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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and anti-inflammatory evaluation of some new 3,6-disubstituted-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles bearing pyrazole moiety

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Abstract In the present study, a new series of 3,6-disubstituted-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles (**4aj**) have been synthesized by condensing 3-substituted-4-amino-5-mercapto-1,2,4-triazoles (**1a–b**) with various 3-substituted-pyrazole-4-carboxylic acids (**3a–e**) in the presence of $POCl_3$. The structures of newly synthesized compounds were characterized by elemental analysis, IR, ^1H NMR, ^{13}C NMR, and mass spectroscopic studies. Structure of the compound **4b** was also confirmed by recording the single crystal X-ray structure. All the synthesized compounds were screened for their anti-inflammatory activities by carrageenan induced paw edema method. Anti-inflammatory screening indicated that, compounds **4d**, **4e**, and **4h** were found to be biologically active whereas remaining compounds showed poor anti-inflammatory activity. Also molecular docking studies were also performed for compounds which showed good anti-inflammatory activity.

Keywords Triazolothiadiazoles · Pyrazole · Anti-inflammatory activity

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Introduction

In the last few decades, triazoles and their derivatives have gained significant importance in the field of medicinal chemistry due to their diversified biological properties like antibacterial (Holla *et al.*, 2001; Suresh *et al.*, 2007; Prakash *et al.*, 2004), antifungal (Hirpara *et al.*, 2003), antiviral (Tarmas *et al.*, 2006), anti-inflammatory (Tozkoparan *et al.*, 2000), analgesic (Turan-Zitouni *et al.*, 1999), antimicrobial (Isloor *et al.*, 2009), antihypertensive (Czarnocka-Janowicz *et al.*, 1991), antitubercular (Kucukguzel *et al.*, 2001), and anticancer (He *et al.*, 2010) properties. The amino and mercapto groups of 1,2,4-triazoles serve as readily accessible nucleophilic centers for the preparation of N-bridged heterocycles. Many commercial drugs such as Ribavirin and Taribavirin (antiviral), Fluconazole and Itraconazole (antifungal) are of triazole derivatives only.

Inflammation and analgesia underlies a wide variety of physiological and pathological processes involving a well established and co-ordinated program between many different immune cells, enzymes, and mediators. Cyclooxygenase (COX; prostaglandin G/H synthase, EC 1.14.99.1) catalyzes the first two steps in the biosynthesis of biological mediator prostaglandin, and there lies a great interest in COX-2 as a key therapeutic target for inflammation (Rouzer and Marnett, 2009; McKellar *et al.*, 2008; Jankowski and Hunt, 2008).

Many pyrazole derivatives are reported to possess a wide range of bioactivities. The pyrazole motif makes up the core structure of numerous biologically active compounds. Thus, some representatives of this heterocycle exhibit analgesic (Isloor *et al.*, 2000), anti-inflammatory (Kalluraya *et al.*, 2004), and antimicrobial activity (Isloor *et al.*, 2009). Much attention was paid to pyrazole as a potential antimicrobial agent after the discovery of the natural pyrazole C-glycoside, pyrazofurin which demonstrated a broad spectrum of antimicrobial activity (Comber *et al.*, 1992). Our previous studies also indicate that few of the pyrazole derivatives (Kalluraya *et al.*, 2001; Isloor *et al.*, 2010) are pharmacologically active.

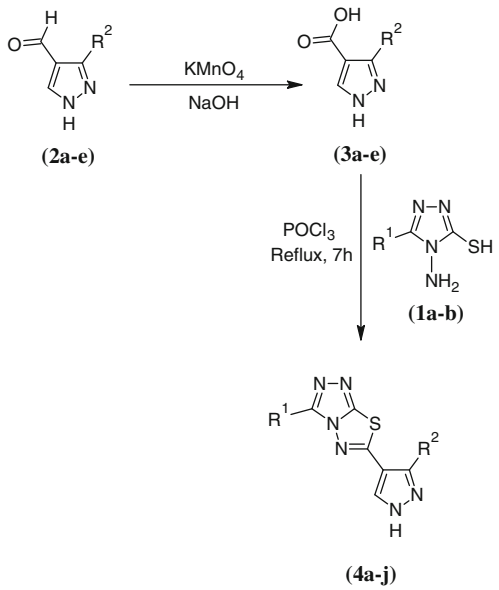
1,3,4-Thiadiazoles exhibit wide spectrum of biological activities, possibly due to presence of toxophoric >N–C–S– moiety (Kamotra *et al.*, 2007). They find applications as antibacterial and anti-inflammatory agents (Mathew *et al.*, 2007). A triazolo thiadiazole system may be viewed as a cyclic analog of two very important components, thiosemicarbazide and biguanide which often display diverse biological activities. The 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole derivatives obtained by reacting the bio-labile 1,2,4-triazole and pyrazole rings together are reported to be pharmacologically active.

