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ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 276–283
Bioorganic & Medicinal Chemistry

Synthesis and biological evaluation of 2-trifluoromethyl/sulfonamido-5,6-diaryl substituted imidazo[2,1-*b*]-1,3,4-thiadiazoles: A novel class of cyclooxygenase-2 inhibitors

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Received 29 June 2007; revised 18 September 2007; accepted 19 September 2007
Available online 25 September 2007

Abstract—A series of 2-trifluoromethyl/sulfonamido-5,6-diarylsubstituted imidazo[2,1-*b*]-1,3,4-thiadiazole derivatives **15a**–**j** have been synthesized by the reaction of 2-amino-5-trifluoromethyl/sulfonamido-1,3,4-thiadiazoles **14a**–**b** and appropriately substituted α-bromo-1,2-(*p*-substituted)diaryl-1-ethanones **13a**–**h**. Structures of these compounds were established by IR, ^{1}H NMR, ^{13}C NMR, Mass, and HRMS data. The selected compounds were evaluated for their preliminary in vitro cyclooxygenase inhibitory activity against COX-2 and COX-1enzymes using colorimetric method. The compounds tested showed selective inhibitory activity toward COX-2 (80.6–49.4%) over COX-1 (30.6–8.6), amongst them compounds **15f** and **15j** showed appreciable COX-2 selective inhibitory activity. These compounds also exhibited significant anti-inflammatory activity (70.09–42.32%), which is comparable to that of celecoxib in the carrageenan-induced rat paw edema method.

1. Introduction

Prostaglandins (PGs) are active mediators of inflammatory responses and also provide cytoprotection in the stomach and intestine. The key enzyme of their biosynthesis is prostaglandin H2 synthase (PGHS or cyclooxygenase, COX), which is a bifunctional enzyme exhibiting both cyclooxygenase and peroxidase activities. The cyclooxygenase component converts arachidonic acid to a hydroperoxy endoperoxide (PGG_2) and the peroxidase component reduces the endoperoxide to the corresponding alcohol (PGH_2), the precursor of PGs, thromboxanes, and prostacyclins.[1] It is now well established that three distinct COX isoforms exist: the constitutive form COX-1 is expressed virtually in all tissues and is involved in the regulation of physiological functions in maintaining platelet aggregation and homeostasis of the GI tract and the kidney.[2] COX-2 is rapidly induced in inflammatory cells in response to cytokines such as tumor necrosis factor-α (TNF-α), interleukines, growth factors, and so on. The PGs produced by COX-2 play an important role in inflammatory symptoms.[3] COX-2 is also indicated in Cancer[4] and Alzheimer's diseases.[5] Recently a third full active isoform, COX-3, and two partial isoforms, pCOX1a and b, in the cerebral cortex and in human heart are reported.[6,7]

Non-steroidal anti-inflammatory drugs (NSAIDs) continue to be one of the more widely used groups of therapeutic agents, which inhibit COX-1, COX-2, and tromboxane synthase with a varying degree of selectivity. Researchers have recently focused on selective COX-2 inhibitors which are believed to reduce inflammation without influencing normal physiologic functions of COX-1. The first COX-2 selective NSAID approved by Food and Drug Administration (FDA) was celecoxib[8] (**1**), which was followed by introduction of rofecoxib[9] (**2**), valdecoxib[10] (**3**), NS-398[11] (**4**), DuP-697[12] (**5**), and SC-57666 (**6**). A common structural

Keywords: Cyclooxygenase; Anti-inflammatory activity; Imidazo[2,1-*b*]-1,3,4-thiadiazoles; NSAIDs.
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doi:10.1016/j.bmc.2007.09.038

backbone of most COX-2 selective inhibitors consists of two aryl groups linked to adjacent atoms of a central ring which can be homocyclic or heterocyclic, one of the aryl groups is substituted in the *para* position with either an aminosulfonyl (SO_2NH_2) or a methyl sulfonyl (SO_2CH_3) group. There are also examples of potent COX-2 inhibitors that possess cycloalkyl, alkoxy or phenoxy moieties in the non-sulfonyl containing 'aryl' region.[13–15] Some of the commonly found central rings within this class of molecules are thiophene, pyrazole, furanone, isoxazole, and cyclopentene as shown in Figure 1.

