at room temperature for 3 h. The precipitate formed was collected by filtration and recrystallized in ethanol. The product was obtained as a yellow solid with 48 % yield. m.p. 170–172 °C. IR (KBr): $\nu_{max}$ ($cm^{-1}$), 3272 (OH), 3129 (NH), 3045 (Ar–H), 2954 (aliphatic C-H) 1688 (amide C=O), 1665 (acid C=O), 1586 (C=C). $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta$ ppm, 2.35 (3H, s, $CH_3$), 3.25 (2H, s, O=C-$CH_2$), 5.76 (1H, d, $J_{AB} = 1.6$ Hz, $H_A$), 6.19 (1H, d, $J_{AB} = 1.6$ Hz, $H_B$), 6.39–8.30 (8H, m, Ar–H), 7.88 (1H, d, $J = 16.4$ Hz, $H_d$), 10.36 (1H, s, NH), 12.56 (1H, s, OH). LCMS (*m/z*): 350 ($M^+ + 1$). Calcd. for $C_{21}H_{19}NO_4$: C, 72.19; H, 5.48; N, 4.01; Found: C, 72.17; H, 5.50; N, 3.99 %.

*4-({3-[3-(4-Methoxyphenyl)prop-2-enoyl]phenyl}amino)-2-methylidene-4-oxobutanoic acid (**4k**)* To a mixture of 4-[(3-acetylphenyl)amino]-2-methylidene-4-oxobutanoic acid (**3b**) (0.1 mol) and 4-substituted benzaldehyde (0.1 mol) in 30 mL ethanol, 10 mL of 10 % sodium hydroxide solution was added and stirred at room temperature for 3 h. The precipitate formed was collected by filtration and recrystallized in ethanol. The product was obtained as a yellow solid with 55 % yield. m.p. 158–160 °C. IR (KBr): $\nu_{max}$ ($cm^{-1}$), 3275 (OH), 3059 (NH), 2926 (Ar–H), 2837 (aliphatic C-H) 1685 (amide C=O), 1656 (acid C=O), 1585 (C=C). $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta$ ppm, 3.44 (2H, s, O=C-$CH_2$), 3.86 (3H, s, $CH_3$), 5.57 (1H, d, $J_{AB} = 1.6$ Hz, $H_A$), 6.50 (1H, d, $J_{AB} = 1.6$ Hz, $H_B$), 6.95 (2H, dt, $J = 2.0$ Hz, 5.2 Hz, Ar–$H_e$), 7.48 (1H, d, $J = 15.6$ Hz, $H_c$), 7.79 (1H, d, $J = 15.6$ Hz, $H_d$), 6.95–7.96 (6H, m, Ar–H), 10.50 (1H, s, NH), 11.55 (1H, s, OH). LCMS (*m/z*): 366 ($M^+ + 1$). Calcd. for $C_{21}H_{19}NO_5$: C, 69.03; H, 5.24; N, 3.83; Found: C, 69.01; H, 5.26; N, 3.81 %.

*4-(3-(3-(4-Isopropylphenyl)acryloyl)phenylamino)-2-methylene-4-oxobutanoic acid (**4l**)* To a mixture of 4-[(3-acetylphenyl)amino]-2-methylidene-4-oxobutanoic acid (**3b**) (0.1 mol) and 4-substituted benzaldehyde (0.1 mol) in 30 mL ethanol, 10 mL of 10 % sodium hydroxide solution was added and stirred at room temperature for 3 h. The precipitate formed was collected by filtration and recrystallized in ethanol. The product was obtained as a yellow solid with 52 % yield. m.p. 168–170 °C. IR (KBr): $\nu_{max}$ ($cm^{-1}$), 3274 (OH), 3109 (NH), 2962 (Ar–H), 2853 (aliphatic C-H) 1686 (amide C=O), 1656 (acid C=O), 1584 (C=C). $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta$ ppm, 1.25 (6H, d, $J_{AB} = 6.8$ Hz), 3.33 (2H, s, O=C-$CH_2$), 3.45 (1H, m, CH), 5.75 (1H, d, $J_{AB} = 1.6$ Hz, $H_A$), 6.18 (1H, d, $J_{AB} = 1.6$ Hz, $H_B$), 7.03–8.30 (8H, m, Ar–H), 7.85 (1H, d, $J = 16.4$ Hz, $H_d$), 10.43 (s, 1H, NH), 11.56 (s, 1H, OH). LCMS (*m/z*): 378 ($M^+ + 1$). Calcd. for $C_{23}H_{23}NO_4$: C, 73.19; H, 6.14; N, 3.71; Found: C, 73.17; H, 6.16; N, 3.69 %.

