Bioorganic Chemistry 54 (2014) 51–59

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

1,3,4-Trisubstituted pyrazole analogues as promising anti-inflammatory agents

S.G. Alegaon [a,*], K.R. Alagawadi [a], M.K. Garg [a], K. Dushyant [b], D. Vinod [c]

[a] Department of Pharmaceutical Chemistry, KLE University's College of Pharmacy, Nehrunagar, Belgaum 590 010, Karnataka, India
[b] Regional Medical Research Centre (ICMR), Nehrunagar, Belgaum, Karnataka, India
[c] Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai 03, India

ARTICLE INFO

Article history:
Received 20 November 2013
Available online 12 April 2014

Keywords:
Pyrazole
1,3,4-Thiadiazole
Thiazolidinone
Thiazole
Anti-inflammatory activity
COX-1 and COX-2
Molecular docking

ABSTRACT

Twenty-two 1,3,4-trisubstituted pyrazole (**3a–d**), (**4a–d**), (**5a–d**), (**6a–l**) derivatives were synthesized and structure of newly synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, and mass spectral analysis. These compounds were screened for the anti-inflammatory activity by carrageenan-induced paw edema method. Compounds **5a**, and **5b** showed excellent anti-inflammatory activity ($\geqslant$84.2% inhibition) and **3a**, **3b**, and **3c** showed good anti-inflammatory activity ($\geqslant$64.6% inhibition) compared to that of the standard drug diclofenac (86.72%) when measured 3 h after the carrageenan injection. Moreover, the cyclooxygenase (COX) enzyme inhibitor activity of selected compounds, which are the excellent anti-inflammatory activities in carrageenan-induced paw edema model, was investigated *in vitro* COX inhibition assay. Molecular docking study further helped in supporting the observed activity. In addition compound **5a** exhibited considerable cytotoxic activity against MCF-7 cell line with IC_{50} value 6.5 μM.

1. Introduction

Trisubstituted pyrazole prototype is one of the privileged structure fragments in modern medicinal chemistry considering its broad biological spectrum and affinity for various bio targets of this class heterocyclic compound. They are reported to exert a number of important biological activities such as anti-inflammatory, antimicrobial, antitubercular, anti-cancer, anti-angiogenic, anti-influenza, antioxidant and antidepressant activities [1–8]. Recently, some novel pyrazole carbohydrazide derivatives were reported to have moderate anticancer activity [9]. Also, substituted pyrazole derivatives represent important key intermediates for the synthesis of therapeutically active drugs. It is well known that 1,3,4-thiadiazole analogues have recently been grown up due to their biological activity [10–13]. They can be used as antimicrobial, antihypertensive, antidepressant, anticonvulsant, antileishmanial, and anti-inflammatory agent. Moreover, 1,3,4-thiadiazole derivatives are widely used in clinical trials for the treatment of patients with different tumors [14].

Thiazolidinone derivatives are an important class of heterocyclic analogues with various pharmacological activities such as anti-inflammatory, antibacterial, antifungal, antitubercular and antipsychotic activities [15–18]. Similarly thiazole analogs exhibit various biological activities such as anti-inflammatory [19], antitumor [20,21], anticancer [22], antimalarial [23], hypolipidemic [24] antimicobacterial [25], and antimicrobial [26] agents. Numerous reports have appeared in the literature describing a broad spectrum of biological properties of thiazoles, and thiazolidinones due to their synthetic and pharmacological flexibility, and more recently for the treatment of pain, as fibrinogen receptor antagonist with antithrombotic activity, and as new inhibitor of bacterial DNA gyrase B [27,28]. In view of the above facts, and in continuation of our search on pharmacologically active heterocyclic compounds, we report herein synthesis, anti-inflammatory activity and molecular docking studies of 1,3,4-trisubstituted pyrazole derivatives.

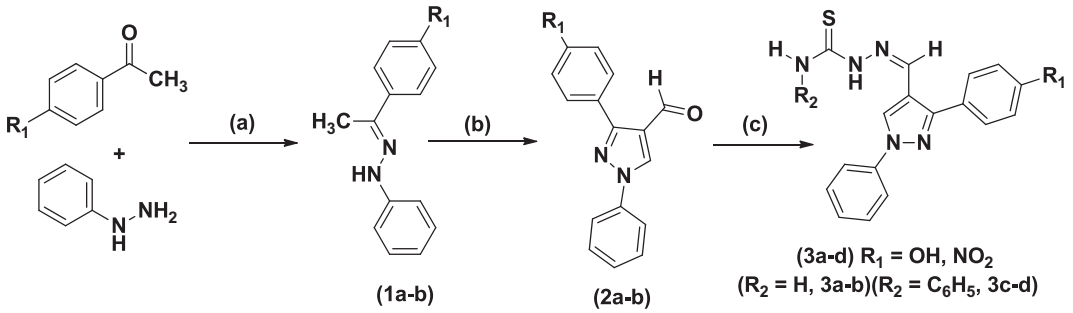
2. Results and discussion

2.1. Chemistry

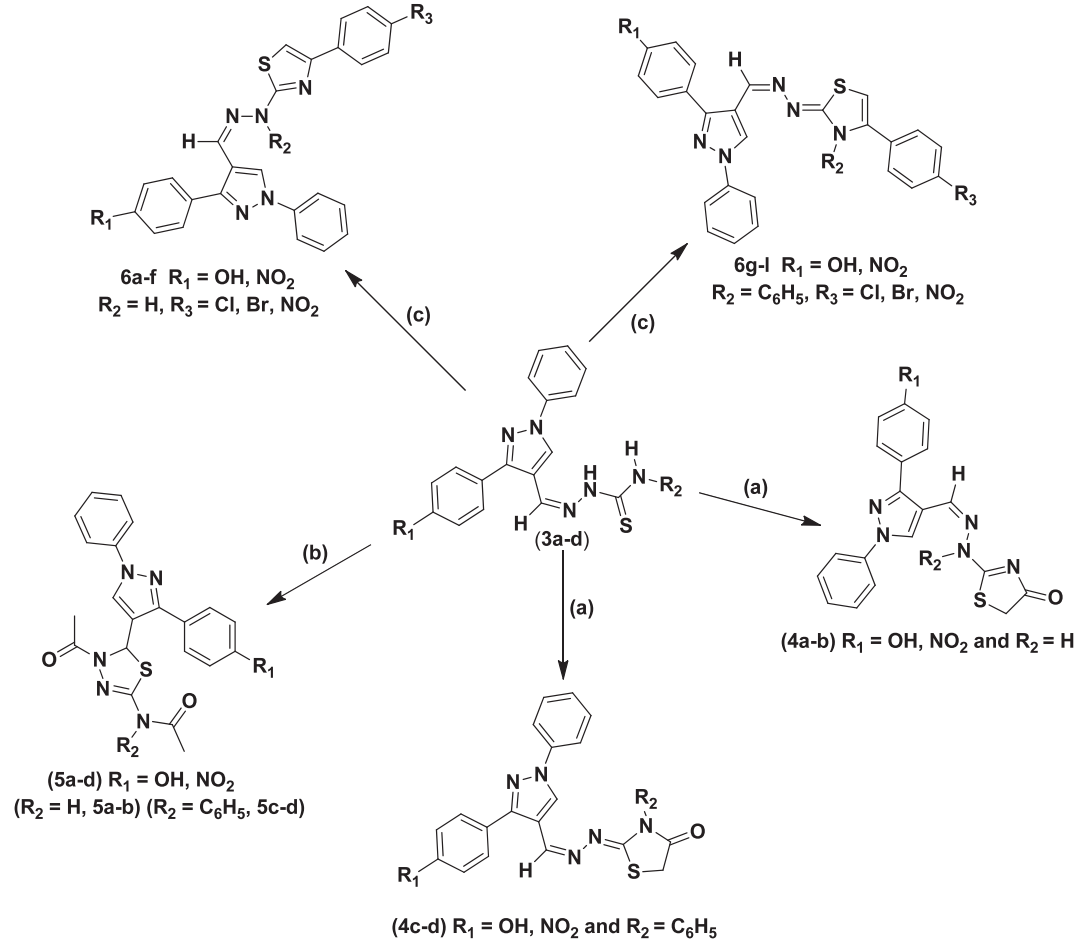
The reaction sequence employed for synthesis of the title compounds (**3a–d**), (**4a–d**), (**5a–d**) and (**6a–l**) are illustrated in Schemes 1 and 2. The two step procedure was adopted for the synthesis of 3-(4-substituted phenly)-1-phenyl-1*H*-pyrazole-4-carbaldehydes **(2a–b)**. The 1-phenyl-2(1-phenylehylidene)hydrazines (**1a–b**) were synthesized by condensation of the phenyl hydrazine with 4-substituted-acetophenones [29]. Treatment of

* Corresponding author. Fax: +91 8312472387.
E-mail addresses: sgalegaon@klepharm.edu, sgalegaon@gmail.com (S.G. Alegaon).

http://dx.doi.org/10.1016/j.bioorg.2014.04.001
0045-2068/



Scheme 1. Reagents and conditions: (a) dry ethanol, glacial acetic acid (1 mL) reflux 5 h; (b) DMF/$POCl_3$, 80 °C; (c) thiosemicarbazide or 4-phenylthiosemicarbazide, ethanol: chloroform (7:3), reflux 10 h.