Recently some bis triazolo-thiadiazoles endowed with excellent anticancer activities have been reported (Holla *et al.*, 2002). Prompted by these literatures and from our previous research on heterocycles (Vijesh *et al.*, 2010), we planned to synthesize new triazolothiadiazole derivatives containing pyrazole, halogen as substituents and to study their anti-inflammatory activity.

Results and discussion

Chemistry

3-Substituted-4-amino-5-mercapto-1,2,4-triazoles (**1**) were synthesized as reported in the literature (Dhaka *et al.*, 1974; Reid and Heindel, 1976). Various 3-substituted-pyrazole-4-carbaldehydes (**2**) were also prepared according to literature method [8]. Oxidation of 3-substituted-pyrazole-4-carbaldehydes using potassium permanganate in the presence of base (Lebedev *et al.*, 2005) yielded corresponding acid. Subsequently condensation of 3-substituted-4-amino-5-mercapto-1,2,4-triazoles (**1**) with various 3-substituted-pyrazole-4-carboxylic acids (**3**) in the presence of phosphorous oxychloride afforded a series of 3,6-disubstituted-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles (**4a–j**). The synthesis of compounds (**4a–j**) followed the general pathway outlined in Scheme 1. The structures of the synthesized compounds (**4a–j**) were confirmed by analytical and spectral data (IR, ^{1}H NMR, ^{13}C NMR, Mass & Elemental). The reaction between **3a–e** and **1a–b** resulted in the formation of cyclized products **4a–j**. The absence of NH_2 ($\sim\delta$ 5.6) and SH ($\sim\delta$ 13.4) peaks in ^{1}H NMR spectra of **4a–j** which were present in **1a–b** can be attributed for the involvement of these functional groups in the formation of cyclized products and also cyclization was further confirmed by the absence of NH_2 ($\sim$3,260 cm^{-1}) absorption band in the IR spectrum of **4a–j**. The ^{1}H NMR of **4f** showed a singlet at δ 13.8 and 8.46 are due to NH and 5H proton of pyrazole ring. A multiplet and a triplet were observed at δ 7.79 and 7.29 (J_{ortho} = 8.6 Hz) which corresponds to Ar–H. Aliphatic protons which resonated as triplet, multiplet, and triplet at δ 2.91, 1.75, and 0.91 can be assigned to $-CH_2-$ CH_2-CH_3, respectively. Similarly, IR spectrum of **4f** exhibited bands at 3403, 3102, 2964, 1603, and 1483 cm^{-1} which corresponds to N–H, aromatic C–H, aliphatic C–H, C=N and C=C, respectively. The mass spectrum of **4f** showed molecular ion peak at m/z = 329 (M + 1), which is in agreement with the molecular formula $C_{15}H_{13}FN_6S$. Among the synthesized compounds the structure of **4b** was confirmed by single crystal X-ray analysis (Fig. 1). The spectral values for all the compounds and C, H, N analyses are given in the experimental part and the characterization data is provided in Table 1. Similarly, crystallographic data (Fun *et al.*, 2010) has been provided in Table 2.



Where R^1 = C_2H_5, C_3H_7

R^2 = C_6H_5, 4-F-C_6H_4, 4-OCH_3-C_6H_4, 4-Cl-C_6H_4, 2,4-Cl_2-C_6H_3

Scheme 1 Synthetic route for 3,6-disubstituted-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles

In silico studies

Considering the well obtained in vitro results, it was thought worthy to perform molecular docking studies, screening for supportive coordination between *in silico* studies with in vitro results. Considering COX-2 as the target receptor, comparative and automated docking studies with newly synthesized candidate lead compounds was performed to determine the best *in silico* conformation. The Lamarckian genetic algorithm, inculcated in the docking program AutoDock 4.2, was employed for the purpose. Figure 2 shows the native crystal structure of Diclofenac bound to the Cyclooxygenase active site of COX-2 obtained from Protein Data Bank (http://www.pdb.org/pdb/home/home.do) with the PDB ID 1PXX (Rowlinson *et al.*, 2003).