*Synthesis of bis-pyrazole derivatives (**5a–l**)*

*N-(4-(5-(4-fluorophenyl)-1-phenyl-1H-pyrazol-3-yl)phenyl-2-(3-hydroxy-1-phenyl-1H-pyrazol-4-yl)acetamide (**5a**)* A mixture of 4-({4-[3-(4-fluorophenyl)prop-2-enoyl]phenyl}amino)-2-methylidene-4-oxobutanoic acid (**4a**) (0.01 mol) and excess of phenyl hydrazine (0.023 mol) in ethanol (20 mL) with the 2–3 drops of HCl were refluxed for 48 h. The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate was collected by filtration and purified by recrystallization from ethanol. The product was obtained as a yellowish brown solid with 42 % yield. m.p. 112–114 °C. IR (KBr): $\nu_{max}$ ($cm^{-1}$), 3296 (OH), 3062 (NH), 2924 (Ar–H), 2854 (aliphatic C-H) 1670 (amide C=O), 1597 (C=C). $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta$ ppm, 3.34 (2H, s, O=C-$CH_2$), 5.43 (1H, s, OH), 6.58 (1H, s, pyrazole A H-4), 6.97–8.05 (20H, m, Ar–H); 8.15 (1H, s, pyrazole B H-5), 10.10 (1H, s, NH). $^{13}$C-NMR (100 MHz, DMSO-$d_6$): $\delta$ ppm,169.76 (amide C=O), 157.64 (pyrazole A C-3), 149.56 (pyrazole A C-5), 147.45 (pyrazole B C-3), 143.23, 140.12, 139.76, 134.66, 132.55, 130.43, 129.54, 128.31, 127.54, 126.43, 125.66, 119.67, 118.33, 114.58, 113.76, 112.25 (aromatic C's), 122.67 (pyrazole B C-5), 119.43 (pyrazole B C-4), 104.49 (pyrazole A C-4), 23.65 (O=C-$CH_2$). LCMS (*m/z*): 530 ($M^+ + 1$). Calcd. for $C_{32}H_{24}FN_5O_2$: C, 72.58; H, 4.57; N, 13.22; Found: C, 72.56; H, 4.59; N, 13.20 %.

*N-(4-(5-(4-bromophenyl)-1-phenyl-1H-pyrazol-3-yl)phenyl-2-(3-hydroxy-1-phenyl-1H-pyrazol-4-yl)acetamide (**5b**)* A mixture of 4-({4-[3-(4-bromophenyl)prop-2-enoyl]phenyl}amino)-2-methylidene-4-oxobutanoic acid (**4b**) (0.01 mol) and excess of phenyl hydrazine (0.023 mol) in ethanol (20 mL) with the 2–3 drops of HCl were refluxed for 48 h. The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate was collected by filtration and purified by recrystallization

from ethanol. The product was obtained as a brown solid with 46 % yield. m.p. 132–134 °C. IR (KBr): $\nu_{max}$ ($cm^{-1}$), 3269 (OH), 3026 (NH), 2942 (Ar–H), 2845 (aliphatic C-H) 1674 (amide C=O), 1596 (C=C). $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta$ ppm, 3.35 (2H, s, O=C-$CH_2$), 5.38 (1H, s, OH), 6.65 (1H, s, pyrazole A H-4), 6.96–8.06 (20H, m, Ar–H); 8.14 (1H, s, pyrazole B H-5), 10.16 (1H, s, NH). $^{13}$C-NMR (100 MHz, DMSO-$d_6$): $\delta$ ppm, 168.79 (amide C=O), 157.74 (pyrazole A C-3), 149.88 (pyrazole A C-5), 147.57 (pyrazole B C-3), 143.94, 140.43, 139.82, 134.45, 132.56, 130.23, 129.17, 128.10, 127.50, 126.32, 125.65, 119.73, 118.73, 114.85, 113.36, 112.51 (aromatic C's), 122.76 (pyrazole B C-5), 119.24 (pyrazole B C-4), 104.93 (pyrazole A C-4), 23.54 (O=C-$CH_2$). LCMS (*m/z*): 591 ($M^+ + 1$). Calcd. for $C_{32}H_{24}BrN_5O_2$: C, 65.09; H, 4.10; N, 11.86; Found: C, 65.07; H, 4.12; N, 11.84 %.