Since their introduction, COX-2 specific inhibitors have become rapidly growing segment of prescription drug market, especially for osteoarthritis and rheumatoid arthritis patients. However, recently there is a controversy regarding hepatic toxicity of nimusulide and cardiovascular complications of rofecoxib. FDA has banned the use of nimusulide in pediatric patients and rofecoxib in both adults and childrens.[16] In spite of these facts, there is a growing need for the development of safer COX-2 selective inhibitors. During recent past several attempts have been made to develop safer COX-2 selective inhibitors containing fused heterocyclic ring system in place of a regular central single heterocycle. Now several different classes of COX-2 inhibitors have been reported, like 5,6-diarylspiro heptenes[17] (**7**) and 5,6-diarylsubstituted thiazolotriazole derivatives (**8**).[18] Recently, a novel series of 5,6-diarylimidazo[2,1-*b*]thiazole derivatives (**9**) have been reported as potent, orally active, selective COX-2 inhibitors[19] indicating the increasing scope of alteration in the central ring that may lead to development of newer, safer, selective COX-2 inhibitors (see Fig. 2).

Thiazole (sulfathiazole/cefixime),[20] imidazo[2,1-*b*]thiazole and their bio-isosteric derivatives thiadiazole (acetazolamide),[21] imidazo[2,1-*b*]1,3,4-thiadiazole[22] are regarded as safer and better drug molecules that are found to possess diversified biological activities like antibacterial, diuretic, antifungal, leismaniacidal, antitubercular, anticancer, anticonvulsant, etc. In addition, levamisole,[23] a well-known anthelmintic drug, contains imidazo[2,1-*b*]thiazole moiety and also exhibits profound effect as anti-arthritic and immuno-modulatory agent. The presently described compounds (**15a**–**j**) are bio-isosteric analogs of imidazo[2,1-*b*]thiazole like L-766 112 (**9**).

Therefore, in view of the above facts and in continuation of our search for biologically active imidazo[2,1-*b*]-1,3,4-thiadiazoles,[24] in this paper, we report the synthesis and preliminary biological evaluation of a novel class of 2-trifluoromethyl/sulfonamide-5,6-diarylsubstituted imidazo[2,1-*b*]1,3,4-thiadiazoles as COX-2 inhibitors.

2. Chemistry

Synthesis of 1,2-(*p*-substituted)diaryl-1-ethanones **12a**–**h** was carried out by reacting appropriate phenyl acetic acid **10a**,**b** with various substituted aromatic hydrocarbons **11a**–**e**. Further, **12a** and **12b** were oxidized to corresponding sulfoxides **12f** and **12h**. Subsequently, **12a**–**h** were subjected to bromination using liquid bromine in chloroform to obtain α-bromo-1, 2-(*p*-substituted)diaryl-1-ethanones **13a**–**h** as shown in Scheme 1.[25–27]

The synthesis of 2-trifluoromethyl/sulfonamido-5,6-diarylsubstituted imidazo[2,1-*b*]-1,3,4-thiadiazole derivatives **15a**–**j** was carried out by the condensation of **13a**–**h** with 2-amino-5-substituted-1,3,4-thiadiazole **14a**,**b** under reflux in dry ethanol (as depicted in Scheme 2). This reaction[28] proceeds via intermediate iminothiadiazole **I**, which spontaneously undergoes ring closer to **II** under reflux temperature to afford the desired fused heterocycles **15a**–**j** in good yields.

The substitution at 5th position of 2-amino-5-substituted-1,3,4-thiadiazole is crucial in determining the course of its reaction with substituted α-bromo-1,2-(*p*-substituted)diaryl-1-ethanone, such type of reactions has been studied by deStevens et al.[29] in case of 2-amino-5-substituted-1,3,4-thiadiazole derivatives and Therien et al.[19] in case of 2-amino-5-substituted thiazole derivatives. Pyl et al.[30] have described the synthesis 5,6-unsubstituted diaryl imidazo[2,1-*b*]-1,3,4-thiadiazole derivatives starting