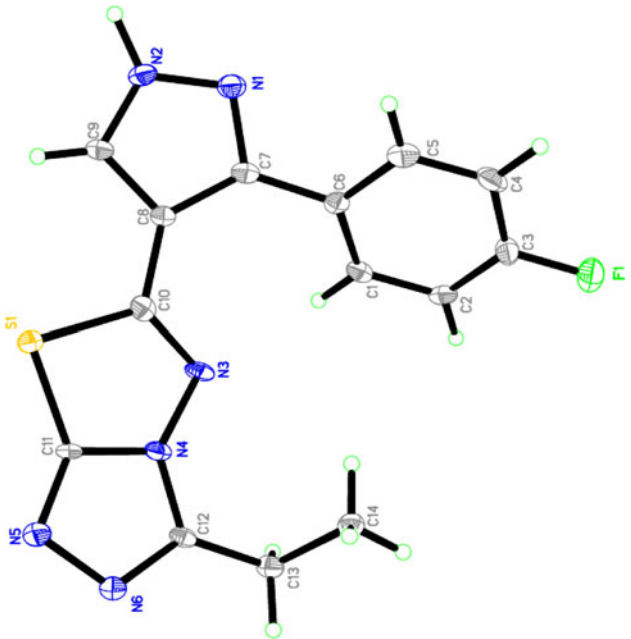


Fig. 1 X-ray crystal structure of compound **4b**

The docking of receptor COX-2 with newly synthesized candidate ligands exhibited well established bonds with one or more amino acids in the receptor active pocket. Docking studies were performed for compounds **4d**, **4e**, **4h**, & Diclofenac. The active pocket was considered to be the site where Diclofenac was complexed in COX-2 of 1PXX. The active pocket consisted of ten amino acid residues as Val349, Ser530, Leu352, Tyr385, Tyr348, Trp387, Gly526, Ala527, Met522, and Leu384 as shown in Fig. 3. The synthesized ligand molecules having 2D structure were converted to energy minimized 3D structures and were further used for *in silico* protein–ligand docking. Figure 4. Shows the images of ligands docked separately to COX2 including the considered standard Diclofenac. Table 3 shows the Binding Energy and Inhibition Constant of all the four compounds including standard Diclofenac. *In-silico* studies revealed that all three synthesized molecules showed good binding energy toward the target protein ranging from −9.1 to −8.76 kJ mol^{-1}.

Table 2 Crystallographic data for compound **4b**

Empirical formula	$C_{14}H_{11}FN_6S$
Formula weight	314.35
Temperature (K)	100
Wavelength (Å)	0.71073
Crystal system	Orthorhombic
Space group	$Pca2_1$
Cell dimensions	
a (Å)	35.053 (2)
b (Å)	3.8463 (2)
c (Å)	9.9482 (6)
Volume ($Å^3$)	1341.26 (13)
Z	4
Density (Mg m^{-3})	1.557
F (000)	648
Θ range for data collection (*P*)	2.3–30.0

Table 1 Characterization data of the compounds **4a–j**

Compounds	R^1	R^2	Molecular formula (mol. wt)	Yield (%)	M.p. (°C)
4a	C_2H_5	C_6H_5	$C_{14}H_{12}N_6S$ (296)	68	217–219
4b	C_2H_5	4-F–C_6H_4	$C_{14}H_{11}FN_6S$ (314)	74	206–207
4c	C_2H_5	4-OCH_3–C_6H_4	$C_{15}H_{14}N_6OS$ (326)	65	222–224
4d	C_2H_5	4-Cl–C_6H_4	$C_{14}H_{11}ClN_6S$ (330)	72	290–293
4e	C_3H_7	C_6H_5	$C_{15}H_{14}N_6S$ (310)	56	219–221
4f	C_3H_7	4-F–C_6H_4	$C_{15}H_{13}FN_6S$ (328)	61	187–189
4g	C_3H_7	4-OCH_3–C_6H_4	$C_{16}H_{16}N_6OS$ (340)	67	213–215
4h	C_3H_7	4-Cl–C_6H_4	$C_{15}H_{13}ClN_6S$ (344)	69	220–222
4i	C_2H_5	2,4-Cl_2–C_6H_3	$C_{14}H_{10}Cl_2N_6S$ (365)	71	200–205
4j	C_3H_7	2,4-Cl_2–C_6H_3	$C_{15}H_{12}Cl_2N_6S$ (379)	77	203–205

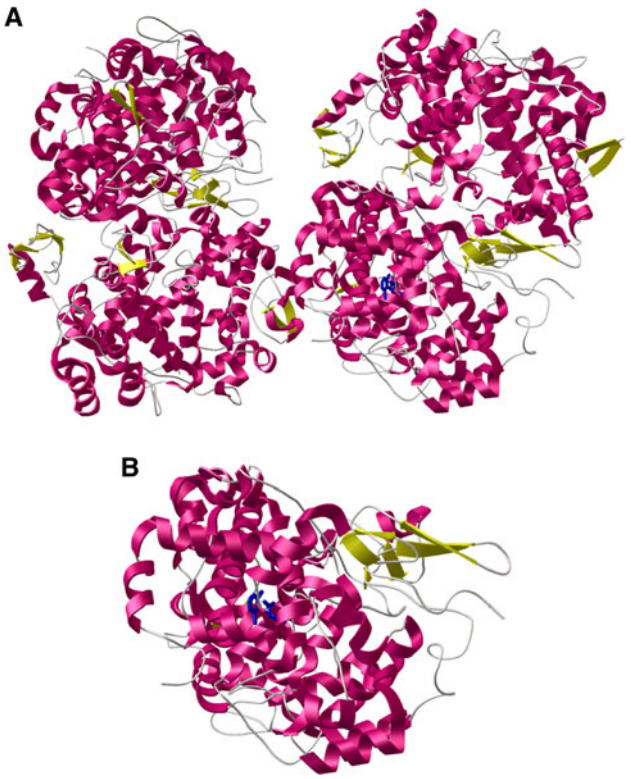


Fig. 2 **a** Secondary structure of COX-2 (PDB ID 1PXX) complexed with Diclofenac (complete protein), showing its dimeric nature with two identical subunits in each monomer. **b** Chain A (part of complete protein) complexed with Diclofenac