*N-(4-(5-(4-chlorophenyl)-1-phenyl-1H-pyrazol-3-yl)phenyl-2-(3-hydroxy-1-phenyl-1H-pyrazol-4-yl)acetamide (**5c**)* A mixture of 4-({4-[3-(4-chlorophenyl)prop-2-enoyl]phenyl}amino)-2-methylidene-4-oxobutanoic acid (**4c**) (0.01 mol) and excess of phenyl hydrazine (0.023 mol) in ethanol (20 mL) with the 2–3 drops of HCl were refluxed for 48 h. The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate was collected by filtration and purified by recrystallization from ethanol. The product was obtained as a yellowish brown solid with 47 % yield. m.p. 136–138 °C. IR (KBr): $\nu_{max}$ ($cm^{-1}$), 3279 (OH), 3046 (NH), 2943 (Ar–H), 2844 (aliphatic C-H) 1672 (amide C=O), 1597 (C=C). $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta$ ppm, 3.35 (2H, s, O=C-$CH_2$), 5.45 (1H, s, OH), 6.56 (1H, s, pyrazole A H-4), 6.96–8.15 (20H, m, Ar–H); 8.25 (1H, s, pyrazole B H-5), 10.21 (1H, s, NH). $^{13}$C-NMR (100 MHz, DMSO-$d_6$): $\delta$ ppm, 168.79 (amide C=O), 157.74 (pyrazole A C-3), 149.88 (pyrazole A C-5), 147.57 (pyrazole B C-3), 143.94, 140.43, 139.82, 134.45, 132.56, 130.23, 129.17, 128.10, 127.50, 126.32, 125.65, 119.73, 118.73, 114.85, 113.36, 112.51 (aromatic C's), 122.76 (pyrazole B 5C), 119.34 (pyrazole B C-4), 105.93 (pyrazole A C-4), 24.54 (O=C-$CH_2$). LCMS (*m/z*): 547 ($M^+ + 1$). Calcd. for $C_{32}H_{24}ClN_5O_2$: C, 70.39; H, 4.43; N, 12.83; Found: C, 70.37; H, 4.45; N, 12.81 %.

*2-(3-Hydroxy-1-phenyl-1H-pyrazol-4-yl)-N-(4-(1-phenyl-5-p-tolyl-1H-pyrazol-3-yl)phenyl) acetamide (**5d**)* A mixture of 2-methylidene-4-({4-[3-(4-methylphenyl)prop-2-enoyl]phenyl}amino)-4-oxobutanoic acid (**4d**) (0.01 mol) and excess of phenyl hydrazine (0.023 mol) in ethanol (20 mL) with the 2–3 drops of HCl were refluxed for 48 h. The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate was collected by filtration and purified by recrystallization from ethanol. The product was obtained as a yellowish brown solid with 43 % yield. m.p. 108–110 °C. IR (KBr): $\nu_{max}$ ($cm^{-1}$), 3415 (OH), 3081 (NH), 2929 (Ar–H), 2837 (aliphatic C-H) 1670 (amide C=O), 1595 (C=C), $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta$ ppm, 1.99 (3H, s, Ar-$CH_3$), 3.51 (2H, s, O=C-$CH_2$), 6.11 (1H, s, OH), 7.00–8.14 (20H, m, Ar–H); 10.70 (1H, s, NH). $^{13}$C-NMR (100 MHz, DMSO-$d_6$): $\delta$ ppm, 168.78 (amide C=O), 159.74 (pyrazole A C-3), 149.86 (pyrazole A C-5), 147.54 (pyrazole B C-3), 144.94, 140.46, 139.82, 134.95, 132.56, 130.27, 129.27, 128.15, 127.55, 126.22, 125.67, 119.83, 118.75, 114.85, 113.36, 112.51 (aromatic C's), 122.76 (pyrazole B C-5), 119.24 (pyrazole B C-4), 104.93 (pyrazole A 4C), 27.45 ($CH_3$) 24.56 (O=C-$CH_2$). LCMS (*m/z*): 526 ($M^+ + 1$). Calcd. for $C_{33}H_{27}N_5O_2$: C, 75.41; H, 5.18; N, 13.32; Found: C, 75.39; H, 5.20; N, 13.30 %.

*2-(3-Hydroxy-1-phenyl-1H-pyrazol-4-yl)-N-(4-(5-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-3-yl) phenyl)acetamide (**5e**)* A mixture of 4-({4-[3-(4-methoxyphenyl)prop-2-enoyl]phenyl}amino)-2-methylidene-4-oxobutanoic acid (**4e**) (0.01 mol) and excess of phenyl hydrazine (0.023 mol) in ethanol (20 mL) with the 2–3 drops of HCl were refluxed for 48 h. The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate was collected by filtration and purified by recrystallization from ethanol. The product was obtained as a yellowish brown solid with 45 % yield. m.p. 102–104 °C. IR (KBr): $\nu_{max}$ ($cm^{-1}$), 3294 (OH), 3035 (NH), 2929(Ar–H), 2835 (aliphatic C-H) 1672 (amide C=O), 1597 (C=C), $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta$ ppm, $\delta$ 3.33 (2H, s, O=C-$CH_2$), 3.76 (3H, s, O-$CH_3$), 5.34 (1H, s, OH), 6.57 (1H, s, pyrazole A H-4), 6.68–7.75 (20H, m, Ar–H); 7.82 (1H, s, pyrazole B H-5), 10.09 (1H, s, NH). $^{13}$C-NMR (100 MHz, DMSO-$d_6$): $\delta$ ppm, 168.78 (amide C=O), 164.07 (C-O-$CH_3$), 159.74 (pyrazole A C-3), 149.86 (pyrazole A C-5), 147.54 (pyrazole B C-3), 144.90, 140.41, 139.82, 134.98, 130.27, 129.27, 128.15, 127.55, 126.22, 125.67, 119.85, 118.77, 114.80, 113.37, 112.54 (aromatic C's), 122.78 (pyrazole B C-5), 119.25 (pyrazole B C-4), 104.95 (pyrazole A C-4), 55.65 (O-$CH_3$), 24.55 (O=C-$CH_2$). LCMS (*m/z*): 542 ($M^+ + 1$). Calcd. for $C_{33}H_{27}N_5O_3$: C, 73.18; H, 5.02; N, 12.93; Found: C, 73.16; H, 5.04; N, 12.91 %.