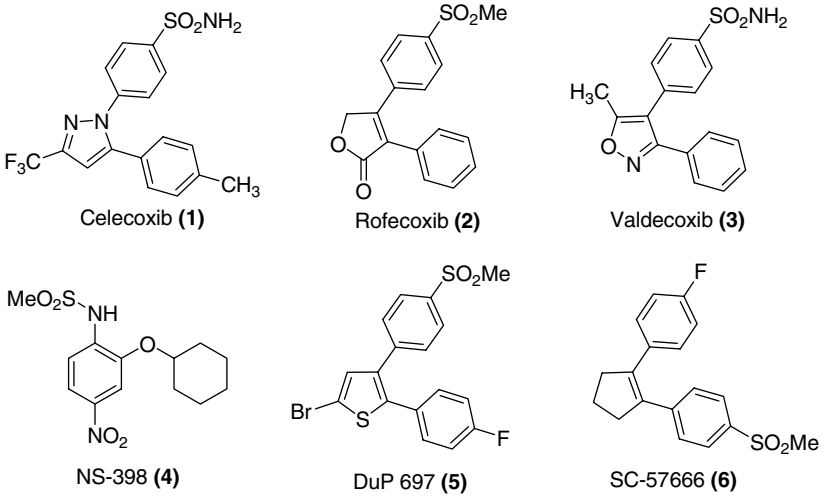


Figure 1. Structures of some selective COX-2 NSAIDs characterized by diaryl carbocyclic or heterocyclic 5-membered ring (**1**–**6**).

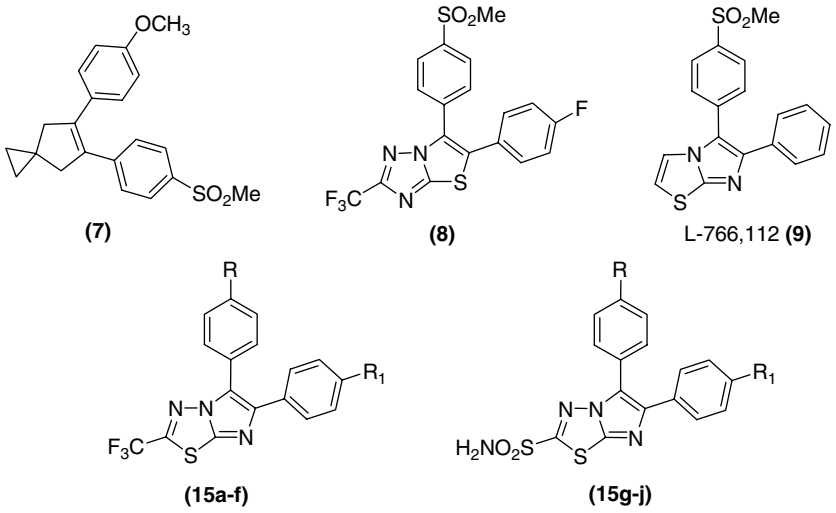
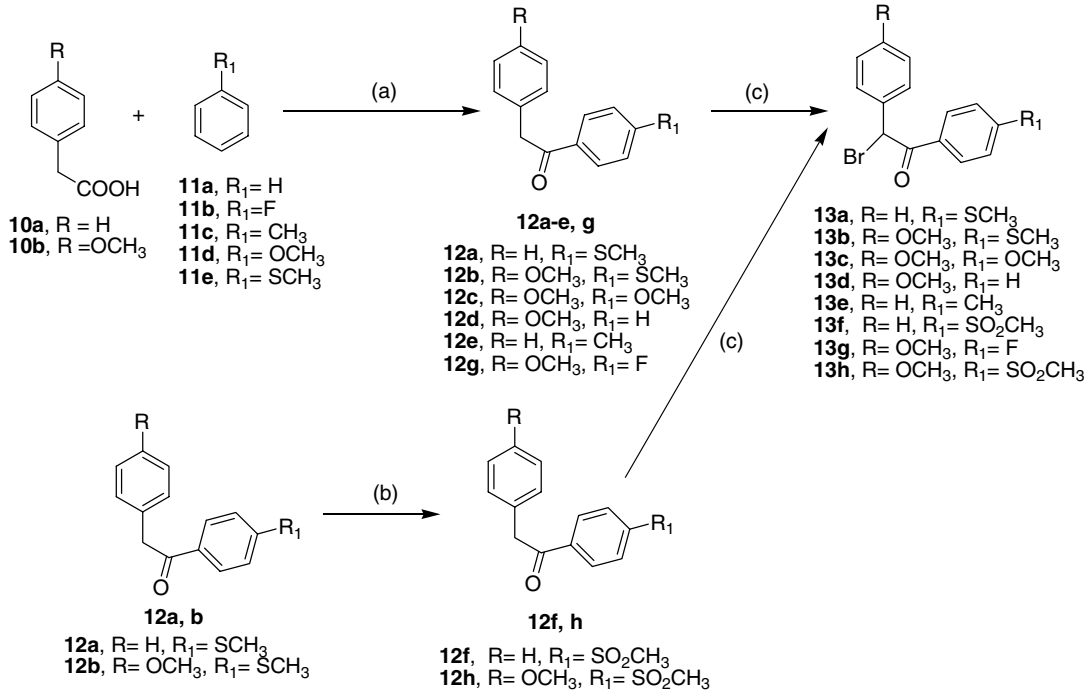