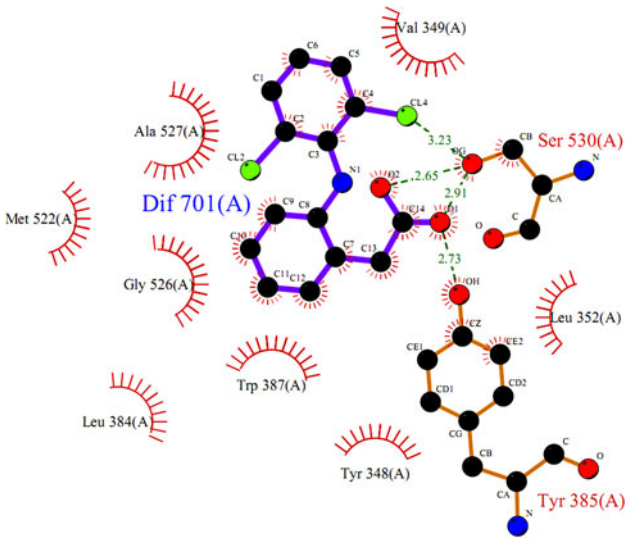


Fig. 3 PDB sum's ligplot results for 1PXX, showing all ten amino acid residues of active pocket

Finally considering both in vitro and *in silico* molecular docking results, among the synthesized molecules **4h** showed the best result and can be considered as the best inhibitor of COX-2.

Conclusion

A series of new 3,6-disubstituted-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles bearing pyrazole derivatives were synthesized in reasonable yields. They were characterized by ^{1}H NMR, ^{13}C NMR, mass spectrometry, IR studies, and elemental analyses. Compound **4b** was analyzed for its molecular structure by single crystal X-ray crystallography. All the newly synthesized compounds were screened for anti-inflammatory activity by Carrageenan induced paw edema method. Among the screened compounds, **4h** showed significant anti-inflammatory activity at percentage inhibition of 64.7, compared to the standard drug Diclofenac sodium which showed the percentage inhibition at 80.4. Similarly compounds **4d** and **4e** have showed percentage inhibition of 56.9. However remaining compounds showed poor anti-inflammatory activity. Docking studies were performed for compounds **4d, 4e, 4h**, & Diclofenac among which **4h** showed the best result and can be considered as the best inhibitor of COX-2.

Compound **4h** has propyl and *p*-chlorophenyl substituents, which is accounted for their significant anti-inflammatory activity. Compounds **4d** has ethyl and *p*-chlorophenyl moiety and **4e** has propyl and phenyl moieties, which has accounted for the moderate activity.

Experimental

Chemistry

Melting points were determined by open capillary method and were uncorrected. The IR spectra (in KBr pellets) were recorded on a Thermo Nicolet avatar 330-FT-IR spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded (DMSO-d_6) on a Bruker (400 MHz) and Varian (400 MHz) spectrometer using TMS as internal standard. Chemical shift values are given in δ scales. The mass spectra were recorded on LC–MS-Agilent 1100 series and API 2000 LC/MS/MS system. Single crystal X-ray structure was recorded using Bruker instrument. Elemental analyses were performed on a Flash EA 1112 series CHNS-O Analyzer. The completion of the reaction was checked by thin layer chromatography (TLC) on silica gel coated aluminum sheets (silica gel 60 F254). Commercial grade solvents and reagents were used without further purification.

General procedure for the synthesis of 3-(4-substituted phenyl)-1*H*-pyrazole-4-carboxylic acid (**3a–e**)

Suitable 3-substituted-pyrazol-4-carbaldehyde (**2a–e**) (0.01 mol) was dissolved with stirring in a solution of 2 g of NaOH in 40 ml of water. The mixture was cooled to