*2-(3-Hydroxy-1-phenyl-1H-pyrazol-4-yl)-N-(4-(5-(4-isopropylphenyl)-1-phenyl-1H-pyrazol-3-yl) phenyl)acetamide (**5f**)* A mixture of 4-(4-(3-(4-isopropylphenyl)acryloyl)phenylamino)-2-methylene-4-oxobutanoic acid (**4f**) (0.01 mol) and excess of phenyl hydrazine (0.023 mol) in ethanol (20 mL) with the 2–3 drops of HCl were refluxed for 48 h. The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate was collected by filtration and purified by recrystallization from ethanol. The product was obtained as a yellowish brown solid with 48 % yield. m.p. 114–116 °C. IR (KBr): $\nu_{max}$ ($cm^{-1}$), 3290 (OH), 3065 (NH), 2927(Ar–H), 2835 (aliphatic C-H) 1671 (amide C=O), 1596 (C=C). $^1$H-NMR (400 MHz, DMSO-

$d_6$): δ ppm, 1.35 (6H, d, $J_{AB}$ = 6.8 Hz, $(CH_3)_2$), 3.36 (2H, s, O=C-$CH_2$), 3.48 (1H, m, CH), 5.47 (1H, s, OH), 6.56 (1H, s, pyrazole A H-4), 6.89–8.15 (20H, m, Ar–H); 8.25 (1H, s, pyrazole B H-5), 10.19 (1H, s, NH). $^{13}$C-NMR (100 MHz, DMSO-$d_6$): δ ppm, 168.75 (amide C=O), 159.74 (pyrazole A C-3), 149.88 (pyrazole A C-5), 147.56 (pyrazole B C-3), 144.84, 140.41, 139.72, 134.94, 132.56, 130.21, 129.26, 128.17, 127.55, 126.24, 125.65, 119.82, 118.75, 114.83, 113.35, 112.51 (aromatic C's), 122.77 (pyrazole B C-5), 119.23 (pyrazole B C-4), 104.95 (pyrazole A C-4), 36.30 (CH), 27.45 ($(CH_3)_2$), 24.56 (O=C-$CH_2$). LCMS (*m/z*): 554 ($M^+ + 1$). Calcd. for $C_{35}H_{31}N_5O_2$: C, 75.93; H, 5.64; N, 12.65; Found: C, 75.91; H, 5.66; N, 12.63 %.

*N-(3-(5-(4-fluorophenyl)-1-phenyl-1H-pyrazol-3-yl)phenyl-2-(3-hydroxy-1-phenyl-1H-pyrazol-4-yl)acetamide (**5g**)* A mixture of 4-({3-[3-(4-fluorophenyl)prop-2-enoyl]phenyl} amino)-2-methylidene-4-oxobutanoic acid (**4g**) (0.01 mol) and excess of phenyl hydrazine (0.023 mol) in ethanol (20 mL) with the 2–3 drops of HCl were refluxed for 48 h. The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate was collected by filtration and purified by recrystallization from ethanol. The product was obtained as a yellowish brown solid with 38 % yield. m.p. 102–104 °C; IR (KBr): $\nu_{max}$ ($cm^{-1}$), 3375 (OH), 3057 (NH), 2987 (Ar–H), 2942 (aliphatic C-H) 1706 (amide C=O), 1596 (C=C). $^1$H-NMR (400 MHz, DMSO-$d_6$): δ ppm, 3.32 (2H, s, O=C-$CH_2$), 5.59 (1H, s, –OH), 6.78 (1H, s, pyrazole A H-4), 7.03–8.15 (20H, m, Ar–H); 8.25 (1H, s, pyrazole B H-5), 10.19 (1H, s, NH). $^{13}$C-NMR (100 MHz, DMSO-$d_6$): δ ppm, 168.58 (amide C=O), 151.74 (pyrazole A 3C), 147.76 (pyrazole A C-5), 144.84 (pyrazole B C-3), 143.44, 142.62, 140.52, 140.36, 139.97, 133.89, 132.68, 129.73, 128.77, 125.80, 123.80, 122.13, 121.23, 120.46, 119.98, 119.92, 116.61, 113.24 (aromatic C's), 122.90 (pyrazole B C-5), 120.93 (pyrazole B C-4), 105.93 (pyrazole A C-4), 24.51 (O=C-$CH_2$). LCMS (*m/z*): 530 ($M^+ + 1$). Calcd. for $C_{32}H_{24}FN_5O_2$: C, 72.58; H, 4.57; N, 13.22; Found: C, 72.56; H, 4.59; N, 13.20 %.

