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Synthesis, pharmacological activities and molecular docking studies of pyrazolyltriazoles as anti-bacterial and anti-inflammatory agents

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Anti-inflammatory activity

ABSTRACT

A series of novel pyrazolyl alcohols (**5a-h**), pyrazolyl azides (**6a-h**), and pyrazolyltriazoles (**8a-h**, **10a-p** and **12a-l**) were prepared and evaluated for their bioactivity (anti-bacterial and anti-inflammatory) profile. The compound **5c** displayed the potent anti-bacterial activity against *Micrococcus luteus* (MIC 3.9 and MBC 7.81 μg/mL). *In vitro* anti-inflammatory activity data denoted that compound **8b** is effective among the tested compounds against IL-6 (IC_{50} 6.23 μM). Docking analysis of compounds **5f**, **8a-b**, **8e-f** and **8h** displayed high binding energies for the compounds **8a-b** and **8h** towards TNF-α dimer (2AZ5 protein) and IL-6 (1ALU protein). *In vivo* anti-inflammatory activity of compounds **8b** and **8h** with respect to LPS induced mice model indicated that compound **8h** showed significant reduction in TNF-α.

1. Introduction

Pyrazoles and its derivatives are important nitrogen containing heterocyclic compounds.[1] Pyrazoles constitute the core structures of natural products and pyrazole based well known drugs are available in the market such as Celebrex, Viagra and Acomplia.[2] On the other hand 1,2,3-triazoles have emerged as an important heterocyclic compounds, have been displayed various biological activities and widely used as pharmaceuticals.[3] Therefore, the development of synthetic methods for the preparation of pyrazoles and triazoles are continuous to be an active area of research.

Inflammation is a process with the tumor necrosis factor (TNF-α) and interleukin-6 (IL-6) play a major role in pathogenesis.[4] Inflammation is a condition of body immunity, swelling, redness, heat, ache and pain associated with damaged tissue or organ. Further, it is associated with diseases including allergy, arthritis, atherosclerosis and auto-immune diseases. The major pharmacological action of nonsteroidal anti-inflammatory drugs (NSAIDs) is the enzymatic inhibition of cyclooxygenase (COX) mediated production of pro-inflammatory prostaglandins and thromboxanes.[5] NSAIDs are used for the treatment of acute inflammation. The long term use of NSAIDs leads to the side effects such as bleeding, gastrointestinal ulceration and kidney damage. Celecoxib (Fig. 1), Valdecoxib and Rofecoxib are the potent and gastrointestinal safe anti-inflammatory drugs. Recently, pyrazole and triazole linked derivatives were reported as potential PDE4, inhibitors.[6]

As part of our ongoing work on synthesis, biological activities of triazoles[7–10] and pyrazoles,[11–14] authors found that both these molecules are effective bioactive compounds. Literature search against pyrazolyltriazoles revealed no information on synthesis and biological activities. In view of the above, the present manuscript describes the preparation of pyrazole based pyrazolyl alcohols, pyrazolyltriazoles and evaluation of their anti-microbial as well as anti-inflammatory activities against TNF-α and IL-6 (*in vitro*) and LPS induced mice (*in vivo*). The structure activity relationships and molecular modeling studies are also discussed.

2. Results and discussion

2.1. Chemistry

The preparation of target compounds pyrazolyl-1*H*-1,2,3-triazolyl alcohols **8a-h**, pyrazolyl-1*H*-1,2,3-triazoles **10a-p** and pyrazolyl-1*H*-1,2,3-triazolyl carboxylates **12a-l** were depicted in Schemes 1–3. Condensation of acetophenones **1a-h** with henylhydrazine

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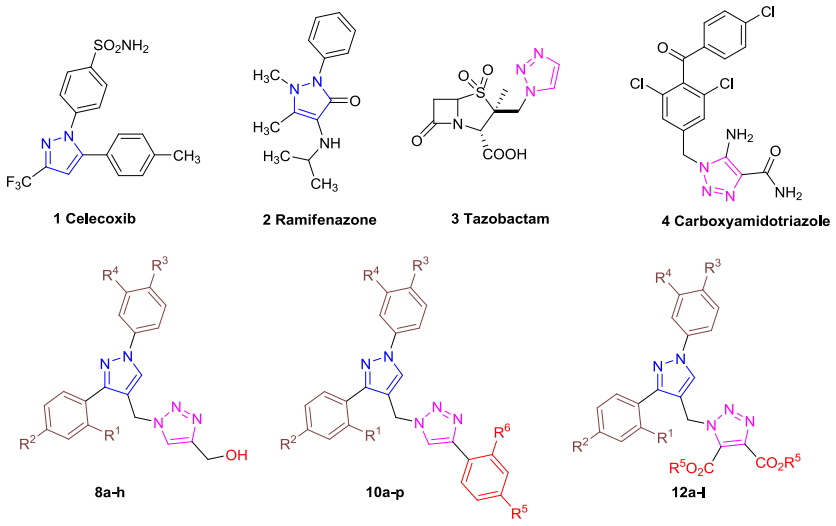
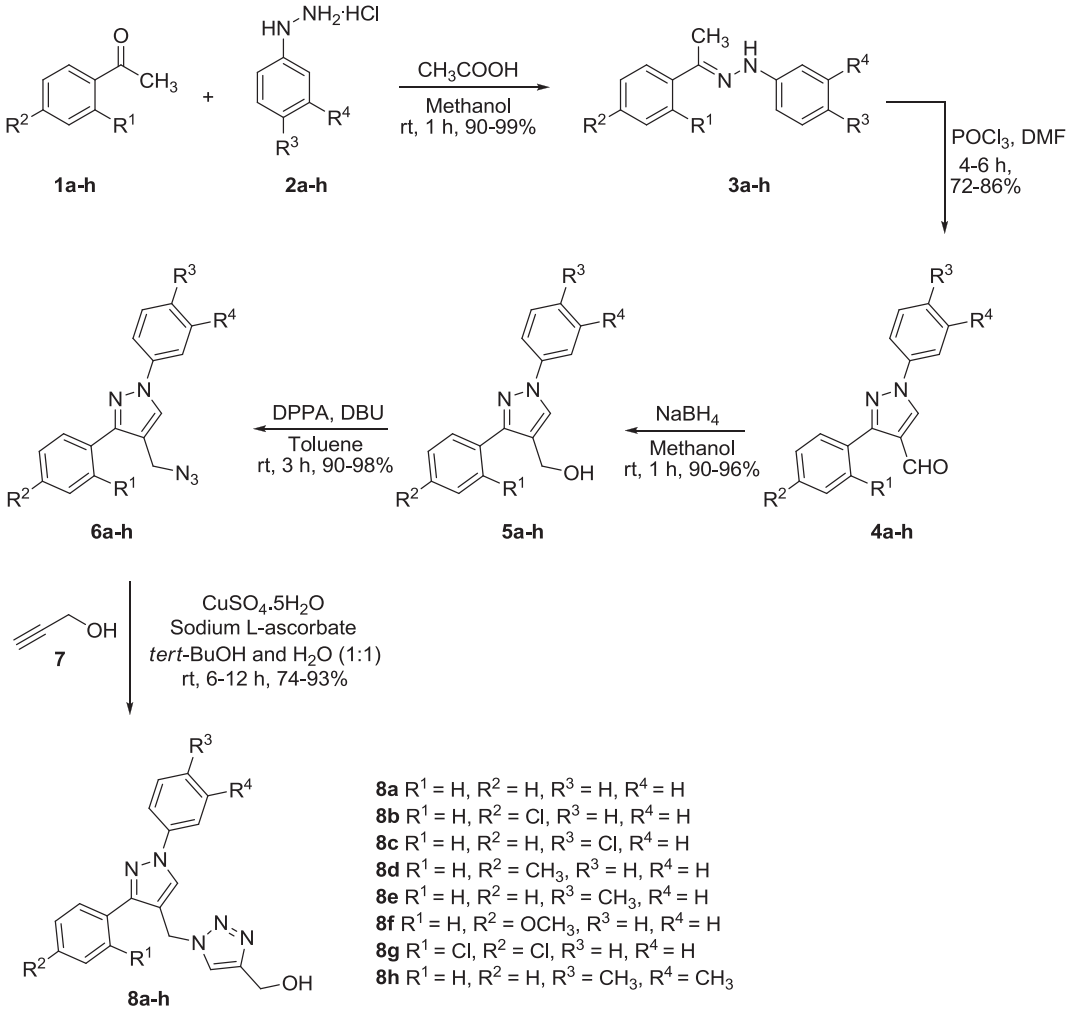
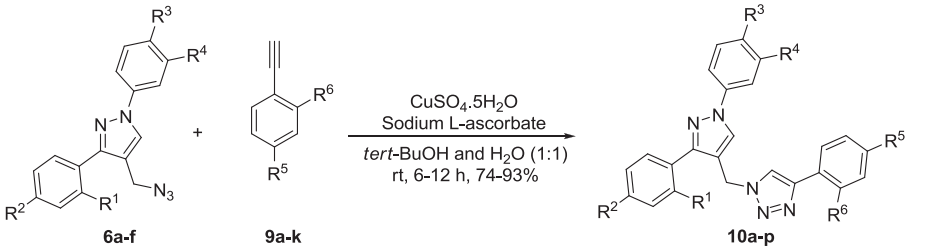


Fig. 1. Structures of pyrazole (**1**-**2**) and triazole (**3**-**4**) drugs; present work on pyrazole based heterocyclic compounds (**8a-h**, **10a-p** and **12a-l**).



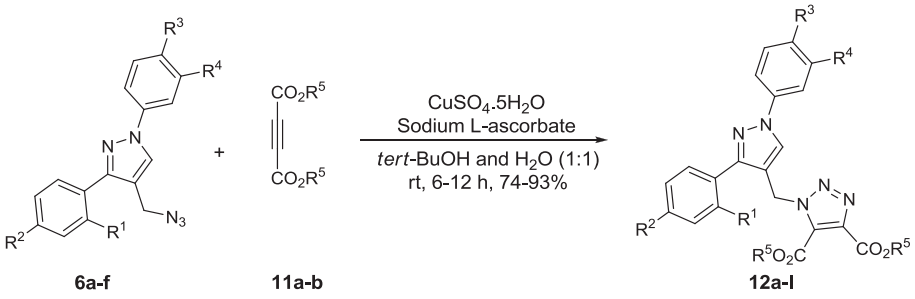
Scheme 1. Synthesis of substituted 1*H*-1,2,3-triazolyl alcohols **8a-h**.



6a-f 9a-k 10a-p

10a R^1 = H, R^2 = H, R^3 = H, R^4 = H, R^5 = H, R^6 = H
10b R^1 = H, R^2 = Cl, R^3 = H, R^4 = H, R^5 = H, R^6 = H
10c R^1 = H, R^2 = H, R^3 = Cl, R^4 = H, R^5 = H, R^6 = H
10d R^1 = H, R^2 = CH_3, R^3 = H, R^4 = H, R^5 = H, R^6 = H
10e R^1 = H, R^2 = H, R^3 = CH_3, R^4 = H, R^5 = H, R^6 = H
10f R^1 = H, R^2 = OCH_3, R^3 = H, R^4 = H, R^5 = H, R^6 = H
10g R^1 = H, R^2 = H, R^3 = H, R^4 = H, R^5 = H, R^6 = CF_3
10h R^1 = H, R^2 = H, R^3 = H, R^4 = H, R^5 = CF_3, R^6 = CF_3
10i R^1 = H, R^2 = H, R^3 = H, R^4 = H, R^5 = NH_2, R^6 = H
10j R^1 = H, R^2 = H, R^3 = H, R^4 = H, R^5 = $N(CH_3)_2$, R^6 = H
10k R^1 = H, R^2 = H, R^3 = H, R^4 = H, R^5 = F, R^6 = H
10l R^1 = H, R^2 = H, R^3 = H, R^4 = H, R^5 = Cl, R^6 = H
10m R^1 = H, R^2 = H, R^3 = H, R^4 = H, R^5 = Br, R^6 = H
10n R^1 = H, R^2 = H, R^3 = H, R^4 = H, R^5 = CH_3, R^6 = H
10o R^1 = H, R^2 = H, R^3 = H, R^4 = H, R^5 = OCH_3, R^6 = H
10p R^1 = H, R^2 = H, R^3 = H, R^4 = H, R^5 = OCH_3, R^6 = CH_3

Scheme 2. Synthesis of substituted 1*H*-1,2,3-triazoles **10a-p**.