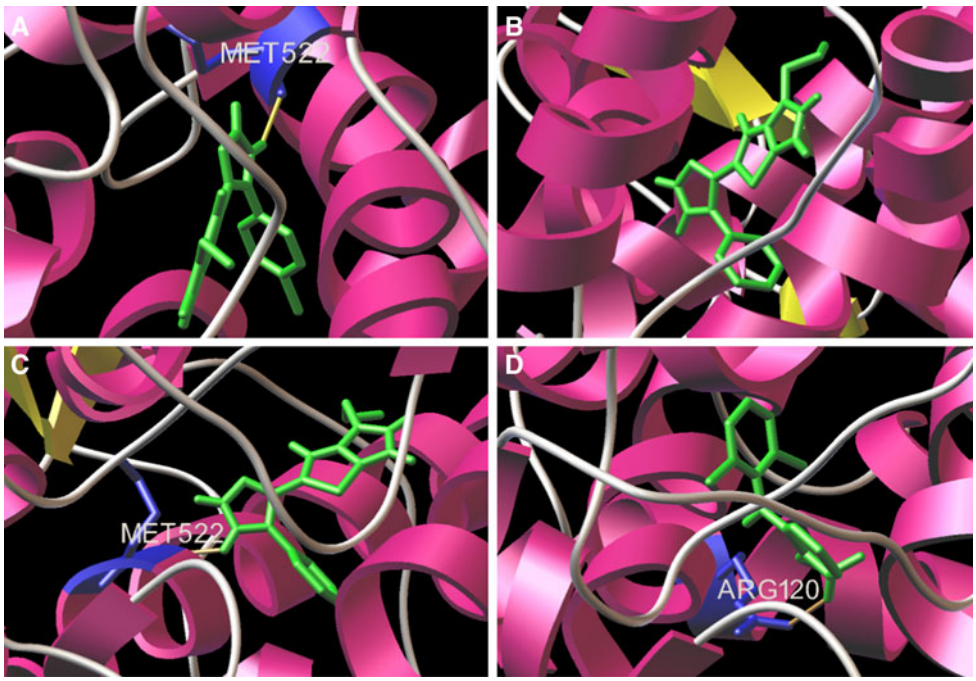


Fig. 4 Showing all ligands docked in best of its conformation. **a 4d** forming 1H bond with Met522. **b 4e** forming no H bonds. **c 4h** forming 1H bond with Met522. **d** Diclofenac forming 1H bond with Arg120

Table 3 Binding energy (kJ mol^{-1}) and inhibition constant of all the four compounds including standard Diclofenac

Compound	Binding energy (kJ mol^{-1})	Inhibition constant
4d	−8.76	406.41 nM
4e	−8.92	287.8 nM
4h	−9.1	212.03 nM
Diclofenac	−7.49	3.22 nM

15°C, and a solution of $KMnO_4$ (0.0088 mol) in 40 ml of water was quickly added. The mixture was stirred for 30 min at 20°C and then heated to 95–100°C until the solution becomes completely decolorized. The solution was cooled and filtered to remove MnO_2 precipitate. Then, the filtrate was acidified with Conc. HCl to pH 3. The resulting solid was filtered off, washed with water and dried.

General procedure for the synthesis of 3,6-disubstituted-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles (**4a**–**j**)

An equimolar mixture of respective triazole (**1**) (0.001 mol) and 3-(4-substitutedphenyl)-1H-pyrazole-4-carboxylic acid (**3**) (0.001 mol) was dissolved in 5 ml of dry phosphorous oxychloride. The resulted solution was further refluxed for 8–9 h. Excess phosphorous oxychloride was then distilled off and the mixture was gradually poured onto crushed ice with stirring. The mixture was allowed to stand overnight and the solid was separated. The separated solid was filtered, washed thoroughly with cold water, 20% $NaHCO_3$ solution and recrystallised from a mixture of dioxane and ethanol.

3-Ethyl-6-(3-phenyl-1*H*-pyrazol-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**4a**)

IR (KBr, υ_{max} cm^{-1}): 3,390 (N–H-str), 3,066 (aromatic C–H-str), 2,909 (aliphatic C–H-str), 1,604 (C=N), 1,481 (C=C); ^{1}H NMR (DMSO-d_6): δ 1.31(t, 3H, CH_3), 2.99 (q, 2H, CH_2), 7.49–7.72 (m, 5H, Ar–H), 8.43 (s, 1H, pyrazole-5H), 13.70 (s, 1H, Pyrazole N–H). ^{13}C NMR: 159.9, 152.8, 148.7, 130.2, 129.8, 129.5, 128.9, 109.3, 18.5, 11.1. MS: $m/z = 297$ (M + 1). Anal. calcd. for $C_{14}H_{12}N_6S$: C, 56.74; H, 4.08; N, 28.36. Found: C, 56.69; H, 4.04; N, 28.39%.