Figure 2. Structures of some selective COX-2 inhibitors containing a central fused heterocyclic ring system (**7**–**9**) and synthesized compounds (**15a**–**f** and **15g**–**j**).



Scheme 1. Reagents and conditions: (a) H_3PO_4, $(CF_3CO)_2O$, 25 °C, 1 min; (b) H_2O_2/AcOH, 50–60 °C, 3–4 h, in case of **12a** and **12b**; (c) Br_2, $CHCl_3$, 50 °C, 0.5 h.

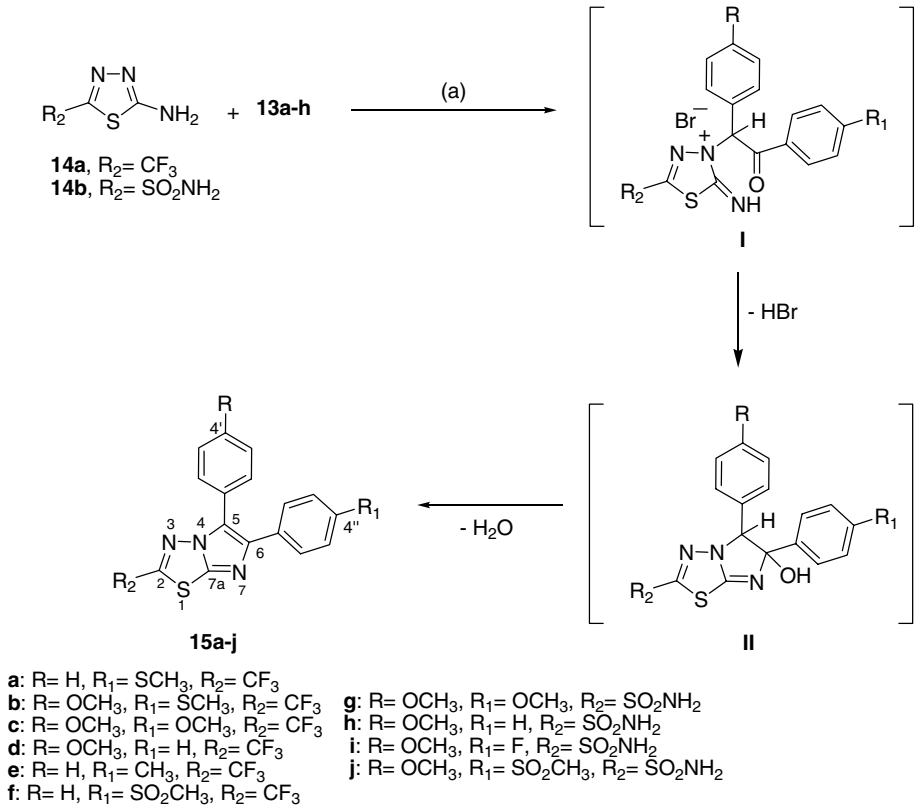
with 2-amino-5-substituted-1,3,4-thiadiazole with bulkier (electron donating) group at 5th position.

3. Biological evaluation

The synthesized compounds **15a**–**d**, **f**, **g**, **i**, and **j** were evaluated for their ability to inhibit COX-2 and COX-1 by in vitro colorimetric COX (ovine) inhibitor assay method,[31] which utilizes the peroxidase component of cyclooxygenase. The peroxidase activity is assayed colorimetrically by monitoring the appearance of oxidized *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylene diamine (TMPD) during the reduction of PGG_2 to PGH_2, at 590 nm. The IC_{50} values for **15f** and **15j** were calculated using non-linear regression analysis. The in vivo anti-inflammatory activity for compounds **15a**–**d**, **f**, **g**, **i**, and **j** was also evaluated using carrageenan-induced rat paw edema model by adopting the earlier reported method of Winter et al.[32]

4. Results and discussion

We have synthesized a series of 2-trifluoromethyl/sulfonamido-5,6-diarylsubstituted imidazo[2,1-*b*]-1,3,4-thiadiazole derivatives by reacting 2-amino-5-substituted-1,3,4-thiadiazole with an appropriately substituted α-bromo-1,2-(*p*-substituted)diaryl-1-ethanone as illustrated in Scheme 2. Structures of the synthesized compounds were established on the basis of IR, ^{1}H NMR, ^{13}C NMR Mass, and HRMS data.