Scheme 2. Reagents and conditions: (a) ethyl-bromoacetate, anhydrous sodium acetate, glacial acetic acid, reflux 8 h; (b) acetic anhydride, reflux 5 h; (c) substituted phenacyl bromide, dry ethanol, reflux 24 h.

the hydrazone derivatives (**1a–b**) with Vilsmeir–Haack reagent (DMF–$POCl_3$) afforded the corresponding 4-formylpyrazole derivatives (**2a–b**). Thiosemicarbazone derivatives (**3a–d**) were obtained via condensation of 1,3-diarylpyrazole-4-carbaldehyde derivatives (**2a–b**) with thiosemicarbazide or 4-phenylthiosemicarbazide in presence of a catalytic amount of conc. HCl in ethanol: chloroform (7:3) as the solvent affording in satisfactory yield. The synthesized thiosemicarbazone derivatives **3a–d** were characterized by IR and ^{1}H NMR, The IR spectra of (**3a–d**) showed the appearance of characteristic bands at 3397 and 3295 cm^{-1} assignable for amino (NH) group, while its proton NMR spectrum indicated singlet signals at around δ 8.3 ppm for hydrazid (—C**H**=N—), and singlet signals at δ 11.76–11.16 ppm for the =N—N**H**— proton. The two amino (—NH_2) protons appeared as two singlets at 7.7 and 8.2 ppm due to enthiol tautomerism, while these protons disappeared in compounds **4a–d**, **5a–d,** and **6a–l**.

The reaction of thiosemicarbazone derivatives (**3a–d**) with ethyl bromoacetate in glacial acetic acid containing anhydrous sodium acetate afforded the corresponding 4-thiazolidinone derivatives **4a–d**. The IR spectra of the compounds (**4a–d**) showed new band at 1700 cm^{-1} attributed to a carbonyl group of 4-thiazolidinone, their proton NMR spectra showed characteristic new singlet at δ 4 ppm attributed to CH_2 thiazolidinone. Also, the compounds were confirmed on the basis of ^{13}C NMR which revealed signals

at δ 35 for C2-thiazolidinone and δ 169 ppm (C4-thiazolidinone). The formation of 4-thiazolidinone compounds (**4a–d**) was also evidenced by mass spectral data.

On the other hand reaction of thiosemicarbazone derivatives (**3a–d**) with acetic anhydride afforded the corresponding 1,3,4-thiadiazole derivatives **5a–d**. Their IR spectra showed new band at 1660–1700 cm^{-1} attributed to an acetyl group of 1,3,4-thiadiazol-2-yl, and their proton NMR spectra showed characteristic new singlet at δ 7 ppm attributed to CH of 1,3,4-thiadiazolyl in addition to signals due to methyl groups of the CH_3CO at δ 2.2–2.5 ppm. Also the structures of (**5a–d**) were conformed on the basis of ^{13}C NMR spectrum which revealed two signals at δ about 21, 22 ppm for methyl carbon, and 168, 170 for carbonyl carbon group. The mass spectra of the synthesized compounds showed M+1 peak, in agreement with their molecular formula. Cyclocondensation of thiosemicarbazone derivatives (**3a–d**) with substituted phenacyl bromide in refluxing ethanol containing catalytic amount of acetic acid resulted in the formation of (*Z*)-2-(2-((1,3-diphenyl-1-*H*-pyrazol-4-yl)methylene)hydrazinyl)-4-phenylthiazole derivatives (**6a–l**). IR spectra of (**6a–f**) exhibited a sharp band between 3310 and 3395 cm^{-1}, characteristic for NH stretching. The stretching band of the C=N appeared between 1550 and 1585 cm^{-1}, and their proton NMR spectroscopic analysis revealed diagnostic singlet signal at around δ 8.2 ppm attributed to H5-thiazole. ^{13}C NMR spectrum of compounds (**6a–l**) revealed signals at δ 104.9 (thiazole-C5) and 175.6 (thiazole-C2). Finally, formation of these compounds was also evidenced by mass spectral data.

2.2. Pharmacological screening

2.2.1. Anti-inflammatory activity

All the synthesized (**3a–d**), (**4a–d**), (**5a–d**) and (**6a–l**) compounds were tested for their ant-inflammatory activity using carrageenan-induced rat hind paw edema method of Winter et al. [30]. The protocol of animal experiments was approved by the Institutional Animal Ethics Committee (IAEC). The edema hind paw was induced by injection of 0.1 mL of 1% carrageenan solution into subplanter region of right hand paw. Diclofenac sodium was used as a reference anti-inflammatory drug and anti-inflammatory activity was calculated at hourly intervals up to 4 h after injection and presented in Table 1 as the mean paw volume (mL) as well as the percentage anti-inflammatory activity (%AI). Most of the synthesized compounds showed appreciable inhibition of the edema size in comparison with diclofenac sodium. The thiosemicarbazone derivatives **3a**, **3b**, **3c**, and **3d** showed good anti-inflammatory activity with a percent inhibition of 67.61%, 66.90%, 66.72%, and 64.6% respectively, the anti-inflammatory activity of **4a**, **4b**, **4c**, and **4d** showed least inhibition of the edema size in comparison with diclofenac sodium. The 1,3,4-thiadiazole derivatives **5a–d** showed excellent protection against inflammation. The acetamide compounds **5a** and **5b** displayed consistently excellent anti-inflammatory activity (87.25% and 85.66% inhibition, respectively) up to 4 h that was comparable to that of the standard drug diclofenac sodium, whereas *N*-phenylacetamide compounds **5c** and **5d** exhibit good systemic anti-inflammatory activity with percent inhibition of 73.45% and 74.33% respectively. Finally, the (*Z*)-2-(2-((1,3-diphenyl-1-*H*-pyrazol-4-yl)methylene)hydrazinyl)-4-phenylthiazole (**6a–l**) derivatives exhibited weak anti-inflammatory activity.

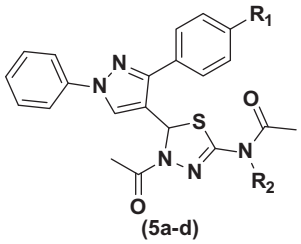
A comparison of the four series (**3a–d**), (**4a–d**), (**5a–d**) and (**6a–l**) reveals that the 1,3,4-thiadiazole derivatives (**5a–d**) exhibited excellent anti-inflammatory activity, whereas thiosemicarbazone (**3a–d**) derivatives shows moderate anti-inflammatory activity. The rest of the compounds showed weak anti-inflammatory activity. A detail study of the results showed satisfactory anti-inflammatory activity of **5a**, **5b**, **5c**, and **5d** in second phase of the biphasic carrageenan induced edema assay demonstrating the capability of the tested compounds to inhibit prostaglandin

Table 1
Anti-inflammatory activity of 1,3,4-trisubstituted pyrazole derivatives.