*N-(3-(5-(4-bromophenyl)-1-phenyl-1H-pyrazol-3-yl)phenyl-2-(3-hydroxy-1-phenyl-1H-pyrazol-4-yl)acetamide (**5h**)* A mixture of 4-({3-[3-(4-bromophenyl)prop-2-enoyl]phenyl}amino)-2-methylidene-4-oxobutanoic acid (**4h**) (0.01 mol) and excess of phenyl hydrazine (0.023 mol) in ethanol (20 mL) with the 2–3 drops of HCl were refluxed for 48 h. The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate was collected by filtration and purified by recrystallization from ethanol. The product was obtained as a brown solid with 46 % yield. m.p. 112–114 °C. IR (KBr): $\nu_{max}$ ($cm^{-1}$), 3373 (OH), 3057 (NH), 2978(Ar–H), 2924 (aliphatic C-H) 1708 (amide C=O), 1595 (C=C); $^1$H-NMR (400 MHz, DMSO-$d_6$): δ ppm, 3.34 (2H, s, O=C-$CH_2$), 5.54 (1H, s, –OH), 6.72 (1H, s, pyrazole A H-4), 6.73–8.05 (20H, m, Ar–H); 8.15 (1H, s, pyrazole B H-5), 10.09 (1H, s, NH). $^{13}$C-NMR (100 MHz, DMSO-$d_6$): δ ppm, 168.85 (amide C=O), 151.47 (pyrazole A C-3), 147.67 (pyrazole A C-5), 144.48 (pyrazole B C-3), 143.44, 142.26, 140.25, 140.06, 139.91, 133.39, 132.38, 129.83, 128.67, 125.80, 122.80, 122.43, 121.23, 120.46, 119.98, 119.29, 116.16, 113.42 (aromatic C's), 122.10 (pyrazole B C-5), 120.93 (pyrazole B C-4), 105.93 (pyrazole A C-4), 24.51 (O=C-$CH_2$). LCMS (*m/z*): 591 ($M^+ + 1$). Calcd. for $C_{32}H_{24}BrN_5O_2$: Calcd. for $C_{32}H_{24}BrN_5O_2$: C, 65.09; H, 4.10; N, 11.86; Found: C, 65.07; H, 4.12; N, 11.84 %.

*N-(3-(5-(4-chlorophenyl)-1-phenyl-1H-pyrazol-3-yl)phenyl-2-(3-hydroxy-1-phenyl-1H-pyrazol-4-yl)acetamide (**5i**)* A mixture of 4-({3-[3-(4-cholrophenyl)prop-2-enoyl]phenyl}amino)-2-methylidene-4-oxobutanoic acid (**4i**) (0.01 mol) and excess of phenyl hydrazine (0.023 mol) in ethanol (20 mL) with the 2–3 drops of HCl were refluxed for 48 h. The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate was collected by filtration and purified by recrystallization from ethanol. The product was obtained as a yellowish brown solid with 43 % yield. m.p. 130–132 °C. IR (KBr): $\nu_{max}$ ($cm^{-1}$), 3376 (OH), 3055 (NH), 2977(Ar–H), 2925 (aliphatic C-H) 1709 (amide C=O), 1595 (C=C). $^1$H-NMR (400 MHz, DMSO-$d_6$): δ ppm, 3.36 (2H, s, O=C-$CH_2$), 5.45 (1H, s, -OH), 6.76 (1H, s, pyrazole A H-4), 7.07–8.15 (20H, m, Ar–H); 8.23 (1H, s, pyrazole B H-5), 10.14 (1H, s, NH). $^{13}$C-NMR (100 MHz, DMSO-$d_6$): δ ppm, 168.78 (amide C=O), 158.69 (pyrazole A C-3), 151.42 (pyrazole A C-5), 144.54 (pyrazole B C-3), 142.15, 140.63, 140.54, 134.28, 133.56, 133.16, 130.30, 129.32, 128.17, 127.54,125.67, 123.87, 121.16, 119.22, 116.37, 114.98, 114.57, 113.58 (aromatic C's), 122.64 (pyrazole B C-5), 120.60 (pyrazole B C-4), 105.15 (pyrazole A C-4), 24.36 (O=C-$CH_2$). LCMS (*m/z*): 547 ($M^+ + 1$). Calcd. for $C_{32}H_{24}ClN_5O_2$: C, 70.39; H, 4.43; N, 12.83; Found: C, 70.37; H, 4.45; N, 12.81 %.