Scheme 2. Reagents and conditions: (a) EtOH, P_2O_5, reflux, 12–14 h, Na_2CO_3.

In ^{1}H NMR spectra the synthesized compounds showed prominent signals for the aromatic protons between δ 6.83 and 8.26 ppm. Compounds **15g**–**j** showed a singlet between δ 8.63 and 8.8 ppm indicating the presence of SO_2NH_2 group. The peaks appearing at around δ 1.22, 1.96–2.03, 3.10, and 3.78–3.88 ppm confirm the presence of CH_3, SCH_3, SO_2CH_3, and OCH_3 groups, respectively. In ^{13}C NMR spectrum we have observed most characteristic signals appeared at around δ 14.9, 24.3, 46.3, 54.9–60.0, and 120.0 ppm, for SCH_3, CH_3, SO_2CH_3, OCH_3, and CF_3, respectively. The signals appeared at around δ 107.0, 114.0, 143.0, 162.0 ppm for C-5, C-6, C-7a, C-2 and carbons of aromatic rings at δ 127.0–134.0 ppm, respectively. Electron Impact mass spectra showed accurate molecular ion peaks at *m*/*z* 392.3, 421.9, 406.6, 375.5, 424.2, 415.9, 386.2, and 403.1 for compounds **15a**–**d** and **15f**–**i**, respectively.

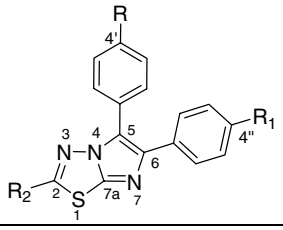
The results of in vitro COX enzyme inhibition assay studies are summarized in Table 1. The results showed that the compounds **15f** and **15j** exhibited effective inhibition against COX-2 (73.6% and 80.6%), compared to the inhibition of COX-1 (8.6% and 13.3%), indicating that the SO_2CH_3 group is accountable for the diaryl heterocyclic inhibitors' selective inhibition against COX-2. Further, compounds **15f** and **15j** showed the IC_{50} values 4.79 and 3.24 μM against COX-2 and more than 50 μM against COX-1, respectively (Celecoxib IC_{50} value is 0.04 and 13 μM against COX-2 and COX-1, respectively). Compounds **15a** (65.8%, 14.2%), **15b** (63.2%, 10.5%), **15c** (66.5%, 15.5%), and **15i** (66.2%, 12.9%), containing different functional groups like SCH_3, OCH_3 and F, showed moderate inhibitory activity toward both COX-2 and COX-1, respectively, at 10 μM concentration.

The structure–activity relationship (SAR) studies demonstrated that the presence of OCH_3, SCH_3, SO_2CH_3, and F groups at the 4″-position of the 6-phenyl ring contributes for selective COX-2 inhibitory activity and the unsubstituted phenyl ring as in case of **15d** (49.4%, 30.6%) was found to inhibit both COX-2 and COX-1. However, we observed that replacement of SO_2NH_2 for CF_3 group at second position did not alter the selectivity and enzyme inhibitory activity to a greater extent. The synthesized compounds were further assessed for in vivo anti-inflammatory activity in carrageenan-induced rat paw edema model. The results presented in Table 1 show that compounds **15a** (37.6%, 54.1%), **15b** (42.3%, 66.1%), **15c** (38.0%, 48.7%), **15f** (46.7%, 70.0%), **15i** (43.4%, 51.72%), and **15j** (54.2%, 61.2%) exhibited good anti-inflammatory activity at the dose of 10 mg/kg at 2 and 4 h, respectively, which is comparable to celecoxib (48.3%, 64.8%).