3-Ethyl-6-(3-(4-fluorophenyl)-1*H*-pyrazol-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**4b**)

IR (KBr, υ_{max} cm^{-1}): 3,397(N–H-str), 3,091 (aromatic C–H-str), 2,929 (aliphatic C–H-str), 1,605 (C=N), 1,479 (C=C); ^{1}H NMR (DMSO-d_6): δ 1.30 (t, 3H, CH_3), 2.98 (q, 2H, CH_2), 7.75–7.79 (m, 2H, Ar–H), 7.30–7.35 (t, 2H, $J_{ortho} = 9$ Hz, Ar–H), 8.46 (s, 1H, pyrazole-5H), 13.70 (s, 1H, Pyrazole N–H). ^{13}C NMR: 159.6, 148.7, 131.8, 131.8, 115.9, 115.7, 109.4, 18.5, 11.2. MS: $m/z = 315$ (M + 1). Anal. calcd. for $C_{14}H_{11}FN_6S$: C, 53.49; H, 3.53; N, 26.74. Found: C, 53.45; H, 3.58; N, 26.77%.

3-Ethyl-6-(3-(4-methoxyphenyl)-1*H*-pyrazol-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**4c**)

IR (KBr, υ_{max} cm^{-1}): 3,350 (N–H-str), 3,111 (aromatic C–H-str), 2,931 (aliphatic C–H-str), 1,613 (C=N), 1,472 (C=C); ^{1}H NMR (DMSO-d_6): δ 1.33 (t, 3H, CH_3), 2.99 (q, 2H, CH_2), 3.83 (s, 3H, –OCH_3), 7.05–7.07 (d, 2H,

$J = 8.0$ Hz, Ar–H), 7.62–7.65 (d, 2H, $J = 8.8$ Hz, Ar–H), 8.34 (s, 1H, pyrazole-5H), 13.70 (s, 1H, Pyrazole N–H). ^{13}C NMR: 159.7, 131.0, 114.4, 109.1, 55.7, 18.5, 11.3. MS: $m/z = 327$ (M + 1). Anal. calcd. for $C_{15}H_{14}N_6OS$: C, 55.20; H, 4.32; N, 25.75. Found: C, 55.17; H, 4.36; N, 25.78%.

3-Ethyl-6-(3-(4-chlorophenyl)-1*H*-pyrazol-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**4d**)

IR (KBr, υ_{max} cm^{-1}): 3,360 (N–H-str), 3,173 (aromatic C–H-str), 2,940 (aliphatic C–H-str), 1,579 (C=N), 1,498 (C=C); ^{1}H NMR (DMSO-d$_6$): δ 1.16 (t, 3H, CH_3), 2.50 (q, 2H, CH_2), 7.46–7.48 (d, 2H, $J = 8.8$ Hz, Ar–H), 7.78–7.80 (d, 2H, $J = 8.4$ Hz, Ar–H), 8.37 (s, 1H, pyrazole-5H), 13.70 (s, 1H, Pyrazole N–H). MS: $m/z = 331$ (M + 1). Anal. calcd. for $C_{14}H_{11}ClN_6S$: C, 50.83; H, 3.35; N, 25.41. Found: C, 50.86; H, 3.31; N, 25.45%.

3-Propyl-6-(3-phenyl-1*H*-pyrazol-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**4e**)

IR (KBr, υ_{max} cm^{-1}): 3,389 (N–H-str), 3,091 (aromatic C–H-str), 2,918 (aliphatic C–H-str), 1,613 (C=N), 1,479 (C=C); ^{1}H NMR (DMSO-d$_6$): δ 0.93 (t, 3H, CH_3), 1.77 (m, 2H, CH_2), 2.93 (t, 2H, CH_2), 7.47–7.71 (m, 5H, Ar–H), 8.43 (s, 1H, pyrazole-5H), 13.70 (s, 1H, Pyrazole N–H). MS: $m/z = 311$ (M + 1). Anal. calcd. for $C_{15}H_{14}N_6S$: C, 58.05; H, 4.55; N, 25.41. Found: C, 50.86; H, 3.31; N, 27.08%.

3-Propyl-6-(3-(4-fluorophenyl)-1*H*-pyrazol-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**4f**)

IR (KBr, υ_{max} cm^{-1}): 3,403 (N–H-str), 3,102 (aromatic C–H-str), 2,964 (aliphatic C–H-str), 1,603 (C=N), 1,483 (C=C);^{1}H NMR (DMSO-d$_6$): δ 0.92 (t, 3H, CH_3), 1.75 (m, 2H, CH_2), 2.91 (t, 2H, CH_2), 7.74–7.77 (m, 2H, Ar–H), 7.29–7.33 (t, 2H, $J_{ortho} = 8.6$ Hz, Ar–H), 8.46 (s, 1H, pyrazole-5H), 13.71 (s, 1H, Pyrazole N–H). MS: $m/z = 329$ (M + 1). Anal. calcd. for $C_{15}H_{13}FN_6S$: C, 54.87; H, 3.99; N, 25.59. Found: C, 54.82; H, 3.94; N, 25.56%.