*2-(3-Hydroxy-1-phenyl-1H-pyrazol-4-yl)-N-(3-(1-phenyl-5-p-tolyl-1H-pyrazol-3-yl)phenyl) acetamide (**5j**)* A mixture of 2-methylidene-4-({3-[3-(4-methylphenyl)prop-2-enoyl]phenyl}amino)-4-oxobutanoic acid (**4j**) (0.01 mol) and excess of phenyl hydrazine (0.023 mol) in ethanol (20 mL) with the 2–3 drops of HCl were refluxed for 48 h. The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate was collected by filtration and purified by recrystallization from ethanol. The product was obtained as a yellowish brown solid with 33 % yield. m.p. 116–118 °C; IR (KBr): $\nu_{max}$ ($cm^{-1}$), 3367 (OH), 3065 (NH), 2976(Ar–H), 2926 (aliphatic C-H) 1701 (amide C=O), 1596 (C=C); $^1$H-NMR (400 MHz, DMSO-$d_6$): δ

ppm, 1.89 (3H, s, Ar-$CH_3$), 3.34 (2H, s, O=C-$CH_2$), 5.54 (1H, s, OH), 6.72 (1H, s, pyrazole A H-4), 6.73–8.05 (20H, m, Ar–H); 8.20 (1H, s, pyrazole B C-5), 10.09 (1H, s, NH). $^{13}$C-NMR (100 MHz, DMSO-$d_6$): $\delta$ ppm, 168.87 (amide C=O), 158.59 (pyrazole A C-3), 151.24 (pyrazole A C-5), 144.45 (pyrazole B C-3), 141.95, 140.65, 140.50, 134.28, 133.56, 133.16, 130.30, 129.32, 128.17, 127.54, 125.67, 123.87, 121.15, 119.12, 116.31, 114.94, 114.54, 113.54 (aromatic C's), 122.65 (pyrazole B C-5), 120.64 (pyrazole B C-4), 105.18 (pyrazole A C-4), 55.65 (O-$CH_3$), 27.52 ($CH_3$), 24.35 (O=C-$CH_2$). LCMS (*m/z*): 526 ($M^+ + 1$). Calcd. for $C_{33}H_{27}N_5O_2$: C, 75.41; H, 5.18; N, 13.32; Found: C, 75.39; H, 5.20; N, 13.30 %.

*2-(3-Hydroxy-1-phenyl-1H-pyrazol-4-yl)-N-(3-(5-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-3-yl) phenyl)acetamide (**5k**)* A mixture of 4-({3-[3-(4-methoxyphenyl)prop-2-enoyl]phenyl}amino)-2-methylidene-4-oxobutanoic acid (**4k**) (0.01 mol) and excess of phenyl hydrazine (0.023 mol) in ethanol (20 mL) with the 2–3 drops of HCl were refluxed for 48 h. The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate was collected by filtration and purified by recrystallization from ethanol. The product was obtained as a yellowish brown solid with 39 % yield. m.p. 106–108 °C. IR (KBr): $\nu_{max}$ ($cm^{-1}$), 3332 (OH), 3059 (NH), 2926 (Ar–H), 2854 (aliphatic C-H) 1670 (amide C=O), 1597 (C=C). $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta$ ppm, 3.43 (2H, s, O=C-$CH_2$), 3.76 (3H, s, –O–$CH_3$), 5.41 (1H, s, OH), 6.68 (1H, s, pyrazole A H-4), 6.78–7.92 (20H, m, Ar–H); 7.82 (1H, s, pyrazole B H-5), 10.10 (1H, s, NH). $^{13}$C-NMR (100 MHz, DMSO-$d_6$): $\delta$ ppm, 168.78 (amide C=O), 165.07 (C-O-$CH_3$), 158.95 (pyrazole A C-3), 151.22 (pyrazole A C-5), 144.49 (pyrazole B C-3), 140.95, 140.36, 140.05, 134.80, 133.62, 133.24, 130.30, 129.32, 128.17, 127.54, 125.77, 121.15, 119.12, 116.13, 114.94, 114.54, 113.45 (aromatic C's), 122.65 (pyrazole B C-5), 120.82 (pyrazole B C-4), 105.13 (pyrazole A C-4), 55.65 (O-$CH_3$), 24.50 (O=C-$CH_2$). LCMS (*m/z*): 542 ($M^+ + 1$). Calcd. for $C_{33}H_{27}N_5O_3$: C, 73.18; H, 5.02; N, 12.93; Found: C, 73.16; H, 5.04; N, 12.91 %.