Compound[a]	Volume of oedema (ml)[b]			
	1 h	2 h	3 h	4 h
Control	0.516 ± 0.0021	0.545 ± 0.0022	0.565 ± 0.0022	0.565 ± 0.0022
Diclofenac	0.096 ± 0.0021*** (81.39)[c]	0.085 ± 0.0022*** (84.4)	0.075 ± 0.0022*** (86.72)	0.051 ± 0.0016*** (90.97)
3a	0.205 ± 0.003*** (60.27)	0.201 ± 0.003*** (63.11)	0.186 ± 0.002*** (67.07)	0.183 ± 0.002*** (67.61)
3b	0.220 ± 0.002*** (57.36)	0.203 ± 0.002*** (62.75)	0.20 ± 0.001*** (64.60)	0.187 ± 0.0016*** (66.9)
3c	0.205 ± 0.003*** (60.27)	0.201 ± 0.003*** (63.11)	0.186 ± 0.002*** (67.07)	0.188 ± 0.001*** (66.72)
3d	0.228 ± 0.007*** (55.81)	0.0883 ± 0.001*** (83.85)	0.208 ± 0.001*** (63.18)	0.20 ± 0.001*** (64.6)
4a	0.346 ± 0.004*** (32.94)	0.225 ± 0.003*** (58.71)	0.338 ± 0.002*** (40.17)	0.331 ± 0.003*** (41.41)
4b	0.346 ± 0.003*** (32.94)	0.341 ± 0.001*** (37.43)	0.336 ± 0.001*** (40.53)	0.321 ± 0.002*** (43.18)
4c	0.355 ± 0.003*** (31.20)	0.350 ± 0.002*** (35.77)	0.339 ± 0.002*** (40.00)	0.333 ± 0.003*** (41.06)
4d	0.366 ± 0.003*** (29.06)	0.358 ± 0.001*** (34.31)	0.348 ± 0.002*** (38.40)	0.345 ± 0.003*** (38.93)
5a	0.105 ± 0.002*** (79.65)	0.186 ± 0.002*** (65.87)	0.087 ± 0.001*** (84.60)	0.072 ± 0.002*** (87.25)
5b	0.202 ± 0.003*** (60.85)	0.186 ± 0.002*** (65.87)	0.088 ± 0.001*** (84.42)	0.081 ± 0.001*** (85.66)
5c	0.223 ± 0.0016*** (56.78)	0.203 ± 0.002*** (62.75)	0.188 ± 0.0016*** (66.72)	0.150 ± 0.002*** (73.45)
5d	0.205 ± 0.003*** (60.27)	0.201 ± 0.003*** (63.11)	0.186 ± 0.002*** (67.07)	0.145 ± 0.001*** (74.33)
6a	0.366 ± 0.002*** (29.06)	0.36 ± 0.001*** (33.94)	0.355 ± 0.002*** (37.16)	0.353 ± 0.002*** (37.52)
6b	0.371 ± 0.001*** (28.10)	0.365 ± 0.002*** (33.02)	0.358 ± 0.001*** (36.63)	0.361 ± 0.001*** (36.1)
6c	0.345 ± 0.005*** (33.13)	0.227 ± 0.004*** (58.34)	0.337 ± 0.001*** (340.35)	0.332 ± 0.004*** (41.23)
6d	0.344 ± 0.002*** (33.33)	0.351 ± 0.001*** (35.59)	0.335 ± 0.002*** (40.70)	0.323 ± 0.001*** (42.83)
6e	0.340 ± 0.002*** (34.10)	0.340 ± 0.001*** (37.61)	0.336 ± 0.001*** (40.53)	0.331 ± 0.002*** (41.41)
6f	0.375 ± 0.002*** (27.32)	0.368 ± 0.001*** (32.47)	0.346 ± 0.001*** (38.76)	0.344 ± 0.002*** (39.11)
6g	0.367 ± 0.0012*** (28.87)	0.341 ± 0.001*** (37.43)	0.335 ± 0.001*** (40.70)	0.330 ± 0.002*** (41.59)
6h	0.377 ± 0.002*** (26.99)	0.358 ± 0.001*** (34.31)	0.346 ± 0.001*** (38.76)	0.340 ± 0.001*** (39.82)
6i	0.31 ± 0.002*** (33.92)	0.351 ± 0.001*** (35.59)	0.351 ± 0.001*** (38.87)	0.341 ± 0.002*** (39.64)
6j	0.306 ± 0.0012*** (40.69)	0.300 ± 0.001*** (44.95)	0.331 ± 0.001*** (41.41)	0.331 ± 0.002*** (41.41)
6k	0.301 ± 0.002*** (41.66)	0.301 ± 0.001*** (44.77)	0.332 ± 0.001*** (41.23)	0.346 ± 0.001*** (38.76)
6l	0.311 ± 0.003*** (39.72)	0.331 ± 0.001*** (39.26)	0.330 ± 0.001*** (41.59)	0.320 ± 0.002*** (43.36)

*** Significantly different compared to respective control values, $p < 0.0001$.
[a] Dose levels: test compounds (50 mg/kg b.w), diclofenic sodium (50 mg/kg b.w).
[b] $N = 6$, values are expressed as mean ± SEM and analysed by ANOVA.
[c] Values in parentheses (percentage anti-inflammatory activity, AI%).

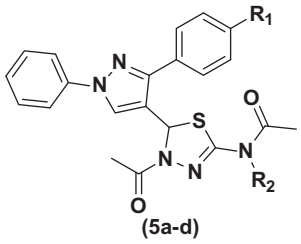
Table 2
Cyclooxygenase inhibitory activity of 1,3,4-thiadiazole derivatives (**5a–d**)



Compound	R_1	R_2	COX-1 IC_{50} (μM)[a]	COX-2 IC_{50} (μM)[a]	COX-1/COX-2 (SI)[b]
5a	OH	H	73	14	5.21
5b	NO_2	H	70	20	3.50
5c	OH	C_6H_5	64	30	2.13
5d	NO_2	C_6H_5	65	30	2.16

[a] The *in vitro* test compound concentration required to produce 50% inhibition of enzymatic activity. The results (IC_{50}) μM is the mean of duplicate, using the COX inhibitor screening assay kit (Cayman chemicals Inc., Ann arbor, MI, USA).
[b] *In vitro* COX-2 selectivity index (COX-1 IC_{50}/COX-2 IC_{50}).

Table 3
Cytotoxic activity of 1,3,4-thiadiazole derivatives (**5a–d**) against cancer cell lines[a]



IC_{50} (μM)[b]				
Compound	R_1	R_2	HeLa	MCF7
5a	OH	H	12 ± 1.5	6.5 ± 1.2
5b	NO_2	H	20 ± 1.9	38 ± 3.5
5c	OH	C_6H_5	28.6 ± 3.2	34.5 ± 3.9
5d	NO_2	C_6H_5	31 ± 4.1	96 ± 7.3
Doxorubicin	–	–	3.33 ± 0.57	2.0 ± 0.8

[a] Cell lines include cervical carcinoma (HeLa); breast adenocarcinoma (MCF-7).
[b] IC_{50} (μM) values are presented as the Mean ± S.D.

synthesis [1,31]. This can be attributed to their ability to bind cyclooxygenase (COX) enzyme, which is responsible for the synthesis of prostaglandins. Consistently exhibited anti-inflammatory activity up to 4 h proposes that these compounds (**5a–d**) do not get easily metabolized in the system. Compounds **5a**, **5b**, **5c**, and **5d** were further evaluated for their inhibitory potency against COX-1 and COX-2.

2.2.2. Cyclooxygenase inhibitory activity

In order to examine whether the anti-inflammatory effect observed in the carrageenan-induced rat paw edama assay is caused by inhibition of COX enzyme (one of the mechanisim for anti-inflammatory activity) [1] the 1,3,4-thiadiazole derivatives (**5a–d**) were evaluated for their ability to inhibit the COX-1 and COX-2 enzymes using COX Inhibitor screening assay kit (Cayman chemicals Inc) [32]. The assay (enzyme immunoassay) includes both ovine COX-1 and human recombinant COX-2 enzymes allowing screen isozyme-specific inhibitors. This methodology is excellent tool which can be used for general cyclooxygenase inhibitory activity [33–38]. The results are given in Table 2. It was observed that 1,3,4-thiadiazole derivatives showed fairly good inhibiting COX-2 activities, but weak to COX-1. Among the four

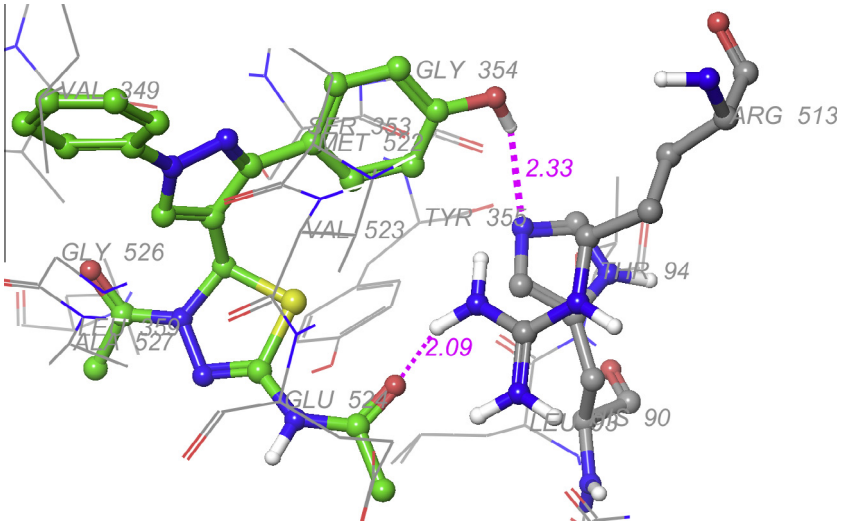


Fig. 1. Orientation of **5a** in COX-2 active pocket: the oxygen atom of acetamide group forms hydrogen bond with amino hydrogen atom of ARG513 (O···H—N, distance: 2.09 Å), and O—H of aryl group forms hydrogen bond with oxygen atom of HIS90 (O—H···N, distance: 2.33 Å).