6a-f 11a-b 12a-l

12a R^1 = H, R^2 = H, R^3 = H, R^4= H, R^5 = CH_3
12b R^1 = H, R^2 = Cl, R^3 = H, R^4= H, R^5 = CH_3
12c R^1 = H, R^2 = H, R^3 = Cl, R^4= H, R^5 = CH_3
12d R^1 = H, R^2 = CH_3, R^3 = H, R^4= H, R^5 = CH_3
12e R^1 = H, R^2 = H, R^3 = CH_3, R^4= H, R^5 = CH_3
12f R^1 = H, R^2 = OCH_3, R^3 = H, R^4= H, R^5 = CH_3
12g R^1 = H, R^2 = H, R^3 = H, R^4= H, R^5 = C_2H_5
12h R^1 = H, R^2 = Cl, R^3 = H, R^4= H, R^5 = C_2H_5
12i R^1 = H, R^2 = H, R^3 = Cl, R^4= H, R^5 = C_2H_5
12j R^1 = H, R^2 = CH_3, R^3 = H, R^4= H, R^5 = C_2H_5
12k R^1 = H, R^2 = H, R^3 = CH_3, R^4= H, R^5 = C_2H_5
12l R^1 = H, R^2 = OCH_3, R^3 = H, R^4= H, R^5 = C_2H_5

Scheme 3. Synthesis of substituted 1*H*-1,2,3-triazole-4,5-dicarboxylates **12a-l**.

hydrochlorides **2a-h** in the presence of acetic acid at room temperature provided the corresponding phenylhydrazones **3a-h** (Scheme 1). Vilsmeier-Haack reaction of **3a-h** in presence of DMF with $POCl_3$ afforded pyrazole carboxaldehydes **4a-h**.[15] $NaBH_4$ reduction of carboxaldehydes **4a-h** provided the corresponding alcohols **5a-h**. Alcohols **5a-h** were converted to corresponding azides **6a-h** in the presence of DPPA and DBU (Scheme 1).[16]

Click chemistry has been explored a new approach for the preparation of various heterocyclic molecules that can accelerate the drug discovery process. Thus prepared azides **6a-h** were subjected to cycloaddition with propargyl alcohol **7**, phenyl acetylenes **9a-k** and dimethyl/diethyl acetylenedicarboxylate **11a-b** to prepare series of target compounds (Schemes 1–3). Initially, we have carried out the reaction of azides **6a-h** with propargyl alcohol **7** in the presence of $CuSO_4{\cdot}5H_2O$/sodium ascorbate in aqueous alcohol medium. This provided the corresponding pyrazolyl-1*H*-1,2,3-triazolyl alcohols **8a-h** (Scheme 1). The cycloaddition of azides **6a-f** and phenyl acetylenes **9a-k** under optimized conditions provided pyrazolyl-1*H*-1,2,3-triazoles **10a-p** (Scheme 2).

Similarly, cycloaddition of azides **6a-f** with dimethyl/diethyl acetylenedicarboxylate **11a-b** provided the corresponding pyrazolyl-1*H*-1,2,3-triazolyl carboxylates **12a-l** (Scheme 3). Thus prepared compounds **8a-h**, **10a-p** and **12a-l** are new and well characterized by spectral data (Supplementary information).

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2.2. Biology

The intermediate compounds pyrazolyl alcohols **5a-h**, pyrazolylazides **6a-h** and target compounds pyrazolyl-1*H*-1,2,3-triazolyl alcohols **8a-h**, pyrazolyl-1*H*-1,2,3-triazoles **10a-p**, pyrazolyl-1*H*-1,2,3-triazolylcarboxylates **12a-l** were screened for their anti-microbial and *in vitro* anti-inflammatory activities. Based on the *in vitro* anti-inflammatory data, the molecular modeling studies were performed for compounds **5f**, **8a-b**, **8e-f** and **8h** while for *in vivo* anti-inflammatory activity, compounds **8b** and **8h** selected based on activity profile data and experiments were carried out using LPS induced mice.

2.2.1. In vitro anti-microbial activity

The *in vitro* anti-microbial activity, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of all the synthesized compounds (**5a-h**, **6a-h**, **8a-h**, **10a-p** and **12a-l**) were analyzed and the data presented in Table 1. The *in vitro* anti-bacterial activity of compounds and internal standard, streptomycin, was tested against two Gram-positive organisms (*Micrococcus luteus* and *Staphylococcus aureus*) and two Gram-negative organisms (*Salmonella typhi* and *Salmonella paratyphi*) by agar well plate method.[17] The *in vitro* anti-fungal activity of compounds (**5a-h**, **6a-h**, **8a-h**, **10a-p** and **12a-l**) was tested by agar plate method against two fungal strains (*Aspergillus niger* and *Aspergillus fumigates*) with fluconazole as standard. The MIC and MBC studies were performed by using tube dilution.

The data presented in Table 1 describes that the chloro pyrazolyl alcohol **5c** and pyrazolyl triazole alcohol **8f** displayed better anti-bacterial activity (26.5 and 26 mm zone of inhibition) against *M. luteus* compared to standard (22.5 mm); while, none of the synthesized compounds showed anti-bacterial activity against the other Gram positive bacterium, *S. aureus*. With respect to Gram negative bacteria, though some of the synthesized compounds revealed anti-bacterial activity however, which is less than standard (Table 1). This data denoted that the synthesized compounds role in growth inhibition of bacterial strains differ with chemical nature of the compounds. Critical evaluation of Table 1 data further indicated that the compound **5c** and **8f** are effective as anti-bacterial agents in comparison with other synthesized compounds. Hence, these compounds (**5c** and **8f**) were further studied for evaluation of concentration dependent anti-bacterial activity profile by measuring the MIC and MBC against *M. luteus*, *S. typhi* and *S. paratyphi* (Table 1). Between **5c** and **8f**, compound **5c** observed to be most potent against *M. leteus* and the observed MIC value is 3.9 and 7.81 μg/ml whereas standard (streptomycin) showed 6.25 μg/ml (for MIC and MBC) (Table 1).

2.2.2. In vitro anti-inflammatory activity

The *in vitro* anti-inflammatory activities of compounds **5a-h**, **6a-h**, **8a-h**, **10a-p** and **12a-l** were tested by using protein (egg albumin) denaturation method[18] along with the standard drug, diclofenac sodium (Table 2) while LPS-induced TNF-α and IL-6 (based on enzyme-linked immunosorbent assay (ELISA)[19] was carried out for those compounds which revealed better activity profile and compared with rolipram (Table 3). Analysis of the structure activity relationship of compounds **5a-h** denoted IC_{50} in the range of 54.21–105.45 μg/ml with respect to *in vitro* protein denaturation profile. The compound with chlorine substitution (**5c**, IC_{50} 54.21 μg/ml) at *N*-aryl displayed equipotency with respect to the standard compound (IC_{50} 53.70 μg/ml; Table-2). However, compound **5a**, **5b** (chlorine substitution at C-aryl) and **5h** (dimethyl substitution at *N*-aryl) displayed moderate activity, while **5d-e** (methyl substitution) and **5g** (dichloro) reduced the activity. Methoxy substituted derivative (**5f**) drastically reduced the activity. Addition of triazole moiety to pyrazolyl derivative (**8a-h**) denoted IC_{50} in the range of 58.00 –128.89 μg/ml. Among these, the compound **8e** showed similar trend as that of **5a-b** and **5h**. Comparing the activity profile of **5f** and **8f** (both have the methoxy substitution) with triazole moiety (**8f**) improved the activity (Table 2).

In vitro anti-inflammatory activity based on TNF-α and IL-6 revealed that pyrazolyltriazole alcohol compounds showed better activity compared to pyrazole alcohols (Table 3, Fig. 2). Among pyrazole alcohols, compound with methoxy substitution (**5f**) showed activity, while the same compound denoted weak activity at protein denaturation (Table 2); while analysis of activity at TNF-α and IL-6, compound **5f** denoted fourfold improved activity with IL-6 (Table 3). In case of pyrazolyltriazole alcohols, the compounds **8a-b**, **8e-f** and **8h** showed activity in TNF-α and IL-6. IL-6 based activity is much better to that of TNF-α, while standard compound showed similar activity profile with TNF-α and IL-6 (Table 3), whereas egg albumin based *in vitro* anti-inflammatory activity was noticed to **8e** and **8f** (Table 2).

Table 1
Anti-bacterial activity of the compounds **5a-h** and **8a-h**.

Compound*	Zone of inhibition in mm (MIC, MBC [μg/ml])			
	Gram positive Bacteria		Gram negative Bacteria	
	M. luteus	*S. aureus*	*S. typhi*	*S. paratyphi*
5a	–	–	11.5 ± 0.71	14 ± 0.00
5b	–	–	–	12 ± 0.00
5c	26.5 ± 0.71 (3.9, 7.81)	–	21.5 ± 0.71 (62.5, 125)	18 ± 0.00 (31.25, 62.5)
5d	16.5 ± 0.71	–	19 ± 0.00	14 ± 0.00
5e	–	–	17 ± 1.41	12.5 ± 0.71
5f	–	–	–	11.5 ± 0.71
5g	–	–	13.5 ± 0.71	11.5 ± 0.71
5h	–	–	14.5 ± 0.71	12 ± 0.00
8a	–	–	–	12.5 ± 0.71
8b	–	–	–	15 ± 0.00
8c	11.5 ± 0.71	–	19.5 ± 0.71	16.5 ± 0.71
8d	–	–	15 ± 0.00	14 ± 0.00
8e	–	–	19 ± 0.00	11.5 ± 0.71
8f	26 ± 0.00 (125, 125)	–	16 ± 0.00 (62.5, 125)	19 ± 0.00 (62.5, 62.5)
8g	–	–	14 ± 1.41	17 ± 0.00
8h	–	–	15.5 ± 0.71	–
Streptomycin	22.5 ± 0.71 (6.25, 6.25)	20.3 ± 0.57	27 ± 1.41 (6.25, 6.25)	30 ± 0.00 (6.25, 6.25)

* Compounds **6a-h**, **10a-p** and **12a-l** were not shown activity; hence they have not incorporated.

Table 2
Anti-inflammatory activity of the compounds **5a-h** and **8a-h** based on protein denaturation method.

Egg Albumin			
Compound*	% inhibition		IC_{50} (μg/ml)
	50 (μg/ml)	100 (μg/ml)	
5a	75.86 ± 0.17	87.93 ± 0.87	56.86 ± 0.76
5b	67.24 ± 0.44	83.62 ± 0.46	59.79 ± 0.34
5c	76.72 ± 0.89	92.24 ± 0.78	54.21 ± 0.87
5d	60.34 ± 0.43	81.90 ± 0.83	61.05 ± 0.65
5e	40.52 ± 0.72	68.10 ± 0.33	73.42 ± 0.54
5f	15.52 ± 0.85	47.41 ± 0.59	105.45 ± 0.85
5g	60.34 ± 0.58	80.17 ± 0.87	62.37 ± 0.43
5h	66.38 ± 0.22	83.62 ± 0.53	59.79 ± 0.12
8a	10.34 ± 0.89	38.79 ± 0.21	128.89 ± 0.74
8b	49.14 ± 0.47	73.28 ± 0.79	68.24 ± 0.32
8c	55.17 ± 0.78	78.45 ± 0.77	63.74 ± 0.84
8d	21.55 ± 0.64	38.79 ± 0.98	128.89 ± 0.75
8e	66.38 ± 0.95	83.62 ± 0.67	59.79 ± 0.39
8f	68.97 ± 0.44	86.21 ± 0.34	58.00 ± 0.71
8g	50.86 ± 0.25	76.72 ± 0.51	65.17 ± 0.82
8h	46.55 ± 0.77	77.59 ± 0.87	64.44 ± 0.55
Diclofenac sodium	66.38 ± 0.06	93.10 ± 0.12	53.70 ± 0.24

* Compounds **6a-h**, **10a-p** and **12a-l** were not shown activity; hence they have not incorporated.

2.2.3. Docking studies on TNF-α and IL-6

Docking studies were performed to those compounds (**5f**, **8a-b**, **8e-f** and **8h**) showed the anti-inflammatory activity at *in vitro* analysis (Table 4; Figs. 3 & 4) along with rolipram as standard towards protein target 2AZ5 (TNF-α dimer) and 1ALU (IL-6).[20] Among the tested compounds, compound **8b** showed the highest (−8.27) binding energy while this value was lowest (−6.64) for **5f** against TNF-α, and rolipram showed binding energy of −8.86 K.cal/mol. In case of IL-6, the compounds **8a** (−6.71) and **8f** (−5.55) showed the highest and lowest binding energy values respectively, and the rolipram showed −6.55 (Table 4). Evaluation of interactive nature (hydrogen bonding) between protein and synthesized compounds suggested that compounds **5f** (Fig. 3 A), **8a** (Fig. 3 B) and **8f** (Fig. 3 E) interacted with protein by single hydrogen bonding while **8b** (Fig. 3 C), **8e** (Fig. 3 D) and **8h** (Fig. 3 F) showed two interactions each with target protein [2AZ5 (TNF-α dimer)] while with respect to IL-6 target protein (1ALU), the compounds **5f** (Fig. 4 A) and **8a-b** (Fig. 4 B-C) denoted two interactions whereas the compounds **8e-f** (Fig. 4 D-E) and **8h** (Fig. 4 F) depicted single interaction.

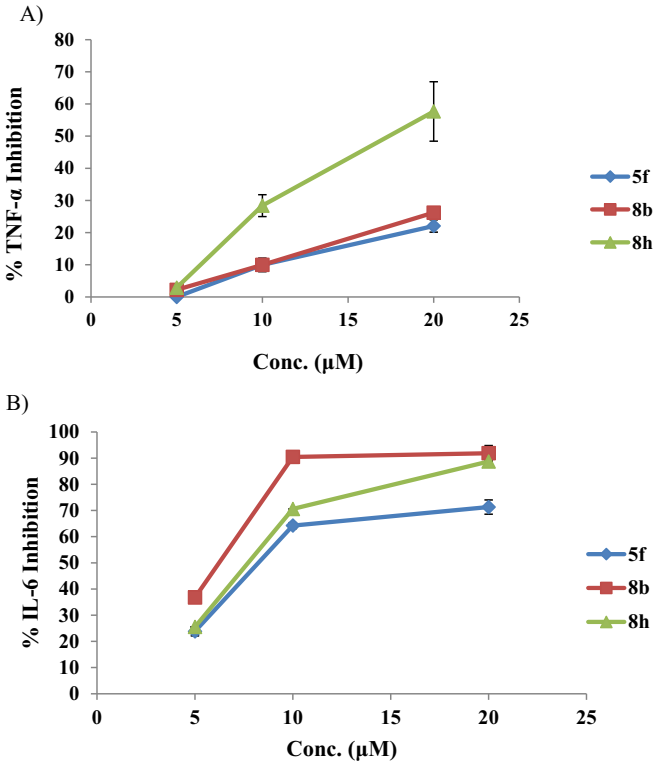

Fig. 2. The active compounds (**5f**, **8b** and **8h**) inhibit PMA/LPS-induced production of IL-6 and TNF-α in U937cells. U937 cells (5×10^4 cells/ml) were pre-incubated for 30 min in the presence of the indicated concentrations of active compounds, and then they were treated with 100 ng/ml LPS for 24 h (A, B). The concentration of TNF-α and IL-6 in the culture supernatants was measured using an ELISA assay. Data shown are typical of three independent experiments with similar results.