*2-(3-Hydroxy-1-phenyl-1H-pyrazol-4-yl)-N-(3-(5-(4-isopropylphenyl)-1-phenyl-1H-pyrazol-3-yl) phenyl)-acetamide (**5l**)* A mixture of 4-(3-(3-(4-isopropylphenyl) acryloyl)phenylamino)-2-methylene-4-oxobutanoic acid (**4l**) (0.01 mol) and excess of phenyl hydrazine (0.023 mol) in ethanol (20 mL) with the 2–3 drops of HCl were refluxed for 48 h. The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate was collected by filtration and purified by recrystallization from ethanol. The product was obtained as a yellowish brown solid with 35 % yield. m.p. 108–110 °C. IR (KBr): $\nu_{max}$ ($cm^{-1}$), 3330 (OH), 3059 (NH), 2925 (Ar–H), 2855 (aliphatic C-H) 1679 (amide C=O), 1595 (C=C). $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta$ ppm, 1.36 (6H, d, $J_{AB} = 6.8$ Hz, $(CH_3)_2$), 3.34 (2H, s, O=C-$CH_2$), 3.48 (1H, m, CH), 5.54 (1H, s, OH), 6.72 (1H, s, pyrazole A H-4), 6.73–8.05 (20H, m, Ar–H); 8.15 (1H, s, pyrazole B H-5), 10.09 (1H, s, NH). $^{13}$C-NMR (100 MHz, DMSO-$d_6$): $\delta$ ppm, 168.87 (amide C=O), 158.59 (pyrazole A C-3), 151.24 (pyrazole A C-5), 144.45 (pyrazole B C-3), 141.95, 140.56, 140.05, 134.82, 133.65, 133.26, 130.33, 129.23, 128.71, 127.45, 125.77, 124.56, 121.51, 119.21, 116.31, 114.94, 114.45, 113.54 (aromatic C's), 122.56 (pyrazole B C-5), 120.62 (pyrazole B C-4), 105.16 (pyrazole A C-4), 55.56 (O-$CH_3$), 36.34 (CH), 27.54 ($(CH_3)_2$), 24.53 (O=C-$CH_2$). LCMS (*m/z*): 554 ($M^+ + 1$). Calcd. for $C_{35}H_{31}N_5O_2$: C, 75.93; H, 5.64; N, 12.65; Found: C, 75.91; H, 5.66; N, 12.63 %.

## Pharmacology

### *Analgesic activity (acetic acid-induced writhing method)*

The male albino mice weighing between 20 and 25 g were selected and divided into 10 groups of six animals each (Vogel and Vogel, 2002a, b). All the animals received 0.1 mL acetic acid 0.6 % v/v. i.p., and the first group served as the control. The second group served as positive control and received aspirin. The third–tenth groups were administrated test compounds **4**(**a**–**d**) and **5**(**a**–**d**) at a dose of 25 mg $kg^{-1}$ bodyweight (suspended in 0.5 % CMC given p.o.) 30 min prior to the administration of acetic acid injection. The writhing effect was indicated by the stretching of the abdomen with simultaneous stretching of at least one hind limb. This was observed for 30 min and the change in the number of writhings in the test group compared to the standard treated and control treated groups.

### *Anti-inflammatory activity (Carrageenan-induced rat paw edema model)*

In carrageenan-induced rat paw edema method (Winter *et al.*, 1962), 1 % carrageenan was used in a dose of 0.1 mL as a phlogistic agent (irritant), injected subcutaneously into the plantar aspect of the left hind paw of the rats. A line was drawn at the tibio–tarsal junction, so that every time the paw was dipped in the mercury column up to the fixed mark to ensure constant paw volume. The paw volume was measured immediately after the carrageenan injection, which served as the initial volume, both in control and drug-treated animals. The paw volume was measured every 30-min interval up to 3 h. The second group was treated with indomethacin 10 mg/kg bodyweight (p.o.). The third–tenth groups were administered test

compounds **5(a–l)** at a dose 10 mg $kg^{-1}$ bodyweight (suspended in 0.5 % CMC given p.o.). After 30 min of the drug administration, the rats were treated with 0.1 mL of 1 % carrageenan by subcutaneous route to the right hind paw. Immediately after the injection, the paw volumes were measured in a mercury plethysmograph. Thereafter, the paw volume was measured at 60, 120 and 180 min. The amount of edema in the drug-treated groups was compared in relation to the control group with the corresponding time intervals. The results were expressed as % inhibition of edema over the untreated control group.

*Acute toxicity study*

Acute toxicity study of selected compounds was determined in Wistar albino rats (150–200 g) according to OECD guidelines No. 425. The animals fasted overnight, and the selected compounds were administered orally with a starting dose of 2000 mg/kg to different animals. Animals were observed continuously for first 3 h and monitored for 14 days for mortality and general behavior of animals, signs of discomfort and nervous manifestations.