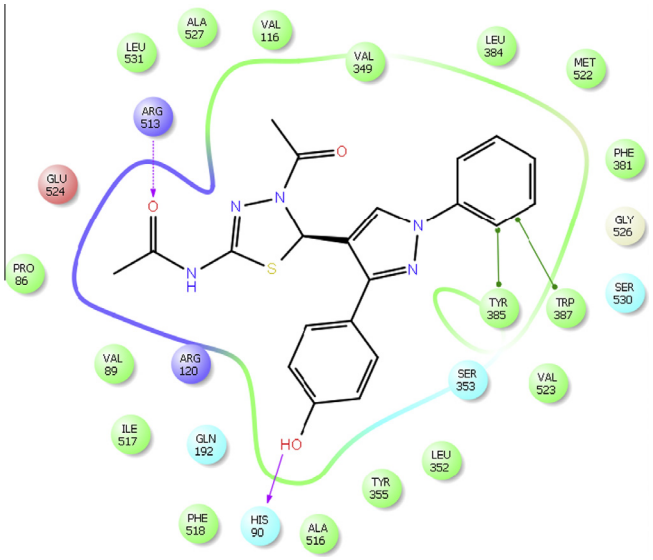


Fig. 2. 2D structure of **5a** in COX-2 active pocket: for clarity, only interacting residues are displayed. The H-bond (pink dotted line) is displayed as arrow, and π–π interactions were shown as green line.

compounds, N-(4-acetyl-5-(3-(4-hydroxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl) acetamide (**5a**) showed good inhibitory activity (IC_{50} = 14 μM) and selectivity (SI = 5.21), whereas the compound (**5b**) exhibit moderate activity (IC_{50} = 20 μM) and selectivity (SI = 3.5).

2.2.3. In vitro cytotoxicity assays

In order to further confirm the cytotoxic activity of the most active anti-inflammatory compounds, we carried out MTT [3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyl-tetrazolium bromide] cell proliferation assay [39]. The 1,3,4-thiadiazole derivatives (**5a–d)** were tested for their cytotoxic activity against HeLa (cervical carcinoma), MCF-7 (breast adenocarcinoma) cell lines. The inhibition of the cell proliferation was determined 24 h after the cells were exposed to the tested compounds. The IC_{50} (the concentration that causes 50% growth inhibition) values were determined and summarized in Table 3. It was observed that compounds **5a, 5b, 5c,** and **5d** showed fairly good inhibitory activity against HeLa with IC_{50} values from 12 to 31 μM, but moderate to MCF-7 cell lines. The N-(4-acetyl-5-(3-(4-hydroxy phenyl)-1-phenyl-1*H*-pyrazol-4-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)acetamide (**5a**) exhibited excellent inhibitory activity against HeLa, and MCF-7 cell lines, with the inhibitory concentration (IC_{50}) values of 12 and 6.5 μM, respectively.

2.2.4. Molecular docking study

To gain better understanding on the strength of the 1,3,4-thiadiazole derivatives (**5a–d)** and guide further structural–activity relationships studies. The molecular docking was performed by putting inhibitor (**5a–d)** into binding site of COX-2 (PDB ID:1CX2). All docking runs were applied by maestro (Schrödinger) [40]. The binding models of compound **5a** with cyclooxygenase-2 were showed in Fig. 1. The amino acid residues which had interaction with **5a** in the active site were characterized. We can see the compound **5a** was satisfactorily bound to the cyclooxygenase-2 via two hydrogen bonds and π–π interaction. It was shown that the oxygen atom of acetamide group forms hydrogen bond with amino hydrogen atom of ARG513 (O···H—N, distance: 2.09 Å), and O—H of aryl group forms hydrogen bond with Oxygen atom of HIS90 (O—H···N, distance: 2.33 Å). In addition π–π interactions were formed by TYR385 and TRP387 with the phenyl ring which enhanced the binding action between cyclooxygenase receptor and inhibitor **5a** as shown in Fig. 2.

3. Conclusion

In our present work, four series of 1,3,4-trisubstituted pyrazole derivatives (**3a–d**), (**4a–d**), (**5a–d**) and (**6a–l**) were synthesized and the structure of newly synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, mass spectral analysis. These compounds were screened for anti-inflammatory activity by carrageenan-induced paw edema method. The cyclooxygenase (COX) enzyme inhibitor activity of selected compounds were evaluated using COX Inhibitor screening assay kit. The assay (enzyme immunoassay) includes both COX-1 and COX-2 enzymes allowing screen isozyme-specific inhibitors. This assay is excellent tool which can be used for general COX-1 and COX-2 enzymes inhibitory screening. Compounds **5a**, and **5b** showed excellent anti-inflammatory activity (⩾84.2% inhibition) and **3a**, **3b**, and **3c** showed good anti-inflammatory activity (⩾64.6% inhibition) when measured 3 h after the carrageenan injection compared to that of the standard drug diclofenac (86.72%). Compound **5a** showed potent anti-inflammatory activity and COX-2 selective inhibition. Molecular docking study further helped in supporting the observed activity. In addition Compound **5a** exhibited considerable cytotoxic activity against MCF-7 cell line with IC_{50} value 6.5 μM. When the results of the docking studies and inhibitory activities are considered together, N-(4-acetyl-5-(3-(4-substituted phenyl)-1-phenyl-1*H*-pyrazol-4-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl) acetamides (**5a–d**) are appropriate scaffolds for the development of new anti-inflammatory agents.

4. Experimental protocols

4.1. Chemistry

All the chemicals used in this study were purchased from Aldrich, Himedia and SD fine chemicals. Melting points were determined in open capillary tubes and are uncorrected. The progress of the reactions was monitored by TLC using Merck precoated silica gel plates (F 254). The IR spectra were recorded on Shimadzu FTIR spectrophotometer. ^{1}H NMR spectra were recorded on AMX-400, Bruker-400 liquid-state NMR spectrometer using tetramethylsilane (TMS) as the internal standard. Chemical shifts were recorded as δ (ppm). Mass spectra were measured on an LCMS-2010A. Spectra facilities were carried out by Sophisticated Analytical Instruments Facility (SAIF) division of Indian Institute of Science, Bangalore, India.

*4.1.1. General procedure for synthesis of 1-phenyl-2(1-phenylehylidene)hydrazine (**1a–b**)*

4-substituted acetophenone (24 mmol) and phenyl hydrazine (24 mmol) were refluxed in presence of glacial acetic acid (1 mL) using ethanol (90 ml) as solvent. Reaction mixture was cooled to room temperature and poured on crushed ice. The solid obtained was filtered, dried and recrystallized from ethanol.

*4.1.2. General procedure for synthesis of 1,3-diphenyl-1H-pyrazole-4-carbaldehyde (**2a–b**)*

1-Phenyl-2(1-phenylehylidene)hydrazine (1a–b) (12 mmol) was added to a cold solution of dimethylformamide (40.0 mmol) then Phosphorylchloride (40.0 mmol) was added and the resulting mixture was stirred at 60 °C for 6 h. The mixture was poured into ice-cold water. A saturated solution of potassium carbonate was

added to neutralize the mixture; the solid precipitated was filter; washed with water; dried and recrystallized from ethanol.

*4.1.2.1. 3-(4-Hydroxyphenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde (**2a**).* Colorless solid powder; Yield: 70%; m.p.: 128–130 °C; IR (KBr, cm^{-1}): 2779 (CH), 1670 (C=O), 1631(C=C), 1569 (C=N). ^1H NMR (400 MHz, δ, ppm, DMSO-d_6): 7.44 (t, 1H, J = 7.2 Hz, Ar—H), 7.58 (t, 2H, J = 7.6 Hz, Ar—H), 7.71 (d, 2H, J = 8.4 Hz, Ar—H), 7.93 (d, 2H, J = 8.4 Hz, Ar—H), 7.99 (d, 2H, J = 8 Hz, Ar—H), 9.35 (s, 1H, pyrazole), 9.72 (s, 1H, CHO), 9.98 (s, 1H, OH).

*4.1.2.2. 3-(4-Nitrophenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde (**2b**).* Light yellowish powder; Yield: 72%, m.p.:165–167 °C; IR (KBr, cm^{-1}): 1734 (C=O), 1567 (C=N). ^1H NMR (400 MHz, δ, ppm, DMSO-d_6): 7.46 (t, 1H, J = 7.2 Hz, Ar—H), 7.64 (t, 2H, J = 7.6 Hz, Ar—H), 7.89 (d, 2H, J = 8.4 Hz, Ar—H), 7.91 (d, 2H, J = 8.6 Hz, Ar—H) 7.94 (d, 2H, J = 8.4 Hz, Ar—H) 9.13 (s, 1H, pyrazole), 9.75 (s, 1H, CHO).

*4.1.3. General procedure for the synthesis of the compounds (**3a–d**)*

To a solution of 3-(4-substituted phenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde (**2a–b**) (10 mmol) in 30 ml of ethanol: chloroform (7:3), thiosemicarbazide or 4-phenylthiosemicarbazide (10 mmol) was added with stirring and the resulting reaction mixture was stirred for 10 h at 80 °C with catalytic amount of concentrated HCl. The reaction was monitored by thin-layer chromatography (TLC) for completion. Reaction mixture was cooled to room temperature the solid obtained was filtered, washed with cold ethanol, and recrystallized from ethanol.

*4.1.3.1. (Z)-2-((3-(4-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl) methylene) hydrazine carbothioamide (**3a**).* Colorless solid powder; Yield: 68%; m.p.: 231–233 °C; IR (KBr, cm^{-1}): 3410 (OH), 3349 (NH), 3018 (Ar—H), 1560 (C=N), 1377 (C=S); ^1H NMR (400 MHz, δ, ppm, DMSO-d_6): 7.22 (t, 1H, J = 7.2 Hz, Ar—H), 7.61–7.39 (m, 4H, Ar—H), 7.71 (s, 1H, NH_2), 7.75 (d, 2H, J = 8.4 Hz, Ar—H), 7.91 (d, 2H, J = 8 Hz, Ar—H), 8.24 (s, 1H, NH_2), 8.32 (s, 1H, CH=N—), 9.25 (s, 1H, pyrazole), 9.80 (s, 1H, OH), 11.76 (s, 1H, NH).