2.2.4. In vivo anti-inflammatory activity by LPS challenged mouse model

LPS has been implicated as an important pathogenic factor for the induction of sepsis, which is characterized by an inflammatory cytokine storm. Hence, *in vivo* anti-inflammatory activity of

Table 3
Anti-inflammatory activity (TNF-α and IL-6) of the compounds **5a-h** and **8a-h**.

Compound	TNF-α		IL-6	
	% Inhibition (10 μM)	IC_{50} (μM)	% Inhibition (10 μM)	IC_{50} (μM)
5a	1.44 ± 0.60	ND	19.57 ± 0.23	ND
5b	3.32 ± 0.04	ND	24.11 ± 6.56	ND
5c	2.61 ± 0.92	ND	21.65 ± 1.48	ND
5d	0.74 ± 0.22	ND	20.87 ± 3.36	ND
5e	ND	ND	23.77 ± 6.95	ND
5f	22.95 ± 1.67	35.53 ± 2.34	68.47 ± 1.64	8.24 ± 1.48
5g	4.92 ± 0.84	ND	18.33 ± 5.23	ND
5h	8.35 ± 1.30	ND	46.36 ± 1.80	ND
8a	15.70 ± 1.10	35.74 ± 2.45	52.99 ± 4.22	8.12 ± 0.69
8b	19.24 ± 3.94	30.92 ± 1.50	76.94 ± 0.01	6.23 ± 0.23
8c	12.73 ± 4.68	ND	38.82 ± 0.86	ND
8d	14.75 ± 2.35	ND	42.04 ± 1.33	ND
8e	31.19 ± 3.51	21.39 ± 1.89	50.40 ± 3.52	8.70 ± 0.51
8f	40.88 ± 0.10	17.18 ± 1.01	46.73 ± 7.34	9.50 ± 1.03
8g	9.90 ± 2.78	ND	39.57 ± 9.06	ND
8h	41.73 ± 5.61	17.40 ± 0.92	87.00 ± 8.36	7.72 ± 0.82
Rolipram	59.13 ± 1.76	1.12 ± 0.03	53.53 ± 3.05	1.17 ± 0.49

ND: Not detected; results summarized in the table, for both % of cytokines inhibition presented as the mean ± SEM from three different experiments.

Table 4
Dock score values for the compounds **5f**, **8a-b**, **8e-f** and **8h**.

Compound	TNF-α		IL-6	
	Binding energy (XP glide score) (K.cal/mol)	Interactions	Binding energy (XP glide score) (K.cal/mol)	Interactions
5f	−6.64	Leu120	−5.57	Lys66, Lys86
8a	−7.51	Tyr151	−6.71	Met67, Ser176
8b	−8.27	Tyr151, Gln61	−6.44	Met67, Ser176
8e	−7.40	Tyr151, Tyr151	−5.79	Lys86
8f	−6.98	Tyr151	−5.55	Lys86
8h	−7.19	Tyr151, Gln61	−6.50	Leu64
Rolipram	−8.86	Tyr151, Ser60	−6.55	Lys66, Ala68
Indomethacin	−8.32	Tyr151, Gly121	−9.97	Arg182, Arg179

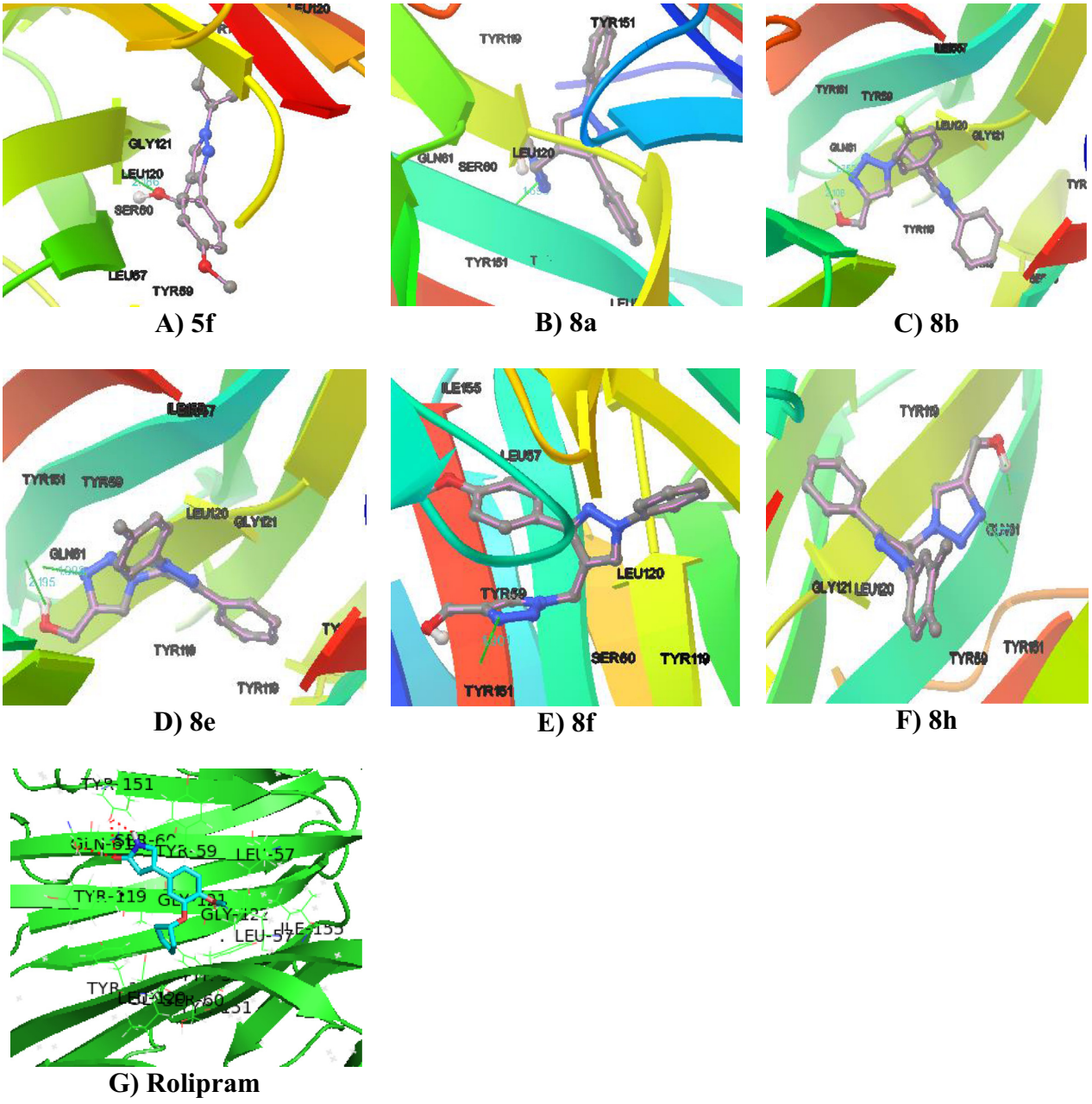

Fig. 3. Docking pose of compounds **5f**, **8a-b**, **8e-f** and **8h** with protein 2AZ5 (TNF-α dimer).

compounds **8b** and **8h** were evaluated on the basis of LPS induced cytokine expression in Balb/c mice (Fig. 5).[21,22] The challenge with LPS in mice showed significant ($P < 0.001$) increase in TNF-α (Fig. 5A) and IL-6 (Fig. 5B) levels compared to the vehicle control group of animals. In the treatment groups, mice were pre-treated with the test compounds **8b** and **8h** at a dose of 100 mg/kg for a

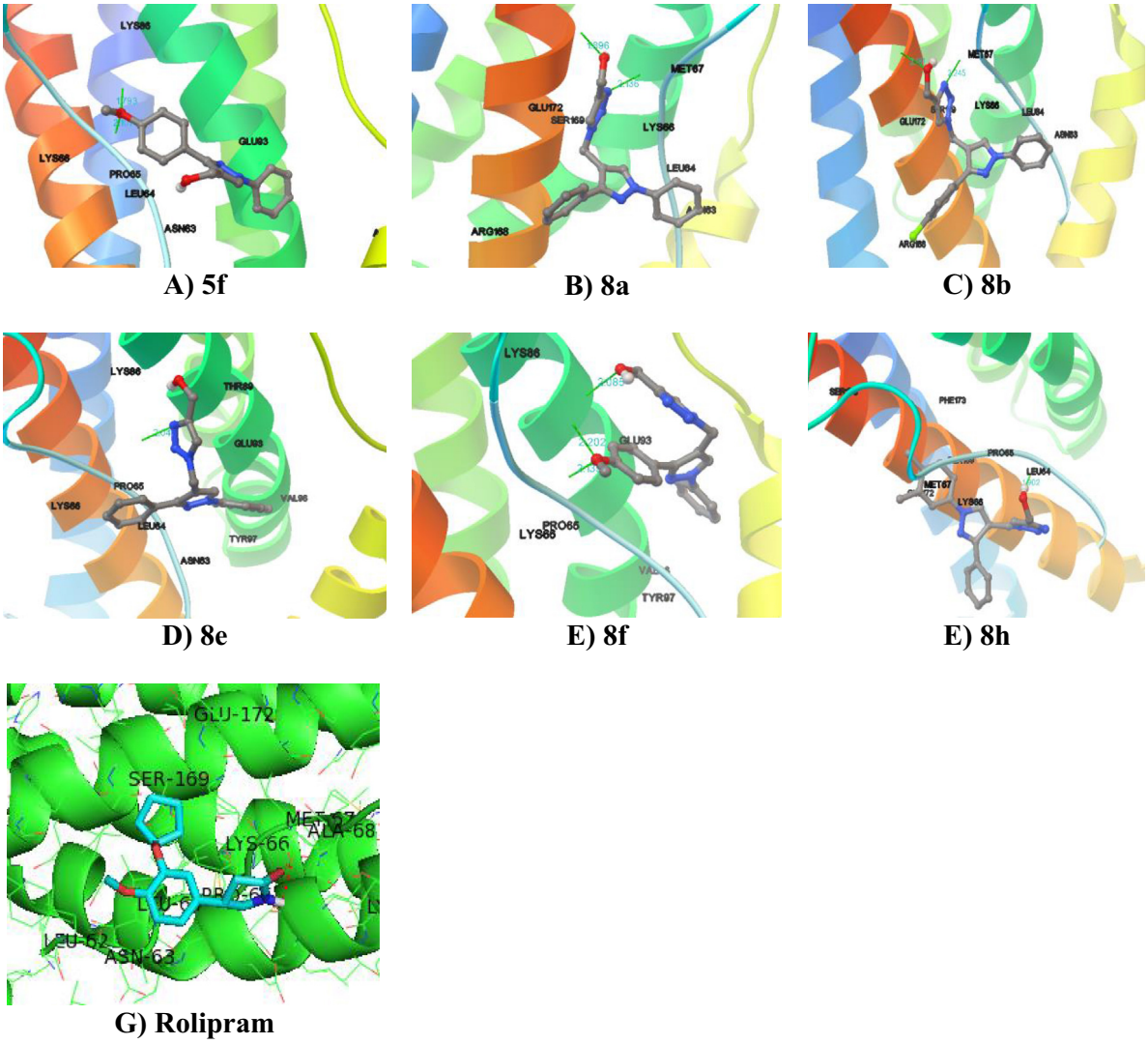

Fig. 4. Docking pose of compounds **5f**, **8a-b**, **8e-f** and **8h** with protein 1ALU (IL-6).

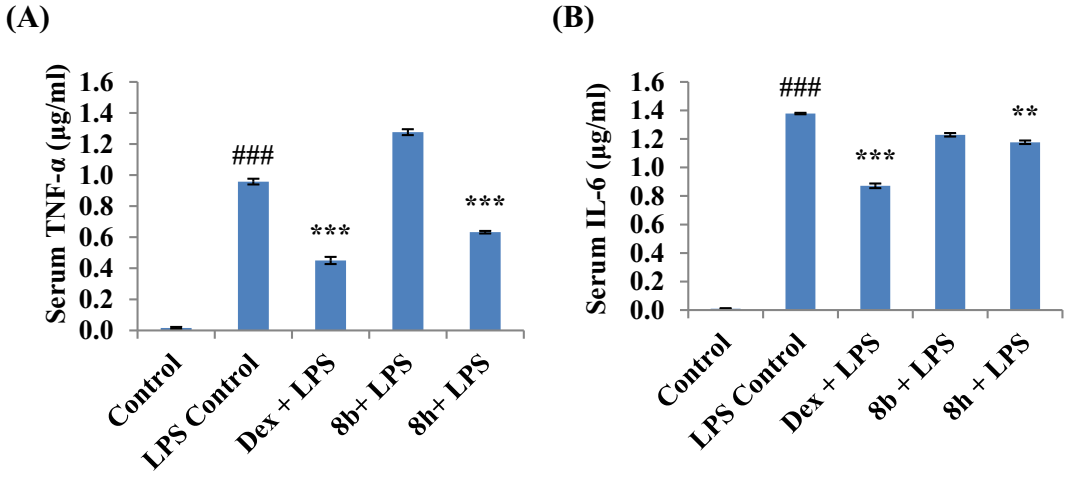

Fig. 5. Effect of test compounds on LPS induced serum inflammatory cytokine secretions. ELISA of tumor necrosis factor-alpha (TNF-α) (A) and interleukin-6 (IL-6) (B). The results were expressed as mean ± S.E.M. of 8 animals in each group. Control, vehicle control mice; LPS control, LPS control mice; LPS + Dex, LPS + Dexamethasone (2 mg/kg) treated mice; **8b** + LPS, LPS + **8b** (100 mg/kg) treated mice; **8h** + LPS, LPS + **8h** (100 mg/kg) treated mice. ### $p < 0.001$ vs. Control; ** $p < 0.01$ vs. LPS Control.

period of 3 consecutive days followed by LPS challenge (10 mg/kg). Results indicated that the test compound **8h** had shown significant ($P < 0.01$) inhibition in the TNF-α levels when compared to the LPS control animals, whereas the levels of IL-6 were not significantly ($P < 0.05$) altered (Fig. 5). Hence, compound **8h** may be considered as a good therapeutic agent to treat the inflammatory disease.