5. Conclusion

In the present paper, we report the synthesis, spectral studies, and cyclooxygenase inhibition (COX-2 and COX-1) and anti-inflammatory activity of a novel series of imidazo-[2,1-*b*]-1,3,4-thiadiazoles. These fused heterocyclic compounds were prepared by the cyclodehydration process between 2-amino-5-trifluoromethyl/

Table 1. In vitro COX-1 and COX-2 enzyme inhibition data and in vivo anti-inflammatory activity data for compounds **15a–j**



Compound	Structure			% Inhibition[a,b]		% Reduction in paw edema[c,d]	
	R	R_1	R_2	COX-1 (10 μM)	COX-2 (10 μM)	2 h	4 h
15a	H	SCH_3	CF_3	14.2	65.8	37.64 ± 1.87	54.16 ± 3.50
15b	OCH_3	SCH_3	CF_3	10.5	63.2	42.38 ± 2.26	66.16 ± 2.87
15c	OCH_3	OCH_3	CF_3	15.5	66.5	38.03 ± 2.80	48.78 ± 5.75
15d	OCH_3	H	CF_3	30.6	49.4	29.62 ± 3.75	42.32 ± 4.96
15e	H	CH_3	CF_3	ND[e]	ND	ND	ND
15f	H	SO_2CH_3	CF_3	8.6 (>50)[f]	73.6 (4.79)	46.73 ± 3.80	70.09 ± 4.65
15g	OCH_3	OCH_3	SO_2NH_2	28.1	58.9	24.68 ± 2.08	37.05 ± 3.72
15h	OCH_3	H	SO_2NH_2	ND	ND	ND	ND
15i	OCH_3	F	SO_2NH_2	12.9	66.2	43.49 ± 5.24	51.72 ± 2.48
15j	OCH_3	SO_2CH_3	SO_2NH_2	13.3 (>50)	80.6 (3.24)	54.28 ± 4.60	61.29 ± 5.69
Celecoxib				2.8 (13)	100 (0.04)	48.31 ± 3.48	64.83 ± 4.49

[a] Values are acquired using in vitro ovine COX-1/COX-2 assay kit (Catalog No. 760 111, Cayman Chemicals Inc., Ann Arbor, MI).
[b] Experiments were carried out in duplicate and have less than 10% error.
[c] Values (Mean ± SE) are obtained using in vivo carrageenan-induced rat paw edema model, using six animal per group of male albino rats.
[d] Test compounds and celecoxib were administered orally at the dose of 10 mg/kg.
[e] Not determined.
[f] Values in bracket are IC_{50} in μM.

sulfonamido-1,3,4-thiadiazoles and α-bromo-1,2-(*p*-substituted)diaryl-1-ethanones.

The preliminary in vitro and in vivo biological activities of these novel series of 2-trifluoromethyl/sulfonamido-5,6-diarylsubstituted imidazo[2,1-*b*]-1,3,4-thiadiazoles have evidenced that 4″-SO_2CH_3 substituted analogs **15f** and **15j** are more selective and active than 4″-OCH_3 (**15c** and **15g)** and 4″-SCH_3 (**15a** and **15b)** analogs. Furthermore, replacement of the 4″-SO_2CH_3 group with F as in case of compound **15i** also has shown moderate selectivity. On the contrary, the replacement of CF_3 by SO_2NH_2 group at second position showed no greater change in selectivity and COX inhibitory activity. The possible improvement of selective COX-2 inhibitory activity of this basic imidazo[2,1-*b*]-1,3,4-thiadiazole structure through modulation of ring substituents and/or additional functionation warrants further investigations. In summary, we have identified a novel series of imidazo[2,1-*b*]-1,3,4-thiadiazoles, which may develop into the potential class of safer and selective COX-2 inhibitors.

6. Experimental

6.1. Chemistry protocols

All research chemicals were purchased from Sigma–Aldrich or Lancaster Co., and used as such for the reactions. Solvents except LR grade were dried and purified according to the literature when necessary. Reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel GF254 plates from E-Merck Co and compounds visualized either by exposure to UV or dipping in 10% aqueous potassium permanganate solution.

Melting points were determined using open capillary tube method and are uncorrected. Infra-red (IR) spectra were recorded using KBr disk on a ThermoNicolet MX-1 FTIR spectrometer; ^1H NMR and ^{13}C NMR spectra were recorded on Bruker AMX-400 and 101 MHz, respectively. Chemical shifts are reported in δ ppm units with respect to TMS as internal standard. Mass spectra were recorded on Autospec Mass Spectrometer under the electron impact at 70 eV.