*4.1.3.2. (Z)-2-((3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)hydrazine carbothioamide (**3b**).* Colorless solid powder; 77%; m.p.: 266–268 °C; IR (KBr) cm^{-1}: 3397 (NH), 3043 (Ar—CH), 1574 (C=N), 1375 (C=S); ^1H NMR (400 MHz, δ, ppm, DMSO-d_6): 7.39 (t, 1H, J = 7.2 Hz, Ar—H), 7.65–7.57 (m, 4H, Ar—H), 7.71 (d, 2H, J = 8.4 Hz, Ar—H), 7.73 (s, 1H, NH_2), 7.90 (d, 2H, J = 7.6 Hz, Ar—H), 8.26 (s, 1H, NH_2), 8.31 (s, 1H, CH=N—), 9.19 (s, 1H, pyrazole), 11.33 (s, 1H, NH).

*4.1.3.3. (Z)-2-((3-(4-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)-N-phenyl hydrazinecarbothioamide (**3c**).* Colorless solid powder; Yield: 67%; m.p.: 243–245 °C; IR (KBr, cm^{-1}): 3295 (NH), 3040 (Ar—CH), 1564 (C=N), 1350 (C=S); ^1H NMR (400 MHz, δ, ppm, DMSO-d_6): 7.37 (t, 1H, J = 7.2 Hz, Ar—H), 7.65–7.51 (m, 9H, Ar—H), 7.67 (d, 2H, J = 8.4 Hz, Ar—H), 7.72 (d, 2H, J = 8.4 Hz, Ar—H), 7.94 (s, 1H, NH), 8.31 (s, 1H, CH=N-), 9.15 (s, 1H, pyrazole), 9.98 (s, 1H, OH), 11.17 (s, 1H, NH). 19.

*4.1.3.4. (Z)-2-((3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)-N-phenyl hydrazinecarbothioamide (**3d**).* Colorless solid powder; Yield: 61%; m.p.: 281–282 °C; IR (KBr, cm^{-1}): 3302 (NH), 3022 (Ar—CH), 1577 (C=N), 1323 (C=S); ^1H NMR (400 MHz, δ, ppm, DMSO-d_6): 7.38 (t, 1H, J = 7.2 Hz, Ar—H), 7.60–7.48 (m, 9H, Ar—H) 7.69 (d, 2H, J = 8.4 Hz, Ar—H), 7.75 (d, 2H, J = 8.4 Hz, Ar—H), 7.92 (s, 1H, NH), 8.31 (s, 1H, CH=N-), 9.12 (s, 1H, pyrazole), 11.75 (s, 1H, NH).

*4.1.4. General procedure for the synthesis of the compounds (**4a–d**)*

A mixture of (**3a–b**) 1-((3-(substituted)-1-phenyl-4,5-dihydro-1*H*-pyrazol-4-yl) methylene) thiosemicarbazide or (**3c–d**) 1-((3-(substituted)-1-phenyl-4,5-dihydro-1*H*-pyrazol-4-yl)methylene)4-phenylthiosemicarbazide (10 mmol), ethylbromoacetate (10 mmol) and anhydrous sodium acetate (10 mmol) in glacialacetic acid (15 mL) was reflux for 8 h. The reaction was monitored by thin-layer chromatography (TLC) for completion. Reaction mixture was cooled to room temperature and poured into ice/water. The resulting precipitate was filtered; washed with water; dried and recrystallized from ethanol.

*4.1.4.1. (Z)-2-(2-((3-(4-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-methylene) hydrazinyl)thiazol-4(5H)-one (**4a**).* Yellow color solid; Yield: 63%; m.p.: 263–265 °C; IR (KBr, cm^{-1}): 3350 (OH), 3265 (NH), 1678 (C=O), 1641 (C=C), 1563 (C=N); ^1H NMR (400 MHz, δ, ppm, DMSO-d_6): 3.90 (s, 2H, thiazolidinone), 7.39 (t, 1H, J = 7.6 Hz, Ar—H) 7.57–7.54 (m, 4H, Ar—H), 7.98–7.95 (m, 4H, Ar—H), 8.44 (s, 1H, CH=N-), 8.98 (s, 1H, pyrazole), 9.97 (s, 1H, OH), 11.88 (s, 1H, NH); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 34.5, 112.3, 118.4, 120.6, 126.1, 126.91, 129.3, 130.4, 139.2, 142.8, 149.7, 155.2, 168.5; MS (ESI) m/z = 378 (M+1).

*4.1.4.2. (Z)-2-(2-((3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene) hydrazinyl) thiazol-4(5H)-one (**4b**).* Yellow color solid; Yield: 71%; m.p.: 266–268 °C; IR (KBr, cm^{-1}): 3329 (N—H), 1708 (C=O), 1639 (C=C), 1575 (C=N); ^1H NMR (400 MHz, δ, ppm, DMSO-d_6): 3.91 (s, 2H, thiazolidinone), 7.36 (t, 1H, J = 7.6 Hz, Ar—H) 7.46–7.37 (m, 4H, Ar—H), 7.71 (d, 2H, J = 8.4 Hz, Ar—H), 7.93 (d, 2H, J = 8.0 Hz, Ar—H), 8.44 (s, 1H, CH=N—), 8.87 (s, 1H, pyrazole), 11.74 (s, 1H, NH); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 36.1, 116.7, 118.8, 120.3, 121.9, 125.4, 127.1, 129.6, 138.0, 139.5, 140.3, 142.8, 149.1, 151.4, 156.8, 169.6; MS (ESI) m/z = 407 (M+1).

*4.1.4.3. (Z)-2-((Z)-((3-(4-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-methylene) hydrazono)-3-phenylthiazolidin-4-one (**4c**).* Yellow color solid; Yield: 51%; m.p.: 273–275 °C; IR (KBr, cm^{-1}): 3025 (Ar—CH), 1714 (C=O), 1631 (C=C), 1569 (C=N); ^1H NMR (400 MHz, δ, ppm, DMSO-d_6): 4.12 (s, 2H, thiazolidinone), 7.21–6.98 (m, 5H, Ar—H), 7.51–7.31 (m, 4H, Ar—H), 7.81–7.63 (m, 3H, Ar—H), 7.94–7.90 (m, 2H, Ar—H), 8.31 (s, 1H, CH=N-), 8.91 (s, 1H, pyrazole), 9.97 (s, 1H, OH); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 37.2, 116.6, 120.6, 121.9, 124.3, 125.4, 127.8, 128.2, 130.2 137.2, 139.8, 140.1, 146.7, 148.6, 151.1, 157.9, 170.2; MS (ESI) m/z = 454 (M+1).

*4.1.4.4. (Z)-2-((Z)-((3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)-methylene) hydrazono)-3-phenylthiazolidin-4-one (**4d**).* Yellow color powder; Yield: 60%; m.p.: 265–266 °C; IR (KBr, cm^{-1}): 3020 (Ar—CH), 1697 (C=O), 1631 (C=C), 1559 (C=N); ^1H NMR (400 MHz, δ, ppm, DMSO-d_6): 4.08 (s, 2H, thiazolidinone), 7.61–7.18 (m, 10H, Ar—H), 7.76 (d, 2H, J = 8.4 Hz, Ar—H), 7.94 (d, 2H, Ar—H, J = 7.6 Hz, Ar—H), 8.31 (s, 1H, CH=N-), 8.92 (s, 1H, pyrazole); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 35.6, 113.5, 120.4, 124.1, 126.7, 129.4, 130.1, 137.9, 139.2, 141.3, 147.6, 149.7, 151.5, 159.3, 169.7; MS (ESI) m/z = 483 (M+1).

*4.1.5. General procedure for the synthesis of the compounds (**5a–d**)*

A solution of (**3a–b**) 1-((3-(substituted)-1-phenyl-4,5-dihydro-1*H*-pyrazol-4-yl)methylene) thiosemicarbazide or (**3c–d**) 1-((3-(substituted)-1-phenyl-4,5-dihydro-1*H*-pyrazol-4-yl) methylene) 4-phenyl thiosemicarbazide (10 mmol) in acetic anhydride (20 mL) was heated under reflux for 5 h. The reaction was monitored by thin-layer chromatography (TLC) for completion. After the reaction mixture was attained at room temperature, excess acetic anhydride was decomposed by water and the reaction mixture was stirred for 30 min. The separated product was collected by

filtration, washed with water, dried, and recrystallized from ethanol.