According to I. M. Garrelds et al[23] there is time dependent production of cytokines (TNF-α and IL-6) by U937 (Human monocytic leukemia) cells mentioning that the production of TNF-α is maximum after 6 h of incubation with gradual decrease at 24 h. It signifies the importance of time points for the analysis of cytokines in *in vitro* models. The independent regulation of the TNF-α and IL-6 induction during differentiation of U937 cells in *in vitro* and their synthesis in *in vivo* plays a prominent role when treated with compounds to suppress the formation of different inflammatory mediators.

Comparative analysis of bioinformatics data and experimental data (*in vitro* and *in vivo* anti-inflammatory) on TNF-α dimer and IL-6 proteins revealed that TNF-α showed more binding energies compared to IL-6 for the compounds **5f**, **8a-b**, **8e-f** and **8h**. Among binding energies of TNF-α, the compound **8b** showed highest binding energy (−8.27), while *in vitro* experimental data indicated **8f** compound displayed effective activity (IC_{50} 17.18 μM). With respect to IL-6 docking data, compounds **8a-b** and **8h** denoted almost similar binding energies (−6.44 to −6.71), whereas, *in vitro* data suggested **8b** shown better activity (IC_{50} 6.23 μM); while *in vivo* analysis indicated that compound **8h** has significant activity against TNF-α ($P < 0.01$). This high selectivity towards TNF-α inhibition in the *in vivo* studies may be due to inhibition of the NF-κB activation which in turn regulated the expression of TNF-α.

3. Conclusion

Series of pyrazolyl alcohols (**5a-h**), pyrazolyl azides (**6a-h**) and pyrazolyltriazoles (**8a-h**, **10a-p** and **12a-l**) were prepared and screened for their anti-microbial and anti-inflammatory (*in vitro* and *in vivo*) activities. The compound **5c** showed potential antibacterial activity against *M. luteus* while, the compounds **5f**, **8b** and **8h** demonstrated potent *in vitro* anti-inflammatory activity, whereas the compound **8h** was found to be effective *in vivo* LPS induced sepsis model in mice.

4. Experimental

4.1. Chemistry

All chemicals and reagents were purchased from Aldrich (Sigma-Aldrich, USA), AVRA Chemicals Pvt. Ltd (Hyderabad, India) and were used without further purification. Reactions were monitored by thin layer chromatography (TLC) on pre-coated silica gel 60 F_{254} (mesh); spots were visualized under UV light. Column chromatography has been carried out by using Merck silica gel (60–120 mesh). Melting points were determined in open glass capillary tubes on a Stuart melting point apparatus and are uncorrected. IR spectrum was recorded with a Thermo Nicollet Nexus 670 FT spectrometer. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker Avance 300, 400 and 500 MHz spectrometers. Chemical shifts (δ) are quoted in parts per million and are referenced to tetramethylsilane (TMS) as internal standard. ESI-MS obtained on quarto micro spectrometer. HRMS were carried out on Agilent 6510, Q-TOFLC/MS instrument ESI-Technique.

4.1.1. General procedure for the preparation of (E)-1-phenyl-2-(1-phenylethylidene)-hydrazines (**3a-h**)

Acetic acid (0.4 mmol) was added to a stirred solution of acetophenone **1a** (8.3 mmol) and phenyl hydrazine hydrochloride **2a** (9.9 mmol) in methanol. The contents were stirred at room temperature for 1 h. After completion of the reaction (TLC), the solvent was removed under reduced pressure and yellow colored solid **3a** was obtained. The other phenylhydrazones **3b-h** were prepared from corresponding acetophenones **1b-h** and phenyl hydrazine hydrochlorides **2b-h** under similar conditions.

*4.1.1.1. (E)-1-phenyl-2-(1-phenylethylidene)hydrazine (**3a**).* Ref. 24.

*4.1.1.2. (E)-1-(1-(4-chlorophenyl)ethylidene)-2-phenylhydrazine (**3b**).* Ref. 24.

*4.1.1.3. (E)-1-(4-chlorophenyl)-2-(1-phenylethylidene)hydrazine (**3c**).* Ref. 24.

*4.1.1.4. (E)-1-phenyl-2-(1-p-tolylethylidene)hydrazine (**3d**).* Ref. 24.

*4.1.1.5. (E)-1-(4-chlorophenyl)-2-(1-phenylethylidene)hydrazine (**3e**).* Ref. 24.

*4.1.1.6. (E)-1-(1-(4-methoxyphenyl)ethylidene)-2-phenylhydrazine (**3f**).* Ref. 25.

*4.1.1.7. (E)-1-(1-(2,4-dichlorophenyl)ethylidene)-2-phenylhydrazine (**3g**).* Ref. 25.

4.1.2. General procedure for the preparation of 1,3-diphenyl-1H-pyrazole-4-carbaldehydes (**4a-h**)

Phenylhydrazone (**3a**, 4.7 mmol) in ethanol was added to a stirred cold solution of Vilsmeier reagent, which is prepared by the addition of DMF (1.1 mL) to $POCl_3$ (2.2 mL) at 0 °C. The contents were brought to room temperature and the stirring was continued at 50–60 °C for 5 h. The reaction mixture was poured into ice cold water and extracted with ethyl acetate (2 × 10 mL). The organic layer was separated, washed with brine, dried over Na_2SO_4 and solvent was removed under reduced pressure. The obtained residue was purified by column chromatography (silicagel 60:120; ethyl acetate/hexane 8:92) provided 1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde **4a**. The 1*H*-pyrazole-4-carbaldehydes **4b-h** were synthesized from corresponding phenylhydrazones **3b-h** under similar conditions.

*4.1.2.1. 1,3-diphenyl-1H-pyrazole-4-carbaldehyde (**4a**).* Ref. 24.

*4.1.2.2. 3-(4-chlorophenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde (**4b**).* Ref. 24.

*4.1.2.3. 1-(4-chlorophenyl)-3-phenyl-1H-pyrazole-4-carbaldehyde (**4c**).* Ref. 24.

*4.1.2.4. 1-phenyl-3-p-tolyl-1H-pyrazole-4-carbaldehyde (**4d**).* Ref. 24.

*4.1.2.5. 3-phenyl-1-p-tolyl-1H-pyrazole-4-carbaldehyde (**4e**).* Ref. 25.

*4.1.2.6. 3-(4-methoxyphenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde (**4f**).* Ref. 15.

*4.1.2.7. 3-(3,4-dichlorophenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde (**4g**).* Ref. 26.

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*4.1.2.8. 1-(3,4-Dimethylphenyl)-3-phenyl-1H-pyrazole-4-carbaldehyde (**4h**).* Yield: 86%; white solid; m.p.: 119–121 °C; ^{1}H NMR ($CDCl_3$): δ 10.03 (s, 1H), 8.48 (s, 1H), 7.83–7.80 (m, 2H), 7.58 (d, J = 2.1 Hz, 1H), 7.51–7.45 (m, 4H), 7.23 (d, J = 8.2 Hz, 1H), 2.33 (s, 3H), 2.30 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 185.12, 154.52, 138.18, 136.89, 136.62, 131.41, 130.79, 130.51, 129.13, 128.92, 128.67, 122.19, 120.93, 116.93, 19.89, 19.34; ESI-MS: m/z, 277 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{18}H_{16}N_2O$ $[M+H]^+$ 277.1335, found 277.1329.

*4.1.3. General procedure for the synthesis of (1,3-diphenyl-1H-pyrazol-4-yl)-methanols (**5a-h**)*

Sodium borohydride (1.2 mmol) was added to a stirred solution of pyrazole aldehyde **4a** (1.0 mmol) in methanol at 0 °C. The reaction mixture was stirred for 1 h at room temperature. After completion of the reaction (TLC), the solvent was removed under reduced pressure; cold water was added and extracted with ethyl acetate (2 × 10 mL). The organic layer was washed with brine, dried over Na_2SO_4 and solvent was removed under reduced pressure provided (1,3-diphenyl-1*H*-pyrazol-4-yl)methanol **5a**. The 1*H*-pyrazolylmethanols **5b-h** were prepared from the corresponding pyrazole aldehydes **4b-h** under similar conditions.

*4.1.3.1. (1,3-Diphenyl-1H-pyrazol-4-yl)methanol (**5a**).* Ref. 27.

*4.1.3.2. (3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)methanol (**5b**).* Ref. 28.

*4.1.3.3. (1-(4-chlorophenyl)-3-phenyl-1H-pyrazol-4-yl)methanol (**5c**).* Ref. 28.

*4.1.3.4. (1-phenyl-3-p-tolyl-1H-pyrazol-4-yl)methanol (**5d**).* Ref. 27.

*4.1.3.5. (3-phenyl-1-p-tolyl-1H-pyrazol-4-yl)methanol (**5e**).* Ref. 27.

*4.1.3.6. (3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)methanol (**5f**).* Ref. 27.

*4.1.3.7. (3-(2,4-Dichlorophenyl)-1-phenyl-1H-pyrazol-4-yl)methanol (**5g**).* Yield: 98%; white solid; m.p.: 116–118 °C; ^{1}H NMR ($CDCl_3$): δ 8.04 (s, 1H), 7.73–7.69 (m, 2H), 7.51 (d, J = 2.1 Hz, 1H), 7.47–7.43 (m, 3H), 7.34–7.28 (m, 2H), 4.57 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 148.64, 139.74, 135.11, 134.42, 133.00, 130.68, 129.54, 129.45, 127.16, 126.86, 126.73, 122.62, 119.10, 56.09; ESI-MS: m/z, 319 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{16}H_{12}Cl_2N_2O$ $[M+H]^+$ 319.0399, found 319.0410.

*4.1.3.8. (1-(3,4-Dimethylphenyl)-3-phenyl-1H-pyrazol-4-yl)methanol (**5h**).* Yield: 96%; white solid; m.p.: 138–140 °C; ^{1}H NMR ($CDCl_3$): δ 7.95 (s, 1H), 7.85 (d, J = 7.4 Hz, 2H), 7.55 (s, 1H), 7.48–7.35 (m, 4H), 7.19 (d, J = 8.1 Hz, 1H), 4.74 (s, 2H), 2.33 (s, 3H), 2.29 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 151.18, 137.92, 135.03, 133.07, 130.38, 128.66, 128.03, 127.82, 120.46, 120.42, 116.34, 56.07, 19.99, 19.34; ESI-MS: m/z, 279 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{18}H_{18}N_2O$ $[M+H]^+$ 279.1492, found 279.1494.

*4.1.4. General procedure for the preparation of 4-(azidomethyl)-1,3-diphenyl-1H-pyrazole (**6a-h**)*

DBU (1.2 mmol) and diphenylphosphoryl azide (1.5 mmol) was added sequentially to a stirred solution of (1,3-diphenyl-1*H*-pyrazol-4-yl)methanol **5a** (1.0 mmol) in dry toluene (10 mL) at room temperature. The reaction mixture was stirred for 3 h at room temperature. After completion of the reaction (TLC), the reaction mixture was quenched with aqueous NH_4Cl solution (8 mL) and extracted with ethyl acetate (2 × 10 mL). The organic layer was separated, washed with brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by column chromatography using silica gel (ethyl acetate/hexane 2:98) provided 4-(azidomethyl)-1,3-diphenyl-1*H*-pyrazole **6a** as colorless liquid. The azide compounds **6b-h** were prepared from corresponding pyrazolyl alcohols **5b-h** under similar conditions.

*4.1.4.1. 4-(Azidomethyl)-1,3-diphenyl-1H-pyrazole (**6a**).* Ref. 29.

*4.1.4.2. 4-(azidomethyl)-3-(4-chlorophenyl)-1-phenyl-1H-pyrazole (**6b**).* Ref. 29.

*4.1.4.3. 4-(Azidomethyl)-1-(4-chlorophenyl)-3-phenyl-1H-pyrazole (**6c**).* Ref. 29.

*4.1.4.4. 4-(Azidomethyl)-1-phenyl-3-p-tolyl-1H-pyrazole (**6d**).* Ref. 29.

*4.1.4.5. 4-(Azidomethyl)-3-phenyl-1-p-tolyl-1H-pyrazole (**6e**).* Ref. 29.

*4.1.4.6. 4-(Azidomethyl)-3-(4-methoxyphenyl)-1-phenyl-1H-pyrazole (**6f**).* Ref. 29.

*4.1.4.7. 4-(Azidomethyl)-3-(2,4-dichlorophenyl)-1-phenyl-1H-pyrazole (**6g**).* Yield: 88%; colorless liquid; ^{1}H NMR ($CDCl_3$): δ 8.04 (s, 1H), 7.72–7.70 (m, 2H), 7.51 (d, J = 2.1 Hz, 1H), 7.47–7.43 (m, 3H), 7.34–7.28 (m, 2H), 4.58 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 152.09, 138.24, 132.16, 129.54, 128.75, 128.48, 127.87, 127.83, 120.12, 115.35, 45.23; ESI-MS: m/z, 344 $[M+H]^+$.