3-Propyl-6-(3-(4-methoxyphenyl)-1*H*-pyrazol-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**4g**)

IR (KBr, υ_{max} cm^{-1}): 3,360 (N–H-str), 3,081 (aromatic C–H-str), 2,951 (aliphatic C–H-str), 1,611 (C=N), 1,463 (C=C); ^{1}H NMR (DMSO-d$_6$): δ 0.94 (t, 3H, CH_3), 1.78 (m, 2H, CH_2), 2.93 (t, 2H, CH_2), 3.82 (s, 3H, –OCH_3), 7.03–7.06 (d, 2H, $J = 8.8$ Hz, Ar–H), 7.61–7.63 (d, 2H, $J = 8.8$ Hz, Ar–H), 8.32 (s, 1H, pyrazole-5H), 13.68 (s, 1H, Pyrazole N–H). MS: $m/z = 341$ (M + 1). Anal. calcd. for $C_{16}H_{16}N_6OS$: C, 56.45; H, 4.74; N, 24.69. Found: C, 56.41; H, 4.78; N, 24.72%.

3-Propyl-6-(3-(4-chlorophenyl)-1*H*-pyrazol-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**4h**)

IR (KBr, υ_{max} cm^{-1}): 3,391 (N–H-str), 3,088 (aromatic C–H-str), 2,928 (aliphatic C–H-str), 1,566 (C=N), 1,476 (C=C); ^{1}H NMR (DMSO-d$_6$): δ 0.92 (t, 3H, CH_3), 1.74 (m, 2H, CH_2), 2.90 (t, 2H, CH_2), 7.52–7.53 (d, 2H, $J = 7.2$ Hz, Ar–H), 7.73–7.75 (d, 2H, $J = 8.4$ Hz, Ar–H), 8.57 (s, 1H, pyrazole-5H), 13.80 (s, 1H, Pyrazole N–H). MS: $m/z = 345$ (M + 1), 347 (M + 2). Anal. calcd. for $C_{15}H_{13}ClN_6S$: C, 52.25; H, 3.80; N, 24.37. Found: C, 52.29; H, 3.77; N, 24.33%.

3-Ethyl-6-(3-(2,4-dichlorophenyl)-1*H*-pyrazol-4-yl)-[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazole (**4i**)

IR (KBr, υ_{max} cm^{-1}): 3,320 (N–H-str), 3,097 (aromatic C–H-str), 2,926 (aliphatic C–H-str), 1,582 (C=N), 1,479 (C=C); ^{1}H NMR (DMSO-d$_6$): δ 1.20 (t, 3H, CH_3), 2.89 (q, 2H, CH_2), 7.59–7.81 (m, 3H, Ar–H), 8.62 (s, 1H, pyrazole-5H). ^{13}C NMR: 158.8, 152.5, 148.6, 135.5, 134.9, 134.1, 129.6, 128.1, 111.2, 18.5, 11.01. MS: $m/z = 365$ (M +), 367 (M + 2), 369 (M + 4). Anal. calcd. for $C_{14}H_{10}Cl_2N_6S$: C, 46.04; H, 2.76; N, 23.01. Found: C, 46.07; H, 2.71; N, 23.08%.

3-Propyl-6-(3-(2,4-dichlorophenyl)-1*H*-pyrazol-4-yl)-[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazole (**4j**)

IR (KBr, υ_{max} cm^{-1}): 3,325 (N–H-str), 3,099 (aromatic C–H-str), 2,875 (aliphatic C–H-str), 1,583 (C=N), 1,472 (C=C); ^{1}H NMR (DMSO-d$_6$): δ 0.86 (t, 3H, CH_3), 1.62 (m, 2H, CH_2), 2.82 (t, 2H, CH_2), 7.59–7.81 (m, 3H, Ar–H), 8.64 (s, 1H, pyrazole-5H), 13.79 (s, 1H, Pyrazole N–H). ^{13}C NMR: 158.8, 152.4, 147.6, 134.9, 134.0, 129.5, 128.0, 111.1, 26.7, 19.9, 13.8. MS: $m/z = 379$ (M +), 381 (M + 2), 383 (M + 4). Anal. calcd. for $C_{15}H_{12}Cl_2N_6S$: C, 47.50; H, 3.19; N, 22.16. Found: C, 47.47; H, 3.14; N, 22.19%.

Anti-inflammatory activity

Screening of anti-inflammatory drugs by Carrageenan induced paw edema method (Hu *et al.*, 2008; Maria *et al.*, 2008)

The anti-inflammatory activity of the test compounds was carried out using carrageenan-induced rat paw edema model according to Winter *et al.* (1962) by employing 1% carrageenan solution as the phlogistic agent. Edema was

induced in the left hind paw of Wistar rats (200–250 g) by the sub-plantar injection of 0.1 ml of 1% Carrageenan in distilled water. Both sexes were used. Each group composed of six animals. The animals which were bred in our laboratory were housed under standard conditions and received a diet of commercial food pellets and water ad libitum during the maintenance but they were entirely fasted during the experiment period. Our studies were conducted in accordance with recognized guidelines on animal experimentation.