*4.1.5.1. N-(4-acetyl-5-(3-(4-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)acetamide (**5a**).* Light yellow color solid; Yield: 49%; m.p.: 223–224 °C; IR (KBr, cm^{-1}): 3430 (OH), 3325 (NH), 1707 (C=O), 1600 (C=C), 1556 (C=N); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 1.84 (s, 3H, —C(=O)—CH_3), 1.86 (s, 3H, —C(=O)—CH_3), 7.09 (s, 1H, thiadiazole), 7.35 (t, 1H, J = 7.6 Hz Ar—H), 7.57–7.47 (m, 4H, Ar—H), 7.73–7.68 (m, 2H, Ar—H), 7.89 (d, 2H, J = 8 Hz Ar—H), 8.37 (s, 1H, pyrazole), 9.98 (s, 1H, OH), 12.59 (s, 1H, NH); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 21.7, 22.2, 79.3, 122.1, 124.6, 125.8, 128.3, 128.9, 129.6, 130.8, 131.3, 132.9, 133.8, 147.8, 166.5, 167.3; MS (ESI) m/z = 442 (M+1).

*4.1.5.2. N-(4-acetyl-5-(3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)acetamide (**5b**).* Light yellow color solid; Yield: 60%; m.p.: 272–274 °C; IR (KBr, cm^{-1}): 3340 (NH), 1701 (C=O), 1598 (C=C), 1574 (C=N); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 1.83 (s, 3H, -C(=O)-CH_3), 1.86 (s, 3H, —C(=O)—CH_3), 7.17 (s, 1H, thiadiazole), 7.43 (t, 1H, J = 7.6 Hz Ar—H), 7.56–7.45 (m, 4H, Ar—H), 7.68 (d, 2H, J = 8.8 Hz, Ar—H), 7.83 (d, 2H, J = 7.6 Hz, Ar—H), 9.21 (s, 1H, pyrazole), 12.45 (s, 1H, NH). ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 22.3, 23.1, 82.4, 122.8, 123.8, 125.5, 128.2, 128.7, 129.6, 130.6, 131.5, 132.9, 134.3, 147.7, 167.4, 168.1; MS (ESI) m/z = 451 (M+1).

*4.1.5.3. N-(4-acetyl-5-(3-(4-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)-N-phenylacetamide (**5c**).* Light yellow color solid; Yield: 61%; m.p.: 192–193 °C; IR (KBr, cm^{-1}): 3247 (OH), 1697 (C=O), 1614 (C=C), 1568 (C=N); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.01 (s, 3H, —C(=O)—CH_3), 2.04 (s, 3H, —C(=O)—CH_3), 7.08 (s, 1H, thiadiazole), 7.12 (d, 2H, J = 8.8 Hz, Ar—H), 7.15 (t, 1H, J = 7.6 Hz, Ar—H), 7.36 (t, 1H, J = 7.6 Hz, Ar—H), 7.65–7.41 (m, 8H, Ar—H), 7.67 (d, 2H, J = 8.4 Hz, Ar—H), 9.17 (s, 1H, pyrazole), 9.98 (s, 1H, OH); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 22.4, 23.6, 76.9, 119.7, 120.2, 122.5, 123.2, 125.6, 126.8, 127.2, 128.2, 129.1, 129.8, 130.1, 133.3, 135.2, 147.1, 150.2, 168.2, 169.8; MS (ESI) m/z = 498 (M+1).

*4.1.5.4. N-(4-acetyl-5-(3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)-N-phenylacetamide (**5d**).* Light yellow color solid; Yield: 64%; m.p.: 205–206 °C; IR (KBr, cm^{-1}): 1691 (C=O), 1600 (C=C), 1584 (C=N); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 1.96 (s, 3H, —C(=O)—CH_3), 1.98 (s, 3H, —C(=O)—CH_3), 7.02 (s, 1H, thiadiazole), 7.15 (d, 2H, J = 8.8 Hz, Ar—H), 7.25 (t, 1H, J = 7.6 Hz, Ar—H), 7.69–7.35 (m, 4H, Ar—H), 7.82–7.60 (m, 3H, Ar—H), 7.84 (d, 2H, J = 8.4 Hz, Ar—H), 7.90 (d, 2H, J = 7.6 Hz, Ar—H), 9.23 (s, 1H, pyrazole); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 22.6, 23.4, 75.1, 119.5, 120.8, 122.7, 124.3, 126.4, 127.6, 129.7, 10.2, 130.9, 135.6, 138.7, 148.1, 149.7, 150.6, 169.2, 170.3; MS (ESI) m/z = 527 (M+1).

*4.1.6. General procedure for the synthesis of the compounds (**6a**–**l**)*

Equimolar amount of (**3a–b**) 1-((3-(substituted)-1-phenyl-4,5-dihydro-1*H*-pyrazol-4-yl) methylene)thiosemicarbazide (5 mmole) or (**3c–d**) 1-((3-(substituted)-1-phenyl-4,5-dihydro-1*H*-pyrazol-4-yl)methylene)4-phenylthiosemicarbazide (5 mmole), substituted phenacyl bromide (5 mmol) were dissolved in 20 mL of in absolute ethanol and stirred 24 h at 80 °C with catalytic amount of acetic acid (200 μL). The reaction was monitored by thin-layer chromatography (TLC) for completion. Reaction mixture was cooled to room temperature; the solid precipitated was filtered; washed with water; dried, and recrystallized from ethanol.

*4.1.6.1. (Z)-4-(4-((2-(4-(4-chlorophenyl)thiazol-2-yl)hydrazono)methyl)-1-phenyl-1H-pyrazol-3-yl)phenol (**6a**).* Brown color solid; Yield: 70%; m.p.: 182–183 °C; IR (KBr, cm^{-1}): 3420 (OH), 3334 (NH), 1566 (C=N); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 7.22 (t, 1H, J = 7.2 Hz, Ar—H), 7.39 (t, 3H, J = 7.6 Hz, Ar—H), 7.59–7.55 (m, 5H, Ar—H and 1H, thiazole), 7.68 (d, 2H, J = 8.4 Hz, Ar—H), 7.74 (d, 2H, J = 8.4 Hz, Ar—H), 8.31 (s, 1H, CH=N—), 9.24 (s, 1H, pyrazole), 9.79 (s, 1H, OH), 11.75 (s, 1H, NH); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 117.3, 117.9, 118.5, 120.3, 121.5, 125.7, 128.1, 128.7, 129.3, 129.8, 133.4, 134.2, 150.0, 177.5; MS (ESI) m/z = 473 (M+1), 474 (M+2).

*4.1.6.2. (Z)-4-(4-((2-(4-(4-bromophenyl)thiazol-2-yl)hydrazono)methyl)-1-phenyl-1H-pyrazol-3-yl)phenol (**6b**).* Yellow color solid; Yield: 57%; m.p.: 266–268 °C; IR (KBr, cm^{-1}): 3425 (OH), 3278 (NH), 1555 (C=N); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 7.76–7.38 (m, 9H, Ar—H), 7.81 (d, 2H, J = 7.6 Hz, Ar—H), 7.90 (d, 2H, J = 8.4 Hz, Ar—H), 8.19 (s, 1H, thiazole), 8.23 (s, 1H, CH=N—), 9.19 (s, 1H, pyrazole), 9.97 (s, 1H, OH), 11.33 (s, 1H, NH); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 116.9, 118.5, 118.7, 120.4, 121.8, 126.9, 127.4, 129.5, 130, 130.5, 131.5, 131.6, 138.9, 149.4, 176.2; MS (ESI) m/z = 518 (M+2).

*4.1.6.3. (Z)-4-(4-((2-(4-(4-nitrophenyl)thiazol-2-yl)hydrazono)methyl)-1-phenyl-1H-pyrazol-3-yl)phenol (**6c**).* Light orange color solid; Yield: 67%; m.p.: >300 °C; IR (KBr, cm^{-1}): 3420 (OH), 3327 (NH), 1585 (C=N); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 7.20 (t, 1H, J = 7.2 Hz, Ar—H), 7.36 (t, 4H, J = 7.6 Hz, Ar—H), 7.72–7.41 (m, 4H, Ar—H), 7.99 (d, 2H, J = 7.6 Hz, Ar—H), 8.11 (d, 2H, J = 8.8 Hz, Ar—H), 8.20 (s, 1H, thiazole), 8.22 (s, 1H, CH=N—), 9.18 (s, 1H, pyrazole), 9.98 (s, 1H, OH), 11.29 (s, 1H, NH). ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 116, 118.8, 125.2, 127.2, 128.1, 128.3, 129.3, 129.8, 130.7, 133.3, 135.4, 138.1, 138.8, 150.4, 175.2; MS (ESI) m/z = 482 (M+1).

*4.1.6.4. (Z)-4-(4-chlorophenyl)-2-(2-((3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene) hydrazinyl)thiazole (**6d**).* Yellow color solid; Yield: 62%; m.p.: 245–247 °C; IR (KBr, cm^{-1}): 3365 (NH), 1574 (C=N); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 7.69–7.38 (m, 9H, Ar—H), 7.71 (d, 2H, J = 8.4 Hz, Ar—H), 7.73 (d, 2H, J = 7.6 Hz, Ar—H), 8.20 (s, 1H, thiazole), 8.27 (s, 1H, CH=N—), 9.19 (s, 1H, pyrazole), 11.33 (s, 1H, NH); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 116.3, 117.3, 118.5, 125.3, 127, 127.9, 128.6, 129.3, 129.8, 130.9, 133.3, 134.6, 138.9, 150, 175.4; MS (ESI) m/z = 502 (M+1).