*4.1.4.8. 4-(Azidomethyl)-1-(3,4-dimethylphenyl)-3-phenyl-1H-pyrazole (**6h**).* Yield: 86%; colorless liquid; ^{1}H NMR ($CDCl_3$): δ 7.94 (s, 1H), 7.78–7.75 (m, 2H), 7.54 (d, J = 1.9 Hz, 1H), 7.46–7.36 (m, 4H), 7.17 (d, J = 8.2 Hz, 1H), 4.38 (s, 2H), 2.31 (s, 3H), 2.26 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 151.42, 137.86, 137.64, 135.20, 132.49, 130.30, 128.63, 128.15, 128.02, 127.81, 120.36, 116.33, 114.36, 45.24, 19.85, 19.22; ESI-MS: m/z, 304 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{18}H_{17}N_5Na$ 326.1376, found 326.1370.

*4.1.5. General procedure for the preparation of 1H-1,2,3-triazolyl methanols (**8a-h**)*

Propargyl alcohol **7** (1.0 mmol) was added to a stirred solution of pyrazole azide **6a** (1.0 mmol) in *tert*-butanol and H_2O (1:1) at room temperature, followed by $CuSO_4{\cdot}5H_2O$ (0.1 mmol) and sodium ascorbate (0.05 mmol). The reaction mixture was stirred for 6–12 h at room temperature. After completion of the reaction (TLC), the reaction mixture was extracted with ethyl acetate (2 × 5 mL). The organic extract was washed with H_2O and dried over anhydrous Na_2SO_4. The residue was purified by column chromatography using silica gel (ethyl acetate/hexane) furnished (1-((1,3-diphenyl-1*H*-pyrazol-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl) methanol **8a**. The 1*H*-1,2,3-triazolyl methanols **8b-h** were synthesized from pyrazole azides **6a-h** under similar conditions.

*4.1.5.1. (1-((1,3-Diphenyl-1H-pyrazol-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methanol (**8a**).* Yield: 96%; white solid; m.p.: 126–128 °C; IR ν_{max} (cm^{-1}): 3451, 2924, 1592, 1500, 1325, 1216, 947, 758; ^{1}H NMR ($CDCl_3$): δ 7.98 (s, 1H), 7.76–7.69 (m, 2H), 7.64–7.58 (m, 2H), 7.51–7.37 (m, 6H), 7.32 (d, J = 7.4 Hz, 1H), 5.60 (s, 2H), 4.72 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 151.65, 148.00, 139.50, 132.03, 129.53, 128.95, 128.66, 128.57, 127.93, 127.02, 121.50, 119.14, 114.47, 56.35, 44.75; ESI-MS: m/z, 332 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{19}H_{17}N_5O$ $[M+H]^+$ 332.1505, found 332.1508.

*4.1.5.2. (1-((3-(4-Chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methanol (**8b**).* Yield: 96%; white solid; m.p.:

156–158 °C; IR ν_{max} (cm^{-1}): 3447, 2924, 1595, 1500, 1327, 1217, 949, 754; ^1H NMR ($CDCl_3$+DMSO-d_6): δ 8.19 (s, 1H), 7.75 (d, J = 7.8 Hz, 2H), 7.64 (d, J = 8.3 Hz, 3H), 7.55–7.40 (m, 4H), 7.33 (t, J = 7.4 Hz, 1H), 5.61 (s, 2H), 4.70 (s, 2H); ^{13}C NMR ($CDCl_3$ + DMSO-d_6): δ 149.62, 148.44, 138.86, 133.70, 130.21, 128.94, 128.63, 128.38, 126.44, 121.16, 118.43, 114.30, 55.62, 43.92; ESI-MS: m/z, 366 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{19}H_{16}ClN_5O$ $[M+H]^+$ 366.1116, found 366.1126.

*4.1.5.3. (1-((1-(4-Chlorophenyl)-3-phenyl-1H-pyrazol-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methanol (**8c**).* Yield: 92%; white solid; m.p.: 154–156 °C; IR ν_{max} (cm^{-1}): 3373, 2862, 1589, 1494, 1392, 1202, 818, 701; ^1H NMR ($CDCl_3$): δ 7.95 (s, 1H), 7.68–7.65 (m, 2H), 7.62–7.58 (m, 2H), 7.49–7.40 (m, 6H), 5.60 (s, 2H), 4.74 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 151.92, 147.97, 138.07, 132.52, 131.85, 129.63, 129.00, 128.82, 128.40, 127.96, 121.47, 120.24, 115.00, 56.50, 44.73; ESI-MS: m/z, 366 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{19}H_{16}ClN_5O$ $[M+H]^+$ 366.1116, found 366.1121.

*4.1.5.4. (1-((1-Phenyl-3-(p-tolyl)-1H-pyrazol-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methanol (**8d**).* Yield: 89%; white solid; m.p.: 140–142 °C; IR ν_{max} (cm^{-1}): 3412, 2919, 1516, 1447, 1320, 1213, 813, 766; ^1H NMR ($CDCl_3$): δ 7.97 (s, 1H), 7.71 (d, J = 7.7 Hz, 2H), 7.53–7.39 (m, 5H), 7.33–7.25 (m, 3H), 5.61 (s, 2H), 4.74 (s, 2H), 2.40 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 151.73, 139.59, 138.59, 129.65, 129.52, 129.18, 128.47, 127.81, 126.94, 121.46, 120.43, 119.13, 114.38, 56.55, 44.82, 21.34; ESI-MS: m/z, 346 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{20}H_{19}N_5O$ $[M+H]^+$ 346.1662, found 346.1665.

*4.1.5.5. (1-((3-Phenyl-1-(p-tolyl)-1H-pyrazol-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methanol (**8e**).* Yield: 85%; white solid; m.p.: 166–168 °C; IR ν_{max} (cm^{-1}): 3447, 2922, 1593, 1499, 1327, 1216, 822, 758; ^1H NMR ($CDCl_3$): δ 7.94 (s, 1H), 7.63–7.57 (m, 4H), 7.50–7.38 (m, 4H), 7.25 (d, J = 8.9 Hz, 2H), 5.60 (s, 2H), 4.73 (d, J = 4.5 Hz, 2H), 2.38 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 151.41, 147.94, 137.32, 136.95, 132.16, 130.04, 128.94, 128.59, 128.49, 127.95, 121.42, 119.13, 114.17, 56.50, 44.79, 20.99; ESI-MS: m/z, 346 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{20}H_{19}N_5O$ $[M+H]^+$ 346.1662, found 346.1664.

*4.1.5.6. (1-((3-(4-Methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methanol (**8f**).* Yield: 95%; white solid; m.p.: 128–130 °C; IR ν_{max} (cm^{-1}): 3427, 2962, 1531, 1461, 1338, 1248, 839, 794; ^1H NMR ($CDCl_3$) δ 7.97 (s, 1H), 7.71 (d, J = 7.7 Hz, 2H), 7.53 (d, J = 8.7 Hz, 2H), 7.45 (t, J = 7.9 Hz, 3H), 7.32–7.27 (m, 1H), 6.98 (d, J = 8.7 Hz, 2H), 5.57 (s, 2H), 4.75 (s, 2H), 3.84 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 159.96, 151.55, 139.57, 129.52, 129.19, 128.53, 126.90, 124.53, 119.09, 114.40, 114.10, 56.51, 55.38, 44.95; ESI-MS: m/z, 362 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{20}H_{19}N_5O_2Na$ 384.1431, found 384.1449.

*4.1.5.7. (1-((3-(2,4-Dichlorophenyl)-1-phenyl-1H-pyrazol-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methanol (**8g**).* Yield: 95%; white solid; m.p.: 128–130 °C; IR ν_{max} (cm^{-1}): 3311, 2924, 1596, 1502, 1346, 1228, 825, 751; ^1H NMR ($CDCl_3$): δ 7.95 (s, 1H), 7.69–7.65 (m, 2H), 7.62–7.58 (m, 2H), 7.50–7.41 (m, 5H), 5.60 (s, 2H), 4.74 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 148.73, 139.34, 135.76, 134.33, 132.97, 129.76, 129.66, 129.49, 127.60, 127.48, 127.18, 121.66, 119.19, 116.63, 56.21, 44.75; ESI-MS: m/z, 400 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{19}H_{15}Cl_2N_5O$ $[M+H]^+$ 400.0726, found 400.0731.

*4.1.5.8. (1-((1-(3,4-Dimethylphenyl)-3-phenyl-1H-pyrazol-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methanol (**8h**).* Yield: 81%; white solid; m.p.: 160–162 °C; IR ν_{max} (cm^{-1}): 3451, 2917, 1509, 1448, 1364, 1214, 865, 767; ^1H NMR ($CDCl_3$): δ 7.94 (s, 1H), 7.61 (dd, J = 5.2, 3.2 Hz, 2H), 7.52 (d, J = 1.9 Hz, 1H), 7.47–7.44 (m, 2H), 7.40 (td, J = 6.1, 1.8 Hz, 3H), 7.19 (d, J = 8.1 Hz, 1H), 5.61 (s, 2H), 4.74 (s, 2H), 2.32 (s, 3H), 2.29 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 151.35, 147.91, 138.05, 137.53, 135.64, 132.20, 130.46, 128.94, 128.56, 128.52, 127.97, 121.41, 120.50, 116.49, 114.04, 56.54, 44.81, 19.95, 19.36; ESI-MS: m/z, 360 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{21}H_{21}N_5O$ $[M+H]^+$ 360.1818, found 360.1821.

*4.1.6. General procedure for the preparation of 1H-1,2,3-triazoles (**10a-p**)*

Phenyl acetylene **9a** (1.0 mmol) was added to a stirred solution of pyrazole azide **6a** (1.0 mmol) in *tert*-butanol and H_2O (1:1) at room temperature, followed by $CuSO_4{\cdot}5H_2O$ (0.1 mmol) and sodium ascorbate (0.05 mmol). The reaction mixture was stirred for 6–12 h at room temperature. After completion of the reaction (TLC), the reaction mixture was extracted with ethyl acetate (2 × 5 mL). The organic extract was washed with H_2O and dried over anhydrous Na_2SO_4. The residue was purified by column chromatography using silica gel (ethyl acetate/hexane) furnished 1-((1,3-diphenyl-1*H*-pyrazol-4-yl)methyl)-4-phenyl-1*H*-1,2,3-triazole **10a**. The 1*H*-1,2,3-triazole compounds **10b-p** were synthesized from pyrazole azides **6a-f** under similar conditions.

*4.1.6.1. 1-((1,3-Diphenyl-1H-pyrazol-4-yl)methyl)-4-phenyl-1H-1,2,3-triazole (**10a**).* Yield: 85%; white solid; m.p.: 156–158 °C; IR ν_{max} (cm^{-1}): 3083, 2925, 1597, 1502, 1455, 1221, 1067, 769, 692; ^1H NMR ($CDCl_3$): δ 8.02 (s, 1H), 7.78–7.73 (m, 4H), 7.67–7.64 (m, 2H), 7.63 (s, 1H), 7.51–7.37 (m, 7H), 7.34–7.29 (m, 2H), 5.69 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 151.70, 148.03, 139.58, 132.15, 130.47, 129.55, 129.00, 128.85, 128.70, 128.49, 128.24, 127.98, 127.02, 125.71, 119.32, 119.15, 114.69, 44.92; ESI-MS: m/z, 400 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{24}H_{19}N_5Na$ $[M+H]^+$ 400.1520, found 400.1532.

*4.1.6.2. 1-((3-(4-Chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)methyl)-4-phenyl-1H-1,2,3-triazole (**10b**).* Yield: 92%; white solid; m.p.: 162–164 °C; IR ν_{max} (cm^{-1}): 3082, 1598, 1501, 1223, 1071, 833, 758, 688; ^1H NMR ($CDCl_3$): δ 8.05 (s, 1H), 7.84–7.71 (m, 4H), 7.69 (s, 1H), 7.62 (d, J = 8.5 Hz, 2H), 7.52–7.31 (m, 8H), 5.68 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 150.38, 148.04, 139.36, 134.63, 130.52, 130.27, 129.51, 129.09, 128.80, 128.62, 128.23, 127.10, 125.63, 119.20, 119.07, 114.53, 44.76; ESI-MS: m/z, 412 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{24}H_{18}N_5Cl$ $[M+H]^+$ 412.1323, found 412.1333.

*4.1.6.3. 1-((1-(4-Chlorophenyl)-3-phenyl-1H-pyrazol-4-yl)methyl)-4-phenyl-1H-1,2,3-triazole (**10c**).* Yield: 90%; white solid; m.p.: 136–138 °C; IR ν_{max} (cm^{-1}): 3082, 2930, 1593, 1486, 1221, 1070, 770, 694; ^1H NMR ($CDCl_3$): δ 8.00 (s, 1H), 7.82 (t, J = 2.0 Hz, 1H), 7.78 (dd, J = 5.2, 3.3 Hz, 2H), 7.66–7.64 (m, 2H), 7.63 (s, 1H), 7.60 (ddd, J = 8.2, 2.1, 0.8 Hz, 1H), 7.51–7.47 (m, 2H), 7.46–7.36 (m, 4H), 7.34–7.27 (m, 2H), 5.68 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 151.65, 146.92, 139.48, 133.91, 132.04, 129.51, 128.95, 128.67, 128.44, 127.90, 127.01, 126.88, 119.29, 119.08, 114.43, 44.89; ESI-MS: m/z, 412 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{24}H_{18}N_5Cl$ $[M+H]^+$ 412.1323, found 412.1332.