*Molecular docking study*

Automated docking was used to determine the orientation of inhibitors bound at the active site of COX-1 (PDB ID: 1CQE) and COX-2 (PDB ID: 1CX2). A Lamarckian genetic algorithm method implemented in the AutoDock 4.2 was employed to identify appropriate binding modes and conformations of the ligand. For docking calculations, Gasteiger–Marsili partial charges (Gasteiger and Marsili, 1980) were assigned to the ligands, and nonpolar hydrogen was merged. All torsions were allowed to rotate during docking. The grid maps were generated with Autogrid using grid values $X = 28.313$, $Y = 9.701$, $Z = 187.515$ for COX-1 and $X = 31.564$, $Y = -0.979$, $Z = -8.786$ for COX-2, respectively. The Lamarckian genetic algorithm and the pseudo-Solis and Wets methods were applied for energy minimization using default parameters. Binding free energy was calculated using the following equation (Sanner *et al.*, 1996).

$$\text{Binding Energy} = [(1) + (2) + (3) + (4)]$$

where (1)—final intermolecular energy, (2)—final total internal energy, (3)—torsional free energy, (4)—unbound system's energy.

*Antimicrobial activity (disk diffusion method)*

*Microbial isolates* The microorganisms used for the microbial sensitivity assay were gram-positive *S. aureus* (NCIM 2079) and gram-negative *E. coli* (NCIM 2931) procured from National Chemical Laboratory, Pune, India. The bacterial strains were maintained on nutrient agar slants.

Antimicrobial assay was carried out by the disk diffusion method (Vardar-Unlu *et al.*, 2003) For in vitro antimicrobial activity, 200 µL of overnight grown culture of each bacteria was dispensed into 20 mL sterile nutrient broth and incubated for 4–5 h at 37 °C to standardize the culture to $10^{-5}$ CFU/mL. For this, 0.1 mL ($10^{-5}$ CFU/mL) of 24-h-old bacterial culture was placed on Muller–Hinton agar medium and spread throughout the plate by spread plate technique. Synthesized compounds (10 mg) were dissolved in 1 mL of DMSO (dimethyl sulphoxide), and 25 µL of respective samples was added to the sterile disks (6 mm diameter) purchased from HIMEDIA laboratories individually and aseptically transferred to the inoculated petri plates and incubated for 24 h. Antimicrobial activity was recorded by measuring the diameter of zone of inhibition. Streptomycin (HIMEDIA) was used as positive standard against bacterial strains.

*Minimum inhibitory concentration (MIC)*

MIC of the DMSO extracts was determined by using different concentrations of extracts in Mueller–Hinton broth for bacteria and potato dextrose broth for fungi by macrodilution method (National Committee for Clinical Laboratory Standards, 2000; Akinyemi *et al.*, 2005). The lowest concentration of the DMSO extract inhibiting the visible growth of microorganisms was considered as MIC.

Freshly prepared nutrient broth was used as diluents. The 24-h-old culture of test bacteria *S. aureus* and *E. coli* was diluted 100-fold in nutrient broth (NB) (100 µL bacterial cultures in 10 mL NB). The stock solution of synthesized compounds was prepared in DMSO by dissolving 10 mg of compound in 1 mL of DMSO. Increasing concentrations of test samples (1.25, 2.5, 5, 10, 20, 40 µL of stock solution contains 12.5, 25, 50, 100, 200, 400 µg of the compounds) were added to test tubes containing the bacterial cultures. All tubes were incubated at 37 °C for 24 h for bacteria. Tubes were examined for visible turbidity and using NB as control. Control without test samples and with solvent was assayed simultaneously. The lowest concentration that inhibited visible growth of the tested organisms was recorded as MIC.

## Conclusions

A new series of *N*-[4-(5-(4-substituted-phenyl)-1-phenyl-1*H*-pyrazol-3-yl)phenyl-2-(3-hydroxy-1-phenyl-1*H*-pyrazol-4-yl)] acetamides **5(a–i)** were synthesized by Michael addition reaction of hydrazine to chalcones **4(a–i)**. All

synthesized compounds were characterized by spectral and evaluated for in vivo analgesic, anti-inflammatory and in vitro antimicrobial activities. The results of in vivo assays showed that compounds containing substituted halogens **5a**, **5b** and **5c** possess better activity in comparison with standard drugs. The other compounds showed moderate-to-less activity. This study was also supported by molecular docking study of the active compounds with mouse COX-1 (PDB ID: 1CQE) and COX-2 (PDB ID: 1CX2) enzymes. By binding energy values, it could be concluded that tested compounds were more selective toward COX-2 enzyme with minimum binding energy than the COX-1 enzyme. The results of in vitro antimicrobial activity showed that compounds **5a, 5b** and **5g** emerged as active compounds with larger zone of inhibition at MIC value 125 μg/mL against *S. aureus* and *E. coli* bacterial strains. Among all assays conducted, *N*-[4-(5-(4-bromophenyl)-1-phenyl-1*H*-pyrazol-3-yl)phenyl-2-(3-hydroxy-1-phenyl-1*H*-pyrazol-4-yl)] acetamide (**5b**) emerged as most potent molecule.

**Acknowledgments** B.N. thanks the UGC for financial assistance through BSR one-time grant for the purchase of chemicals. P.S.N. thanks Manipal Institute of Technology for NMR data and DST-PURSE for financial assistance.

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