*4.1.6.5. (Z)-4-(4-bromophenyl)-2-(2-((3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene) hydrazinyl)thiazole (**6e**).* Light yellow color solid; Yield: 50%; m.p.: 265–267 °C; IR (KBr, cm^{-1}): 3340 (NH), 3029 (Ar—H), 1572 (C=N); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 7.81–7.39 (m, 9H, Ar—H), 7.90 (d, 2H, J = 7.6 Hz, Ar—H), 7.92 (d, 2H, J = 7.6 Hz, Ar—H), 8.20 (s, 1H, thiazole), 8.32 (s, 1H, CH=N—), 9.19 (s, 1H, pyrazole), 12.74 (s, 1H, NH); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 116.7, 118.6, 125.3, 127.4, 128.3, 129.6, 130, 130.8, 131.7, 132.8, 133.5, 134.6, 138.9, 150, 175.4; MS (ESI) m/z = 547 (M+2).

*4.1.6.6. (Z)-4-(4-nitrophenyl)-2-(2-((3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene) hydrazinyl)thiazole (**6f**).* Reddish color solid; Yield: 60%; m.p.: >300 °C; IR (KBr, cm^{-1}): 3327 (NH), 1564 (C=N); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 7.86–7.38 (m, 9H, Ar—H), 7.91 (d, 2H, J = 8.0 Hz, Ar—H), 7.95 (d, 2H, J = 8.4 Hz, Ar—H), 8.21 (s, 1H, thiazole), 8.31 (s, 1H, CH=N—), 9.20 (s, 1H, pyrazole), 12.11 (s, 1H, NH); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 116.4, 117.3, 118.7, 121.8, 124.3, 125.6, 126.7, 127.9, 128.4, 129.6, 130.3, 132.1, 132.8, 145.1, 149.2, 173.8; MS (ESI) m/z = 512 (M+1).

*4.1.6.7. 4-(4-((Z)-((Z)-4-(4-chlorophenyl)-3-phenylthiazol-2(3H)-ylidene) hydrazono)methyl-1-phenyl-1H-pyrazol-3-yl)phenol (**6g**).* Light Brown color solid; Yield: 57%; m.p.: 245–247 °C; IR (KBr, cm^{-1}): 3429 (OH), 1629 (C=C), 1575 (C=N), 1350 (C—S), 758 (C—Cl). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 7.87–7.22 (m, 14H, Ar—H), 7.90 (d, 2H, J = 8.4 Hz, Ar—H), 7.93 (d, 2H, J = 8.0 Hz, Ar—H), 8.19 (s, 1H, thiazole), 8.27 (s, 1H, CH=N—), 9.18 (s, 1H, pyrazole), 9.97 (s, 1H, OH); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 116.9, 118.7, 122.5, 127.8, 128.5, 129.3, 129.9, 130.1, 130.7, 131.6, 132.8, 139.1, 149.2, 173.8; MS (ESI) m/z = 549 (M+1).

*4.1.6.8. 4-(4-((Z)-((Z)-4-(4-bromophenyl)-3-phenylthiazol-2(3H)-ylidene) hydrazono)methyl-1-phenyl-1H-pyrazol-3-yl)phenol (**6h**).* Orange–yellow color solid; Yield: 50%; m.p.: 232–234 °C; IR (KBr, cm^{-1}): 3360 (OH), 1580 (C=N); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 7.87–7.13 (m, 14H, Ar—H), 7.91 (d, 2H, J = 8.0 Hz, Ar—H), 7.94 (d, 2H, J = 8.4 Hz, Ar—H), 8.19 (s, 1H, thiazole), 8.25 (s, 1H, CH=N—), 9.17 (s, 1H, pyrazole), 9.96 (s, 1H, OH); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 116.7, 118.3, 123.9, 125.6, 128.3, 129.1, 129.7, 130.8, 131.4, 132.7, 133.4, 140.1, 149.9, 174.7; MS (ESI) m/z = 594 (M+2),

*4.1.6.9. 4-(4-((Z)-((Z)-4-(4-nitrophenyl)-3-phenylthiazol-2(3H)-ylidene) hydrazono)methyl-1-phenyl-1H-pyrazol-3-yl)phenol (**6i**).* Reddish-brown color solid; Yield: 52%; m.p.: 279–281 °C; IR (KBr, cm^{-1}): 3427 (OH), 1568 (C=N); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 7.91–7.34 (m, 14H, Ar—H), 7.94 (d, 2H, J = 8.0 Hz, Ar—H), 7.96 (d, 2H, J = 8.4 Hz, Ar—H), 8.16 (s, 1H, thiazole), 8.29 (s, 1H, CH=N—), 9.12 (s, 1H, pyrazole), 9.98 (s, 1H, OH); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 116.7, 117.8, 118.4, 124.8, 125.1, 126.2, 128.3, 129.2, 129.8, 130.5, 131.9, 132.8, 134.2, 141.6, 150.2, 173.4; MS (ESI) m/z = 559 (M+1).

*4.1.6.10. (Z)-4-(4-nitrophenyl)-2-((Z)-((3-(4-phenyl)-1-phenyl-1H-pyrazol-4-yl)methylene) hydrazono)-3-phenyl-2,3-dihydrothiazole (**6j**).* Orange–yellow color solid; Yield: 40%; m.p.: 249–251 °C; IR (KBr, cm^{-1}): 3016 (Ar—H), 1574 (C=N); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 7.92–7.22 (m, 14H, Ar—H), 7.95 (d, 2H, J = 8.4 Hz, Ar—H), 7.97 (d, 2H, J = 7.6 Hz, Ar—H), 8.21 (s, 1H, thiazole), 9.24 (s, 1H, CH=N—), 9.17 (s, 1H, pyrazole); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 116.9, 118.7, 122, 125.2, 127.2, 128.2, 128.9, 129.6, 120.1, 131.7, 132.8, 135.4, 138.7, 140.1, 149.8, 175.3; MS (ESI) m/z = 578 (M+1).

*4.1.6.11. (Z)-4-(4-bromophenyl)-2-((Z)-((3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl) methylene) hydrazono)-3-phenyl-2,3-dihydrothiazole (**6k**).* Yellow color solid; Yield: 51%; m.p.: 243–245 °C; IR (KBr, cm^{-1}): 3016 (Ar—H), 1556 (C=N); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 7.87–7.23 (m, 14H, Ar—H), 7.93 (d, 2H, J = 8 Hz, Ar—H), 7.94 (d, 2H, J = 8.4 Hz, Ar—H), 8.22 (s, 1H, thiazole), 9.25 (s, 1H, CH=N—), 9.31 (s, 1H, pyrazole); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 116.2, 117, 125.3, 127.2, 128.2, 128.7, 129.1, 130.7, 131.5, 133.4, 135.5, 138.7, 139.3, 149.6, 175.4; MS (ESI) m/z = 623 (M+2).

*4.1.6.12. (Z)-4-(4-nitrophenyl)-2-((Z)-((3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene) hydrazono)-3-phenyl-2,3-dihydrothiazole (**6l**).* Reddish-brown color solid; Yield: 55%; m.p.: 254–256 °C; IR (KBr, cm^{-1}): 3016 (Ar—H), 1574 (C=N); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 7.91–7.34 (d, 14H, Ar—H), 7.93 (d, 2H, J = 8.4 Hz, Ar—H), 7.95 (d, 2H, J = 7.6 Hz, Ar—H), 8.21 (s, 1H, thiazole), 9.28 (s, 1H, CH=N—), 9.27 (s, 1H, pyrazole); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 116.8, 117.3, 124.5, 125.3, 128.2, 129.7, 130.2, 131.7 133.4, 135.4, 137.5, 139.3, 150.4, 175.3; MS (ESI) m/z = 588 (M+1).

4.2. Anti-inflammatory activity screening

All the synthesized compounds were tested for their anti-inflammatory activity using carrageenan-induced rat hind paw edema method of Winter et al. [30] the edema hind paw was induced by injection of 0.1 mL of 1% carrageenan solution into sub-planter region of right hand paw. The volume of paw was measured plethysmographically immediately and 1 h, 2 h, 3 h and 4 h after the injection of irritant. The difference in volume gave the amount of edema developed. Percent inhibition of the edema between the control group and the compound treated group was calculated and compared with the group receiving standard (diclofenac sodium) drug at 50 mg/kg b.w.

4.2.1. Acute toxicity study

The oral acute toxicity study was done and safe dose was calculated as per OECD test guideline 425 [41].

4.2.2. Cyclooxygenase inhibitory activity

The 1,3,4-thiadiazole derivatives (**5a–d**) were evaluated for their ability to inhibit the COX-1 and COX-2 enzymes using COX Inhibitor screening assay kit (Cayman chemicals Inc.) [32], and NS-398 was used as reference compound.