*4.1.6.4. 4-Phenyl-1-((1-phenyl-3-(p-tolyl)-1H-pyrazol-4-yl)methyl)-1H-1,2,3-triazole (**10d**).* Yield: 80%; white solid; m.p.: 142–144 °C; IR ν_{max} (cm^{-1}): 3082, 2923, 1601, 1454, 1252, 1173, 1021, 829, 755, 688; ^1H NMR ($CDCl_3$): δ 8.00 (s, 1H), 7.80–7.70 (m, 4H), 7.63 (s, 1H), 7.54 (d, J = 8.1 Hz, 2H), 7.46 (t, J = 8.0 Hz, 2H), 7.39 (t, J = 7.6 Hz, 2H), 7.30 (dd, J = 17.3, 7.7 Hz, 4H), 5.67 (s, 2H), 2.41 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 151.75, 147.99, 139.61, 138.62, 130.50, 129.69, 129.54, 129.24, 128.85, 128.42, 128.22, 127.83, 126.93, 125.71, 119.33, 119.11, 114.54, 44.96, 21.36; ESI-MS: m/z, 392 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{25}H_{21}N_5$ $[M+H]^+$ 392.1870, found 392.1880.

*4.1.6.5. 4-Phenyl-1-((3-phenyl-1-(p-tolyl)-1H-pyrazol-4-yl)methyl)-1H-1,2,3-triazole (**10e**).* Yield: 82%; white solid; m.p.: 145–147 °C; IR ν_{max} (cm^{-1}): 3087, 2998, 1605, 1453, 1295, 1047, 752, 695; ^1H NMR ($CDCl_3$): δ 7.98 (s, 1H), 7.81–7.73 (m, 2H), 7.67–7.61 (m, 4H), 7.60 (s, 1H), 7.51–7.25 (m, 8H), 5.68 (s, 2H), 2.39 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 151.44, 148.02, 137.39, 136.91, 132.26, 130.52, 130.02, 128.94, 128.81, 128.59, 128.38, 128.18, 127.97, 125.71, 119.23, 119.12, 114.38, 44.92, 20.95; ESI-MS: *m/z*, 392 $[M+H]^+$; ESI-HRMS: *m/z* calcd for $C_{25}H_{21}N_5$ $[M+H]^+$ 392.1870, found 392.1883.

*4.1.6.6. 1-((3-(4-Methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)methyl)-4-phenyl-1H-1,2,3-triazole (**10f**).* Yield: 81%; white solid; m.p.: 130–132 °C; IR ν_{max} (cm^{-1}): 3001, 2958, 1731, 1461, 1224, 1060, 752, 686; ^1H NMR ($CDCl_3$): δ 8.03 (s, 1H), 7.77 (dd, *J* = 13.9, 7.8 Hz, 4H), 7.66 (s, 1H), 7.59 (d, *J* = 8.6 Hz, 2H), 7.52–7.29 (m, 6H), 7.01 (d, *J* = 8.6 Hz, 2H), 5.68 (s, 2H), 3.87 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 163.67, 161.70, 151.69, 139.55, 132.13, 129.56, 129.01, 128.72, 128.53, 127.97, 127.47, 127.41, 127.05, 126.70, 119.13, 115.91, 115.74, 114.58, 44.92; ESI-MS: *m/z*, 408 $[M+H]^+$; ESI-HRMS: *m/z* calcd for $C_{25}H_{21}N_5O$ $[M+H]^+$ 408.1818, found: 408.1827.

*4.1.6.7. 1-((1,3-Diphenyl-1H-pyrazol-4-yl)methyl)-4-(2-(trifluoromethyl)phenyl)-1H-1,2,3-triazole (**10g**).* Yield: 95%; white solid; m.p.: 76–78 °C; IR ν_{max} (cm^{-1}): 2922, 1709, 1440, 1347, 1162, 818, 662, 548; ^1H NMR ($CDCl_3$): δ 8.01 (s, 1H), 7.93 (d, *J* = 7.8 Hz, 1H), 7.75–7.71 (m, 3H), 7.68 (d, *J* = 0.7 Hz, 1H), 7.66–7.59 (m, 3H), 7.50–7.40 (m, 6H), 7.29–7.33 (m, 1H), 5.71 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 151.74, 145.27, 139.45, 132.56, 132.34, 132.08, 131.96, 129.56, 129.02, 128.79, 128.49, 127.91, 127.13, 126.41, 125.56, 123.29 (d, *J* = 273.37 Hz), 121.57 (q, *J* = 5.44 Hz), 120.29, 119.11, 114.03, 45.09; ESI-MS: *m/z*, 446 $[M+H]^+$; ESI-HRMS: *m/z* calcd for $C_{25}H_{18}N_5F_3Na$ $[M+H]^+$ 468.1389, found 468.1406.

*4.1.6.8. 4-(2,4-Bis(trifluoromethyl)phenyl)-1-((1,3-diphenyl-1H-pyrazol-4-yl)methyl)-1H-1,2,3-triazole (**10h**).* Yield: 92%; white solid; m.p.: 134–136 °C; IR ν_{max} (cm^{-1}): 3116, 2943, 1504, 1278, 1145, 1085, 1145,751, 683; ^1H NMR ($CDCl_3$): δ 8.21 (s, 2H), 8.08 (s, 1H), 7.82–7.71 (m, 4H), 7.64 (dd, *J* = 7.9, 1.4 Hz, 2H), 7.52–7.42 (m, 5H), 7.33 (t, *J* = 7.4 Hz, 1H), 5.73 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 151.61, 144.45, 139.54, 132.07, 131.96, 131.64, 129.47, 129.37, 128.89, 128.61, 128.28, 128.24, 127.94, 127.03 (d, *J* = 29.97 Hz), 126.91, 126.06 (q, *J* = 5.44 Hz), 124.01 (d, *J* = 273.37 Hz), 122.64 (q, *J* = 5.44 Hz), 119.09, 114.70, 44.99; ESI-MS: *m/z*, 514 $[M+H]^+$; ESI-HRMS: *m/z* calcd for $C_{26}H_{17}N_5F_6$ $[M+H]^+$ 514.1451, found 514.1460.

*4.1.6.9. 4-(1-((1,3-Diphenyl-1H-pyrazol-4-yl)methyl)-1H-1,2,3-triazol-4-yl)aniline (**10i**).* Yield: 78%; white solid; m.p.: 168–170 °C; IR ν_{max} (cm^{-1}): 3380, 3319, 2922, 1604, 1501, 1454, 1272, 1069, 830, 752, 695; ^1H NMR ($CDCl_3$): δ 8.00 (s, 1H), 7.76–7.72 (m, 2H), 7.68–7.63 (m, 2H), 7.59–7.54 (m, 2H), 7.50 (s, 1H), 7.49–7.40 (m, 5H), 7.31 (t, *J* = 7.4 Hz, 1H), 6.69 (t, *J* = 5.5 Hz, 2H), 5.66 (s, 2H), 3.72 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 151.64, 146.51, 139.59, 132.17, 129.49, 128.92, 128.60, 128.37, 127.96, 126.91, 119.12, 117.94, 115.19, 114.88, 77.29, 44.82; ESI-MS: *m/z*, 393 $[M+H]^+$; ESI-HRMS: *m/z* calcd for $C_{24}H_{20}N_6$ $[M+H]^+$ 393.1814, found 393.1822.

*4.1.6.10. 4-(1-((1,3-Diphenyl-1H-pyrazol-4-yl)methyl)-1H-1,2,3-triazol-4-yl)-N,N-dimethylaniline (**10j**).* Yield: 74%; white solid; m.p.: 168–170 °C; IR ν_{max} (cm^{-1}): 3419, 3100, 2926, 1618, 1506, 1449, 1224, 1067, 813, 751, 693; ^1H NMR ($CDCl_3$): δ 7.99 (s, 1H), 7.73 (d, *J* = 7.9 Hz, 2H), 7.65 (dd, *J* = 7.7, 4.1 Hz, 4H), 7.51 (s, 1H), 7.50–7.41 (m, 5H), 7.31 (t, *J* = 7.4 Hz, 1H), 6.73 (d, *J* = 8.8 Hz, 2H), 5.65 (s, 2H), 2.97 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 151.60, 150.38, 148.47, 139.54, 132.14, 129.47, 128.91, 128.58, 128.37, 127.94, 126.89, 126.60, 119.08, 118.61, 117.77, 114.92, 112.41, 44.78, 40.44; ESI-MS: *m/z*, 421 $[M+H]^+$; ESI-HRMS: *m/z* calcd for $C_{26}H_{24}N_6$ $[M+H]^+$ 421.2135, found 421.2153.

*4.1.6.11. 1-((1,3-Diphenyl-1H-pyrazol-4-yl)methyl)-4-(4-fluorophenyl)-1H-1,2,3-triazole (**10k**).* Yield: 89%; white solid; m.p.: 156–158 °C; IR ν_{max} (cm^{-1}): 3095, 2925, 1598, 1497, 1224, 1069, 839, 751, 692; ^1H NMR ($CDCl_3$): δ 8.02 (s, 1H), 7.73 (dd, *J* = 8.0, 3.2 Hz, 4H), 7.65 (d, *J* = 7.0 Hz, 2H), 7.59 (s, 1H), 7.53–7.37 (m, 5H), 7.31 (t, *J* = 7.4 Hz, 1H), 7.07 (t, *J* = 8.6 Hz, 2H), 5.67 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 163.60, 161.63, 151.63, 139.48, 132.05, 129.49, 128.93, 128.65, 128.44, 127.89, 127.40, 127.33, 126.98, 119.06, 115.84, 115.67, 114.50, 44.85; ESI-MS: *m/z*, 396 $[M+H]^+$; ESI-HRMS: *m/z* calcd for $C_{24}H_{18}N_5F$ $[M+H]^+$ 396.1619, found 396.1641.

*4.1.6.12. 4-(4-Chlorophenyl)-1-((1,3-diphenyl-1H-pyrazol-4-yl)methyl)-1H-1,2,3-triazole (**10l**).* Yield: 85%; white solid; m.p.: 164–166 °C; IR ν_{max} (cm^{-1}): 3083, 2926, 1501, 1453, 1220, 1095, 834, 751, 693; ^1H NMR ($CDCl_3$): δ 8.03 (s, 1H), 7.73 (d, *J* = 7.7 Hz, 2H), 7.69 (d, *J* = 8.5 Hz, 2H), 7.67–7.63 (m, 2H), 7.60 (s, 1H), 7.50–7.40 (m, 5H), 7.37–7.30 (m, 3H), 5.67 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 151.61, 146.87, 139.44, 133.87, 132.00, 129.48, 128.95, 128.65, 128.44, 127.87, 126.98, 126.85, 119.30, 119.04, 114.40, 44.84; ESI-MS: *m/z*, 412 $[M+H]^+$; ESI-HRMS: *m/z* calcd for $C_{24}H_{18}N_5Cl$ $[M+H]^+$ 412.1323, found 412.1341.

*4.1.6.13. 4-(4-Bromophenyl)-1-((1,3-diphenyl-1H-pyrazol-4-yl)methyl)-1H-1,2,3-triazole (**10m**).* Yield: 93%; white solid; m.p.: 170–172 °C; IR ν_{max} (cm^{-1}): 3108, 3080, 2926, 1596, 1501, 1453, 1219, 1069, 829, 752, 691; ^1H NMR ($CDCl_3$): δ 8.05 (s, 1H), 7.75 (d, *J* = 7.6 Hz, 2H), 7.72–7.58 (m, 6H), 7.56–7.43 (m, 6H), 7.34 (t, *J* = 7.4 Hz, 1H), 5.69 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 151.70, 146.98, 139.53, 132.09, 131.98, 129.56, 129.43, 129.01, 128.73, 128.51, 127.95, 127.21, 127.07, 122.11, 119.39, 119.13, 114.47, 44.94; ESI-MS: *m/z*, 456 $[M+H]^+$; ESI-HRMS: *m/z* calcd for $C_{24}H_{18}N_5Br$ $[M+H]^+$ 456.0818, found 456.0846.

*4.1.6.14. 1-((1,3-Diphenyl-1H-pyrazol-4-yl)methyl)-4-(p-tolyl)-1H-1,2,3-triazole (**10n**).* Yield: 89%; white solid; m.p.: 186–188 °C; IR ν_{max} (cm^{-1}): 3098, 2924, 1596, 1501, 1452, 1218, 823, 752, 693; ^1H NMR ($CDCl_3$): δ 8.02 (s, 1H), 7.74 (d, *J* = 8.0 Hz, 2H), 7.66 (d, *J* = 8.0 Hz, 4H), 7.59 (s, 1H), 7.51–7.41 (m, 5H), 7.30 (dd, *J* = 14.0, 6.6 Hz, 1H), 7.20 (d, *J* = 8.0 Hz, 2H), 5.68 (s, 2H), 2.36 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 151.63, 148.05, 139.52, 138.03, 132.09, 129.48, 129.46, 128.93, 128.62, 128.40, 127.92, 127.57, 126.94, 125.55, 119.08, 118.89, 114.69, 44.82, 21.25; ESI-MS: *m/z*, 392 $[M+H]^+$; ESI-HRMS: *m/z* calcd for $C_{25}H_{21}N_5$ $[M+H]^+$ 392.1869, found 392.1884.