The test compounds were given intraperitoneally 30 min after Carrageenan injection. The paw volume was measured plethysmometrically at 0 and 3 h after the carrageenan injection. The difference in the paw volume of the injected and the control were compared for each animal. The percentage inhibition of edema was calculated using the formula,

$$\% \text{ Oedema inhibition} = 100 - (V_{\text{test}}/V_{\text{control}}) \times 100$$

Statistical analysis

All experimental groups were composed of six animals. Data obtained from animal experiments were expressed as mean ± standard error (S.E.M.). The statistical significance of difference between groups were assessed by means of analysis of variance (ANOVA) followed by Dunnet's test. The results of the anti-inflammatory studies have been presented in Table 4.

In silico studies

The ligands were drawn in ChemDraw Ultra 6.0 (Chem Office package) assigned with proper 2D orientation and the structure of each compound was analyzed for connection error in bond order. OSIRIS, an ADMET based Java library layer that provides reusable cheminformatics functionality and is an entirely in-house developed drug discovery informatics system was used to predict the total drug score via *in silico* (Sander *et al.*, 2009). Energy of the molecules was minimized using Dundee PRODRG2 server (Schuttelkopf and van Aalten, 2004).The energy minimized compounds were then read as input for AutoDock 4.2, in order to carry out the docking simulation (Morris *et al.*, 2009). All the heteroatoms were removed from the 1PXX.pdb, to make complex less receptor free of any ligand before docking. The Graphical User Interface program "AutoDock Tools" was used to prepare, run, and analyze the docking simulations. Kollman united atom charges, solvation parameters and polar hydrogen's were added to the receptor for the preparation of protein in docking simulation. Since ligands are not peptides, Gasteiger charge was assigned and then non-polar hydrogens were merged. AutoDock requires pre-calculated grid maps, one for each atom type, present in the ligand being docked and its stores the potential energy arising from the interaction with macromolecule. This grid must surround the region of interest (active site) in the macromolecule. In the present study, the binding site was selected based on the amino acid residues, which are involved in binding with Diclofenac of COX-2 as obtained from PDB with ID 1PXX which would be considered as the probable best accurate region as it is solved by experimental crystallographic data (Rowlinson *et al.*, 2003). Therefore, the grid was centered at the region including all the ten amino acid residues (Val349, Ser530, Leu352, Tyr385, Tyr348, Trp387, Gly526, Ala527, Met522 and Leu384) that surround active site as in Fig. 2. The grid box size was set at 56, 44, and 54 A° for *x*, *y* and *z*, respectively, and the grid center was set to 26.472, 25.806, and 12.52 for *x*, *y* and *z,* respectively, which covered all the ten amino acid residues in the considered active pocket. AutoGrid 4.0 Program, supplied with AutoDock 4.0 was used to produce grid maps. The spacing between grid points was 0.375 angstroms. The Lamarckian Genetic Algorithm (LGA) was chosen to search for the best conformers. During the

Table 4 Anti-inflammatory activity of compounds (**4a–j**)

Treatment	Dose (mg/kg)	Initial paw volume (ml) (*b*)	Paw volume after 3 h (ml) (*a*)	Edema volume (*a–b*)	Percentage inhibition
Control	Vehicle	0.93 ± 0.01	2.20 ± 0.04	1.27 ± 0.02	
Diclofenac	10	0.80 ± 0.04	1.05 ± 0.02	0.25 ± 0.04	80.4**
4a	20	0.8 ± 0.08	1.78 ± 0.01	0.90 ± 0.09	29.4*
4b	20	0.85 ± 0.04	1.83 ± 0.06	0.98 ± 0.02	23.5*
4c	20	0.85 ± 0.01	1.90 ± 0.08	1.05 ± 0.01	17.6
4d	20	0.83 ± 0.09	1.38 ± 0.02	0.55 ± 0.04	56.9**
4e	20	0.85 ± 0.02	1.40 ± 0.04	0.55 ± 0.08	56.9**
4f	20	0.93 ± 0.09	2.00 ± 0.09	1.05 ± 0.04	12.0
4g	20	0.95 ± 0.04	2.38 ± 0.01	0.93 ± 0.02	13.5
4h	20	0.85 ± 0.01	1.28 ± 0.04	0.45 ± 0.08	64.7**
4i	20	0.98 ± 0.09	1.93 ± 0.02	0.95 ± 0.01	25.5*
4j	20	0.83 ± 0.08	1.80 ± 0.02	0.98 ± 0.09	23.5*

* $P < 0.05$ compared to control

** $P < 0.01$ compared to control

docking process, a maximum of 10 conformers was considered for each compound. All the AutoDock docking runs were performed in Intel CentrinoCore2Duo CPU @ 2.20 GHz of IBM system origin, with 2 GB DDR RAM. AutoDock 4.0 was compiled and run under Windows XP Service Pack 3 operating system.

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