4.3. Cytotoxic activity evaluation

In vitro cytotoxicity was determined using a standard MTT assay with protocol appropriate for the individual test system. Test compounds were prepared prior to the experiment by dissolving in 0.1% DMSO and diluted with medium. The cells were then exposed to different concentrations of the drugs. Cells in the control wells received the same volume of medium containing 0.1% DMSO. After 24 h, the medium was removed and cell cultures were incubated with 100 μM MTT reagent (1 mg/mL) for 5 h at 37 °C. The suspension was placed on microvibrator for 10 min and absorbance was recorded by the ELISA reader.

4.4. Molecular docking study

The molecular docking study was performed by of maestro [35], running on A8 VISION AMD A8-4500M APU with Radeon(tm) HD Graphics 1.90 GHz, RAM Memory 4 GB under Windows 8 pro system. The crystal structure of the COX-2 complexed with a selective inhibitor SC-558 was collected from PDB under code PDB ID: 1CX2.

Acknowledgments

Authors are grateful to Dr. A.D. Taranalli, Principal and Dean Faculty of pharmacy, for providing necessary facilities for the research. Abounded thanks are due to Dr. Raghu for providing the Schrodinger software and also for the encouragement and motivation to complete the computational studies. Authors are also grateful to NMR Research center, IISC, Bangalore, India, for providing the spectral data.

References

[1] N. Chandra, S. Kumar, P. Kaushik, D. Kaushik, S.K. Roy, G.K. Gupta, S.M. Jachak, J.K. Kapoor, P.K. Sharma, Bioorg. Med. Chem. 21 (2013) 4581–4590.
[2] S. Bondock, W. Fadaly, M.A. Metwally, Eur. J. Med. Chem. 45 (2010) 3692–3701.
[3] S.G. Alegaon, K.R. Alagawadi, D.H. Dadwe, Drug Res (Arzneimittelforschung), doi: 10.1055/s-0033-1363976.
[4] G.M. Nitulescu, C. Draghici, A.V. Missir, Eur. J. Med. Chem. 45 (2010) 4914–4919.
[5] M.S. Christodoulou, S. Liekens, K.M. Kasiotis, S.A. Haroutounian, Bioorg. Med. Chem. 18 (2010) 4338–4350.

[6] S.R. Shih, T.Y. Chu, G.R. Reddy, S.N. Tseng, H.L. Chen, W.F. Tang, M.S. Wu, J.Y. Yeh, Y.S. Chao, J.T. Hsu, H.P. Hsieh, J.T. Horng, J. Biomed. Sci. 17 (2010) 13.
[7] B.P. Bandgar, S.S. Gawande, R.G. Bodade, N.M. Gawande, C.N. Khobragade, Bioorg. Med. Chem. 17 (2009) 8168–8173.
[8] D.M. Bailey, P.E. Hansen, A.G. Hlavac, E.R. Baizman, J. Pear, A.F. DeFelice, M.E. Feigenson, J. Med. Chem. 28 (1985) 256–260.
[9] D. Zhang, G. Wang, G. Zhao, W. Xu, L. Huo, Eur. J. Med. Chem. 46 (2011) 5868–5877.
[10] X.H. Yang, Q. wen, T.T. Zhao, J. Sun, X. Li, M. Xing, X. Lu, H.L. Zhu, Bioorg. Med. Chem. 20 (2012) 1181–1187.
[11] S.G. Alegaon, K.R. Alagawadi, P.V. Sonkusare, S.M. Chaudhary, D.H. Dadwe, A.S. Shah, Bioorg. Med. Chem. Lett. 22 (2012) 1917–1921.
[12] S.G. Alegaon, K.R. Alagawadi, Med. Chem. Res. 21 (2012) 3214–3223.
[13] S.G. Alegaon, K.R. Alagawadi, S.M. Pawar, D. Vinod, U. Rajput, Med. Chem. Res. 23 (2014) 987–994.
[14] D. Kumar, N.M. Kumar, K.-H. Chang, K. Shah, Eur. J. Med. Chem. 45 (2010) 4664–4668.
[15] A.K. Jain, A. Vaidya, R. Ravichandran, S.K. Kashaw, R.K. Agrawal, Bioorg. Med. Chem. 20 (2012) 3378–3395.
[16] A. Verma, S.K. Saraf, Eur. J. Med. Chem. 43 (2008) 897–905.
[17] A. Kunzler, P.D. Neuenfeldt, A.M. Das Neves, C.M.P. Pereira, G.H. Marques, P.S. Nascente, M.H.V. Farnandes, S.O. Hubner, W. Cunico, Eur. J. Med. Chem. 64 (2013) 74–84.
[18] S.G. Alegaon, K.R. Alagawadi, Lett. Drug Des. Discovery 8 (2011). pp. 642–618.
[19] M.H.M. Helal, M.A. Salem, M.S.A. El-Gaby, M. Aljahdali, Eur. J. Med. Chem. 65 (2013) 517–526.
[20] S.M. El-Messery, G.S. Hassan, F.A.M. Al-Omary, H.I. El-Subbagh, Eur. J. Med. Chem. 54 (2012) 615–625.
[21] J. Cai, M. Sun, X. Wu, J. Chen, P. Wang, X. Zong, M. Ji, Eur. J. Med. Chem. 63 (2013) 702–712.
[22] E.L. Luzina, A.V. Popov, Eur. J. Med. Chem. 44 (2009) 4944–4953.
[23] A. Cohen, P. Verhaeghe, M.D. Crozet, S. Hutter, P. Rathelot, P. Vanelle, N. Azas, Eur. J. Med. Chem. 55 (2012) 315–324.
[24] S.N. Mokale, P.T. Sanap, D.B. Shinde, Eur. J. Med. Chem. 45 (2010) 3096–3100.
[25] P. Makam, R. Kankanala, A. Prakash, T. Kannan, Eur. J. Med. Chem. 69 (2013) 564–576.
[26] R.P. Karuvalam, K.R. Haridas, S.K. Nayak, T.N. Guru Row, P. Rajeesh, R. Rishikesan, N.S. Kumari, Eur. J. Med. Chem. 49 (2012) 172–182.
[27] M.A. Gouda, M.A. Berghot, G.E. Abd El-Ghani, A.M. Khalil, Eur. J. Med. Chem. 45 (2010) 1338–1345.
[28] J. Rudolph, H. Theis, R. Hanke, R. Endermann, L. Johannsen, F.U. Geschke, J. Med. Chem. 44 (2001) 619–626.
[29] P. Rathelot, N. Azas, H. El-Kashef, F. Delmas, C.D. Giorgio, P.T. David, J. Maldonado, P. Vanelle, Eur. J. Med. Chem. 37 (2002) 671–679.
[30] C.A. Winter, E.A. Risley, G.W. Nuss, Proc. Soc. Exp. Biol. Med. 111 (1962) 544–547.
[31] R. Vinegar, W. Schreiber, R.J. Hugo, J. Pharmacol. Exp. Ther. 166 (1969) 96–103.
[32] COX inhibtor screening assay kit (COX-1 (ovine) and human recombinant COX-2) (Catalog No. 560131) Cayman Chemical Co., USA. 2013.
[33] C. Gundogdu-Hizliates, H. Alyuruk, M. Gocmenturk, Y. Ergun, L. Cavas, Bioorg. Chem. 52 (2014) 8–15.
[34] K.J. Kim, M.J. Choi, J. Shin, M. Kim, H. Choi, S.M. Kang, J.H. Jin, K. Lee, J.Y. Lee, Bioorg. Med. Chem. Lett. (2014) http://dx.doi.org/10.1016/j.bmcl.2014.02.074.
[35] B. Lee, J. Kwak, S. Huang, J. Jang, S. Lim, Y. Kwak, K. Lee, H.S. Kim, S. Han, J. Hong, H. Lee, S. Song, S. Seo, J. Jung, Bioorg. Med. Chem. 20 (2012) 2860–2868.
[36] P. Vitale, M.G. Perrone, P. Malebra, A. Lavecchia, A. Scilimati, Eur. J. Med. Chem. 74 (2014) 606–618.
[37] I.A. Al-Suwaidan, A.M. Alanazi, A.S. El-Azab, A.M. Al-Obaid, K.E.H. Eltahir, A.R. Maarouf, M.A. Abu El-Enin, A.A.M. Abdi-Aziz, Bioorg. Med. Chem. Lett. 23 (2013) 2601–2605.
[38] A. Zarghi, T. Zeberdast, B. Daraie, M. Hedayati, Bioorg. Med. Chem. 17 (2014) 5369–5373.
[39] T. Mosmann, J. Immunol. Methods 65 (1983) 55–63.
[40] Maestro, version 9.3, Schrödinger, LLC, New York, NY, 2012.
[41] OECD Guidelines for the Testing of Chemicals: 425 Acute Oral Toxicity-Upand-Down-Procedure (UDP), OECD, 2008, pp. 1–27.