*4.1.6.15. 1-((1,3-Diphenyl-1H-pyrazol-4-yl)methyl)-4-(4-methoxyphenyl)-1H-1,2,3-triazole (**10o**).* Yield: 86%; white solid; m.p.: 188–190 °C; IR ν_{max} (cm^{-1}): 3086, 2925, 1603, 1499, 1452, 1247, 1025, 829, 751, 689; ^1H NMR ($CDCl_3$): δ 8.03 (s, 1H), 7.77 (dd, *J* = 13.9, 7.8 Hz, 4H), 7.66 (s, 1H), 7.59 (d, *J* = 8.6 Hz, 2H), 7.51–7.28 (m, 6H), 7.01 (d, *J* = 8.6 Hz, 2H), 5.68 (s, 2H), 3.87 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 159.89, 151.46, 147.89, 139.51, 130.39, 129.44, 129.10, 128.75, 128.36, 128.13, 126.78, 125.60, 124.51, 119.23, 118.95, 114.32, 114.17, 55.29, 44.86; ESI-MS: *m/z*, 408 $[M+H]^+$; ESI-HRMS: *m/z* calcd for $C_{25}H_{21}ON_5$ $[M+H]^+$ 408.1818, found 408.1834.

*4.1.6.16. 1-((1,3-Diphenyl-1H-pyrazol-4-yl)methyl)-4-(4-methoxy-2-methylphenyl)-1H-1,2,3-triazole (**10p**).* Yield: 89%; white solid; m.p.: 138–140 °C; IR ν_{max} (cm^{-1}): 3083, 2925 1602, 1455, 1253,

1174, 1022, 831, 756, 689; ^{1}H NMR ($CDCl_3$): δ 8.06 (s, 1H), 7.76 (d, J = 7.7 Hz, 2H), 7.68 (dd, J = 11.4, 4.7 Hz, 3H), 7.54–7.40 (m, 6H), 7.33 (t, J = 7.4 Hz, 1H), 6.86–6.74 (m, 2H), 5.71 (s, 2H), 3.82 (s, 3H), 2.33 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 159.32, 151.61, 147.12, 139.53, 137.04, 132.20, 130.09, 129.48, 128.92, 128.61, 128.40, 127.93, 126.92, 122.50, 120.84, 119.07, 116.16, 114.82, 111.34, 55.19, 44.73, 21.49; ESI-MS: m/z, 422 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{26}H_{23}N_5O$ $[M+H]^+$ 422.1960, found 422.1975.

*4.1.7. General procedure for the preparation of 1H-1,2,3-triazole-4,5-dicarboxylates (**12a-l**)*

Dimethyl but-2-ynedioate **11a** (1.0 mmol) was added to a stirred solution of pyrazole azide **6a** (1.0 mmol) in *tert*-butanol and H_2O (1:1) at room temperature, followed by $CuSO_4{\cdot}5H_2O$ (0.1 mmol) and sodium ascorbate (0.05 mmol). The reaction mixture was stirred for 6–12 h at room temperature. After completion of the reaction (TLC), the reaction mixture was extracted with ethyl acetate (2 × 5 mL). The organic extract was washed with H_2O and dried over anhydrous Na_2SO_4. The residue was purified by column chromatography using silica gel (ethyl acetate/hexane) furnished dimethyl 1-((1,3-diphenyl-1*H*-pyrazol-4-yl)methyl)-1*H*-1,2,3-triazole-4,5-dicarboxylate **12a**. The 1*H*-1,2,3-triazole dicarboxylate compounds **12b-l** were synthesized from pyrazole azides **6a-f** under similar conditions.

*4.1.7.1. Dimethyl 1-((1,3-diphenyl-1H-pyrazol-4-yl)methyl)-1H-1,2,3-triazole-4,5-dicarboxylate (**12a**).* Yield: 82%; white solid; m.p.: 86–88 °C; IR ν_{max} (cm^{-1}): 3123, 2953, 1741, 1448, 1349, 1224, 1134, 1055, 830, 757, 690; ^{1}H NMR ($CDCl_3$): δ 7.93 (s, 1H), 7.73–7.64 (m, 4H), 7.53–7.38 (m, 5H), 7.34–7.25 (m, 1H), 5.88 (s, 2H), 3.94 (s, 3H), 3.74 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 160.23, 158.69, 151.60, 139.81, 139.35, 131.87, 129.87, 129.37, 128.69, 128.48, 128.39, 128.19, 126.86, 119.00, 114.20, 53.29, 52.64, 44.97; ESI-MS: m/z, 418 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{22}H_{19}N_5O_4$ $[M+H]^+$ 418.1510, found 418.1514.

*4.1.7.2. Dimethyl 1-((3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl) methyl)-1H-1,2,3-triazole-4,5-dicarboxylate (**12b**).* Yield: 86%; white solid; m.p.: 86–88 °C; IR ν_{max} (cm^{-1}): 3134, 2953, 1727, 1461, 1344, 1225, 1060, 962, 819, 754, 687; ^{1}H NMR ($CDCl_3$): δ 7.95 (s, 1H), 7.71–7.63 (m, 4H), 7.49–7.41 (m, 4H), 7.31 (t, J = 7.4 Hz, 1H), 5.86 (s, 2H), 3.96 (s, 3H), 3.82 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 160.35, 158.95, 150.68, 140.10, 139.43, 134.68, 130.55, 129.73, 129.64, 129.55, 129.02, 128.79, 127.16, 119.21, 114.30, 53.47, 52.78, 44.96; ESI-MS: m/z, 452 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{22}H_{18}N_5O_4Cl$ $[M+H]^+$ 452.1120, found 452.1131.

*4.1.7.3. Dimethyl 1-((1-(4-chlorophenyl)-3-phenyl-1H-pyrazol-4-yl) methyl)-1H-1,2,3-triazole-4,5-dicarboxylate (**12c**).* Yield: 92%; white solid; m.p.: 96–98 °C; IR ν_{max} (cm^{-1}): 3010, 2955, 1741, 1592, 1478, 1232, 1135, 1059, 779, 689; ^{1}H NMR ($CDCl_3$): δ 7.95 (s, 1H), 7.71–7.64 (m, 4H), 7.46 (ddd, J = 8.4, 4.7, 2.1 Hz, 4H), 7.32 (t, J = 7.4 Hz, 1H), 5.86 (s, 2H), 3.97 (s, 3H), 3.82 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 160.29, 158.74, 152.11, 140.36, 140.03, 135.32, 131.68, 130.47, 129.81, 128.81, 128.74, 128.39, 128.28, 126.87, 119.37, 116.81, 114.93, 53.38, 52.71, 44.95; ESI-MS: m/z, 452 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{22}H_{18}N_5O_4Cl$ $[M+H]^+$ 452.1120, found 452.1126.

*4.1.7.4. Dimethyl 1-((1-phenyl-3-(p-tolyl)-1H-pyrazol-4-yl)methyl)-1H-1,2,3-triazole-4,5-dicarboxylate (**12d**).* Yield: 86%; white solid; m.p.: 84–86 °C; IR ν_{max} (cm^{-1}): 3016, 2923, 1741, 1446, 1227, 1135, 1057, 753, 683; ^{1}H NMR ($CDCl_3$): δ 7.90 (s, 1H), 7.70–7.66 (m, 2H), 7.58–7.55 (m, 2H), 7.46–7.41 (m, 2H), 7.32–7.27 (m, 3H), 5.87 (s, 2H), 3.95 (s, 3H), 3.76 (s, 3H), 2.41 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 160.32, 158.83, 151.75, 139.88, 139.52, 138.41, 129.99, 129.44, 129.07, 128.33, 128.13, 126.85, 119.09, 114.16, 53.34, 52.68, 45.16, 21.29; ESI-MS: m/z, 432 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{23}H_{21}N_5O_4$ $[M+H]^+$ 432.1666, found: 432.1670.

*4.1.7.5. Dimethyl 1-((3-phenyl-1-(p-tolyl)-1H-pyrazol-4-yl)methyl)-1H-1,2,3-triazole-4,5-dicarboxylate (**12e**).* Yield: 84%; white solid; m.p.: 96–98 °C; IR ν_{max} (cm^{-1}): 3036, 2957, 1752, 1517, 1356, 1230, 1230, 1140, 1059, 815, 765, 691; ^{1}H NMR ($CDCl_3$): δ 7.88 (s, 1H), 7.70–7.64 (m, 2H), 7.56 (d, J = 8.4 Hz, 2H), 7.52–7.38 (m, 3H), 7.25 (t, J = 6.4 Hz, 2H), 5.88 (s, 2H), 3.95 (s, 3H), 3.74 (s, 3H), 2.37 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 160.38, 158.87, 151.50, 139.94, 137.31, 136.92, 132.12, 130.02, 128.82, 128.55, 128.45, 128.35, 119.14, 114.03, 53.42, 52.76, 45.16, 20.99; ESI-MS: m/z, 432 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{23}H_{21}N_5O_4$ $[M+H]^+$ 432.1666, found 432.1671.

*4.1.7.6. Dimethyl 1-((3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)methyl)-1H-1,2,3-triazole-4,5-dicarboxylate (**12f**).* Yield: 88%; white solid; m.p.: 108–110 °C; IR ν_{max} (cm^{-1}): 3133, 3057, 2955, 1731, 1461, 1224, 1180, 1060, 819, 752, 685; ^{1}H NMR ($CDCl_3$): δ 7.92 (s, 1H), 7.69 (d, J = 7.7 Hz, 2H), 7.63 (d, J = 8.7 Hz, 2H), 7.45 (t, J = 7.9 Hz, 2H), 7.34–7.27 (m, 1H), 7.02 (d, J = 8.7 Hz, 2H), 5.87 (s, 2H), 3.96 (s, 3H), 3.87 (s, 3H), 3.79 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 160.37, 159.94, 158.94, 151.62, 139.94, 139.56, 130.02, 129.58, 129.49, 128.44, 126.87, 124.51, 119.09, 114.24, 114.01, 55.37, 53.44, 52.74, 45.23; ESI-MS: m/z, 448 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{23}H_{21}N_5O_5$ $[M+H]^+$ 448.1615, found 448.1619.

*4.1.7.7. Diethyl 1-((1,3-diphenyl-1H-pyrazol-4-yl)methyl)-1H-1,2,3-triazole-4,5-dicarboxylate (**12g**).* Yield: 85%; colorless liquid; IR ν_{max} (cm^{-1}): 3065, 2923, 1730, 1453, 1213, 1060, 856, 759, 695; ^{1}H NMR ($CDCl_3$): δ 7.89 (s, 1H), 7.70 (d, J = 7.8 Hz, 4H), 7.47 (dt, J = 11.6, 7.6 Hz, 5H), 7.31 (t, J = 7.4 Hz, 1H), 5.89 (s, 2H), 4.42 (q, J = 7.1 Hz, 2H), 4.24 (q, J = 7.1 Hz, 2H), 1.40 (t, J = 7.1 Hz, 3H), 1.22 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 159.95, 158.52, 151.61, 140.08, 139.47, 131.97, 130.09, 129.41, 128.72, 128.50, 128.34, 128.22, 126.87, 119.06, 114.41, 62.95, 61.82, 45.01, 14.10, 13.65; ESI-MS: m/z, 446 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{24}H_{23}N_5O_4Na$ $[M+Na]^+$ 468.1750, found: 468.1682.

*4.1.7.8. Diethyl 1-((3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl) methyl)-1H-1,2,3-triazole-4,5-dicarboxylate (**12h**).* Yield: 84%; colorless liquid; IR ν_{max} (cm^{-1}): 2985, 1729, 1504, 1272, 1199, 1065, 836, 757, 683 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.95 (s, 1H), 7.69 (dd, J = 8.7, 1.9 Hz, 4H), 7.51–7.41 (m, 4H), 7.31 (dd, J = 13.2, 5.8 Hz, 1H), 5.86 (s, 2H), 4.43 (q, J = 7.1 Hz, 2H), 4.30 (q, J = 7.1 Hz, 2H), 1.40 (t, J = 7.1 Hz, 3H), 1.27 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 160.05, 158.66, 150.61, 140.30, 139.44, 134.63, 130.58, 129.88, 129.61, 129.55, 129.01, 128.78, 127.13, 119.16, 114.43, 63.09, 61.98, 44.91, 14.20, 13.78; ESI-MS: m/z, 480 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{24}H_{22}N_5O_4Cl$ $[M+Na]^+$ 480.1433, found 480.1451.

*4.1.7.9. Diethyl 1-((1-(4-chlorophenyl)-3-phenyl-1H-pyrazol-4-yl) methyl)-1H-1,2,3-triazole-4,5-dicarboxylate (**12i**).* Yield: 92%; colorless liquid; IR ν_{max} (cm^{-1}): 2983, 1729, 1593, 1488, 1210, 1060, 855, 776; ^{1}H NMR ($CDCl_3$): δ 7.90 (s, 1H), 7.77 (t, J = 1.9 Hz, 1H), 7.71–7.67 (m, 2H), 7.55 (dd, J = 8.1, 1.2 Hz, 1H), 7.48 (t, J = 7.4 Hz, 2H), 7.43 (t, J = 7.3 Hz, 1H), 7.35 (t, J = 8.1 Hz, 1H), 7.25 (d, J = 7.9 Hz, 1H), 5.87 (s, 2H), 4.42 (q, J = 7.1 Hz, 2H), 4.25 (q, J = 7.1 Hz, 2H), 1.39 (t, J = 7.1 Hz, 3H), 1.22 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 160.03, 158.55, 152.09, 140.43, 140.26, 135.35, 131.76, 130.53, 130.03, 128.85, 128.77, 128.40, 128.29, 126.87, 119.36, 116.83, 115.12, 63.07, 61.94, 44.97, 14.18, 13.75; ESI-MS: m/z, 480 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{24}H_{22}N_5O_4Cl$ $[M+Na]^+$ 480.1433, found 480.1452.

*4.1.7.10. Diethyl 1-((1-phenyl-3-(p-tolyl)-1H-pyrazol-4-yl)methyl)-1H-1,2,3-triazole-4,5-dicarboxylate (**12j**).* Yield: 88%; colorless liquid; IR ν_{max} (cm^{-1}): 3071, 2983, 1740, 1598, 1462, 1223, 1061, 757, 688; ^{1}H NMR ($CDCl_3$): δ 7.88 (s, 1H), 7.68 (dt, J = 8.9, 1.8 Hz, 2H), 7.58 (d, J = 8.1 Hz, 2H), 7.46–7.41 (m, 2H), 7.29 (ddd, J = 7.6, 3.9, 1.6 Hz, 3H), 5.86 (s, 2H), 4.41 (q, J = 7.1 Hz, 2H), 4.23 (q, J = 7.1 Hz, 2H), 2.40 (s, 3H), 1.38 (t, J = 7.1 Hz, 3H), 1.21 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 159.91, 158.50, 151.59, 139.96, 139.46, 138.28, 130.10, 129.34, 129.04, 128.22, 128.01, 127.67, 126.71, 118.95, 114.22, 62.88, 61.74, 45.04, 21.20, 14.04, 13.58; ESI-MS: m/z, 460 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{25}H_{26}N_5O_4$ $[M+H]^+$ 460.1985, found 460.1983.

*4.1.7.11. Diethyl 1-((3-phenyl-1-(p-tolyl)-1H-pyrazol-4-yl)methyl)-1H-1,2,3-triazole-4,5-dicarboxylate (**12k**).* Yield: 84%; colorless liquid; IR ν_{max} (cm^{-1}): 3063, 2986, 1740, 1516, 1461, 1223, 1150, 1061, 844, 772; ^{1}H NMR ($CDCl_3$): δ 7.85 (s, 1H), 7.68 (d, J = 6.9 Hz, 2H), 7.56 (d, J = 8.4 Hz, 2H), 7.51–7.39 (m, 3H), 7.24 (d, J = 8.3 Hz, 2H), 5.87 (s, 2H), 4.42 (q, J = 7.1 Hz, 2H), 4.23 (q, J = 7.1 Hz, 2H), 2.38 (s, 3H), 1.39 (t, J = 7.1 Hz, 3H), 1.21 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 160.05, 158.63, 151.43, 140.12, 137.34, 136.88, 132.14, 130.23, 130.01, 128.80, 128.52, 128.36, 128.31, 119.12, 114.16, 63.04, 61.92, 45.13, 20.99, 14.20, 13.75; ESI-MS: m/z, 460 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{25}H_{25}N_5O_4$ $[M+H]^+$ 460.1979, found 460.1962.

*4.1.7.12. Diethyl 1-((3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl) methyl)-1H-1,2,3-triazole-4,5-dicarboxylate (**12l**).* Yield: 80%; colorless liquid; IR ν_{max} (cm^{-1}): 2926, 2848, 1727, 1552, 1460, 1197, 1062, 1012, 843, 757, 681; ^{1}H NMR ($CDCl_3$): δ 7.87 (s, 1H), 7.67 (d, J = 7.9 Hz, 2H), 7.61 (t, J = 5.7 Hz, 2H), 7.42 (t, J = 7.9 Hz, 2H), 7.27 (t, J = 5.2 Hz, 1H), 6.99 (d, J = 8.7 Hz, 2H), 5.84 (s, 2H), 4.40 (q, J = 7.1 Hz, 2H), 4.24 (q, J = 7.1 Hz, 2H), 3.84 (s, 3H), 1.38 (t, J = 7.1 Hz, 3H), 1.21 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 160.06, 159.96, 158.71, 151.58, 140.15, 139.61, 130.19, 129.57, 129.49, 128.37, 126.84, 124.56, 119.08, 114.25, 114.16, 63.04, 61.91, 55.38, 45.20, 14.20, 13.75; ESI-MS: m/z, 476 $[M+H]^+$; ESI-HRMS: m/z calcd for $C_{25}H_{25}N_5O_5$ $[M+H]^+$ 476.1928, found 476.1945.

4.2. Biology

4.2.1. Chemicals and kits

Dexamethasone (≥95%), 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide inner salt (XTT sodium salt), phorbol 12-myristate 13-acetate (PMA) and lipopolysaccharides from Escherichia coli 055:B5 (LPS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Human U937cells were procured from American Type Culture Collection (ATCC, Manassas, USA). Dulbecco's Modified Eagle Medium (DMEM), Roswell Park Memorial Institute medium (RPMI), Foetal bovine serum (FBS), Trypsin-EDTA, l-glutathione and Penicillin-Streptomycin (Pen-Strep) were obtained from Gibco, Life Technologies, Scotland, UK. TNF-α and IL-6 ELISA kits (BD Opt EIA) were obtained from BD Biosciences, San Diego, CA, USA. All other chemicals used were of analytical grade unless otherwise specified.

4.2.2. Anti-microbial assay

Well plate method is followed for both antibacterial and antifungal activities for measuring the zone of inhibitions.[17] For antibacterial activity test strains used are *Micrococcus luteus*, *Staphylococcus aureus* (Gram positive) and *Salmonella typhi*, *Salmonella paratyphi* (Gram negative), in nutrient agar. For antifungal studies test strains used are *Aspergillus niger* and *Aspergillus fumigatus* and the medium used is potato dextrose agar. The compounds **5a-h**, **6a-h**, **8a-h**, **10a-p** and **12a-l** were used for activity studies and the concentration of each compound is 1.0 mg/mL along with standard and control. The media, Petri dishes were autoclaved at 121 °C for 15 min. After sterilization the plates were poured with appropriate medium left over for 30 min for solidification, later the plates were inoculated with 60 μL of test inoculum using sterile cotton swabs. An 8 mm width size wells were made with sterile cork borer and in each well exactly 100 μL of sample were loaded. Control and standard also placed in separate wells. The plates were initially incubated for 20–30 min at 4 °C to allow the compounds to diffuse into the agar, and then subsequently incubated for 24 h at 37 °C for bacteria and 48 h at 28 °C for fungi. Zone diameters were expressed in mm using calibrated scale. Experiment was triplicate to minimize the deviations.

4.2.2.1. Minimum inhibitory concentration. The compounds having better *anti-microbial* activity **5c** and **8f** were selected for the MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) studies against the positive strains (*Salmonella typhi*, *Salmonella paratyphi* and *Micrococcus luteus*). The concentrations of test samples were serially diluted from 500 to 1.9 μg/mL and one tube without drug serves as control. All the tubes were inoculated with 1 mL of respective cultures having an OD of 0.2 (∼McFarland standard) and the tubes were incubated at 37 °C for 12–16 h. The turbidity of each tube is measured with respect to control tube. MIC values are defined as the lowest concentration of compound at which growth is completely inhibited. After incubation the culture from each tube was plated in nutrient agar to evaluate the MBC concentration. The concentration at which the cells are completely dead was defined as minimum bactericidal concentration.

4.2.3. Anti-inflammatory activity

4.2.3.1. In-vitro assay by protein denaturation method. Protein denaturation method was followed for performing anti-inflammatory activity of synthesized pyrazole and pyrazolyltriazole compounds **5a-h**, **6a-h**, **8a-h**, **10a-p** and **12a-l** according to Mizushima and Kobayashi 1968.[18] Bovine serum albumin and egg albumin proteins were used in the study and were solubilised in 50 mM sodium phosphate buffer (pH 6.4) at a concentration of 1%. The reaction mixture consists of 0.1 ml of test sample (1 mg/ml), 0.2 ml of albumin protein and make up to a final volume of 5 ml with buffer. Reaction mixture were incubated at 37 °C for 20 min and then heated to 95 °C for 20 min. After cooling the samples to room temperature, the turbidity was measured at 660 nm using UV–visible Spectrophotometer (Model SL 210, Elico India Ltd.). The experiment was performed in triplicate and average values were reported. The percentage inhibition of protein denaturation was calculated as follows:

$$\text{Percentage inhibition} = [(\text{Abs control} - \text{Abs sample})/\text{Abs control}] \times 100$$

4.2.3.2. In vitro anti-inflammatory activity TNF-α and IL-6. Anti-inflammatory activity of the **5a-h** and **8a-h** were assessed using reported protocol[19] with slight modifications by employing LPS induced inflammation model in human U937 cells. Briefly, human U937 cells were incubated in RPMI1640 medium which was supplemented with 10% foetal bovine serum, 2mM l-glutathione and 100U/ml Pen Strep and were incubated at 37 °C and 5% CO_2. The cells were treated with 30 ng/ml of Phorbol 12-myristate 13-acetate (PMA) for 24 h and following differentiation, the U937 cells were incubated with test compounds for 1 h. Subsequently, the cells were challenged with lipopolysaccharide (LPS, 1 μg/ml) and were further incubated for 24 h. The supernatants were then extracted and used for the estimation of the amount of TNF-α and IL-6 secreted using respective ELISA kits (BD Bioscience, San Diego, CA, USA) as per the instructions provided by the manufacturer.

4.2.3.3. Cell viability XTT assay. XTT (2,3-Bis(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide inner salt) assay was performed to assess the cell viability by using mitochondria-dependent reduction of colorless tetrazolium XTT salt to orange colored solution.[30] The cells were seeded at 1×10^4 cells/well in a 96 well plate and were incubated overnight. The cells were then treated with different concentrations of test compounds **5a-h**, **8a-h** and incubated for 24 h. After incubation, the cells were treated with 50 μl of 1 mg/ml XTT in PBS solution and were further incubated in RPMI 1640 medium without phenol red or serum for a period of 4 h. The plates were then briefly mixed and the optical density of the resultant orange colored solution was measured at 450 nm using a Bio-Tek multi mode microplate reader (Bio-Tek Instruments, Inc., Winooski, VT, USA).

4.2.4. Molecular docking studies

Docking of the compounds **5f**, **8a-b**, **8e-f** and **8h** to the both proteins TNF-α (PDB ID- 2AZ5) and IL-6 (PDB ID- 1ALU) (https://www.rcsb.org/pdb/home/home.do) were performed by using auto dock tool (ADT) 4.2 (http://autodock.scripps.edu/resources/adt).[20] The ADT graphics interface was employed for manual preparation of the protein by adding polar hydrogens and merging non-polar hydrogens. In the case of compounds, all compounds were sketched in Tripo's Sybyl6.7 and Gasteiger-Huckel charges were added to minimize the molecules and non-polar hydrogens were merged to give stability. Rotatable bonds were set for synthesized compounds. GPF (grid parameter file) and DPF (docking parameter file) files were prepared and the grid points for autogrid calculations were set as 60∗60∗60A° with the active site residues at the centre of the grid box. Lamarckian genetic algorithm method is used to calculate protein-fixed, ligand-flexible calculations.

4.2.5. Animal experiments

Healthy male Balb/c mice, weighing 20–25 g were acclimatized to the laboratory conditions for about seven days before initiation of the study in the BIOSAFE, an animal quarantine facility of the institute (Registration No: 97/GO/RBi/S/1999/CPCSEA). All animals were given free access to standard pellet diet and fresh drinking water ad libitum. They were housed under standard laboratory conditions with 12 h/12 h of light/dark cycle, 25 ± 2 °C temperature and 56 ± 2% relative humidity throughout the study period. All protocols involving experimental animals were performed as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines under the approval and scrutiny of Institutional Animal Ethics Committee (IAEC) of CSIR-Indian Institute of Chemical Technology (IICT), Hyderabad, India (Approval No: IICT/34/2016).

4.2.5.1. In vivo anti-inflammatory activity. Mice were randomly divided into four groups, as detailed below, each group consisting of 8 mice and were treated as follows.

Group I: (vehicle control, Control): Mice were orally administered with a suspension of 2% gum acacia (10 ml/kg) intragastrically for 3 consecutive days. On day 3, 1 h following oral administration, mice were injected normal saline (10 mg/kg) intraperitoneally.

Group II: (LPS control): Mice were orally administered with a suspension of 2% gum acacia (10 ml/kg) intragastrically for 3 consecutive days. On day 3, 1 h following oral administration, mice were injected LPS (10 mg/kg) intraperitoneally. The dose of LPS was selected on the basis of previously reported literature.[21,22]

Group III: (LPS + dexamethasone (2 mg/kg), LPS + Dex): Mice were orally administered with a suspension of 2 mg/kg of dexamethasone in 2% gum acacia (10 ml/kg) intragastrically for 3 consecutive days. On day 3, 1 h following oral administration, mice were injected LPS (10 mg/kg) intraperitoneally. The dose of dexamethasone was selected based on previously reported literature.[21]

Group IV: (LPS + compound **8b** (100 mg/kg), compound **8b** + LPS): Mice were orally administered with a suspension of 100 mg/kg of test compound **8b**, in 2% gum acacia (10 ml/kg) intragastrically for 3 consecutive days. On day 3, 1 h following oral administration, mice were injected LPS (10 mg/kg) intraperitoneally.

Group V: (LPS + compound **8h** (100 mg/kg), compound **8h** + LPS): Mice were orally administered with a suspension of 100 mg/kg of test compound **8h**, in 2% gum acacia (10 ml/kg) intragastrically for 3 consecutive days. On day 3, 1 h following oral administration, mice were injected LPS (10 mg/kg) intraperitoneally.

Six hours following LPS injection, blood samples were collected from the retro-orbital plexus of the mice and were centrifuged at 4000 RPM for 15 min to separate the serum. The serum obtained was used for the estimation of inflammatory cytokines, TNF-α and IL-6 levels by enzyme linked immunosorbent assay (ELISA) using respective kits (BD Opt EIA, BD Biosciences, San Diego, CA, USA) as per manufacturer's instruction. The % inhibition of inflammatory cytokines by the test compounds was calculated with respect to the LPS control group.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2017.08.042.

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