6.1.1. General procedure for the synthesis of 1-(4″-substituted)phenyl-2-(4′-substituted)phenyl-1-ethanones (12a–e,g, Scheme 1). To a mixture of phenyl acetic acid/*p*-substituted phenyl acetic acid (**10a/b**, 7.3 mmol), substituted aromatic hydrocarbon (one of **11a–e**, 8.8 mmol), and 88–93% orthophosphoric acid (8.8 mmol) was added trifluoroacetic anhydride (29.5 mmol) rapidly with vigorous stirring at 25 °C. The mixture turned into a dark colored solution with vigorous exothermic reaction. The reaction mixture was stirred for 1 min at the same temperature and poured into ice-cold water (50 mL) with stirring. Then it was washed with cold hexane (2× 10 mL) to obtain **12a–e**, **g** as solid.

6.1.2. General procedure for the synthesis of 1-(4″-methylsulfonyl)phenyl-2-(4′-substituted)phenyl-1-ethanones (12f,h). Thirty percent of hydrogen peroxide solution (3.5 mL, 29.41 mmol) was slowly added to a mixture of 1-(4″-methylsulfanyl)phenyl-2-(4′-substituted)phenyl-1-

ethanones (**12a/b**, 8.0 mmol) in glacial acetic acid (12 mL). The reaction mixture was heated at 50 °C for 3–4 h. The cooled mass was poured over ice water and extracted with dichloromethane. The combined organic layer was washed with water, dried, and evaporated. The crude solid was triturated with dichloromethane–petroleum ether mixture to get **12f**,**h** as light yellow/brown solid.

6.1.3. General procedure for the synthesis of α-bromo-1-(4″-substituted)phenyl-2-(4′-substituted)phenyl-1-ethanones (13a–h). To a solution of **12a**–**h** (200 mmol) in chloroform (30 mL) kept at 50 °C was added dropwise bromine (220 mmol) with stirring. After being stirred at 50 °C for 0.5 h, the mixture was washed successively with aqueous 10% sodium thiosulfate solution and water.

The solvent was removed in vacuo to obtain the title compounds (**13a**–**h**) either as oil/solid mass/crystalline compounds.

6.2. General procedure for the synthesis of 2-trifluoromethyl/sulfonamido-5,6-diarylsubstituted imidazo[2,1-*b*]-1,3,4-thiadiazole (15a–j, Scheme 2)

A mixture of 2-amino-5-substituted-1,3,4-thiadiazole (one of **14a**–**b**, 10 mmol) and an appropriate α-bromo-1-(4″-substituted)phenyl-2-(4′-substituted)phenyl-1-ethanone (one of **13a**–**h**, 10 mmol) in dry ethanol (150 mL) was heated to reflux on a water bath for 6–8 h, phosphorus pentoxide (3 mmol) was added, and refluxing was continued for another 4–6 h. The reaction mixture was cooled overnight at room temperature. Excess of solvent was removed under reduced pressure and the solid hydrobromide separated was filtered, washed with cold ethanol, and dried. Neutralization of hydrobromide salts with cold aqueous solution of Na_2CO_3 yielded the corresponding free bases (**15a**–**j**), which were purified by recrystallization from dry ethanol. Further, the compounds were purified by column chromatography using 200–400 mesh silica gel and eluted either with ethyl acetate/hexane (2:8) or chloroform/hexane (1:9) as mobile phase.

6.2.1. 2-Trifluoromethyl-5-phenyl-6-(4″-(methylthio)phenyl)-imidazo[2,1-*b*]-1,3,4-thiadiazole (15a). This was obtained by reacting 2-amino-5-trifluoromethyl-1,3,4-thiadiazole (**14a**, 1.69 g) and α-bromo-1-(4″-(methylthio)phenyl)-2-phenyl-1-ethanone (**13a**, 3.21 g) as described in the general procedure and isolated as dark yellow crystals. Yield: 2.83 g (72.5%); mp 162–164 °C; IR (KBr) ν_{max} 3133, 2925, 2832, 1595, 1514, 1409, 1287, 1156, 746 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.02 (d, J = 8.8 Hz, 2H, aryl-H), 7.98 (d, J = 8.4 Hz, 2H, aryl-H), 7.65–7.26 (m, 3H, aryl-H), 6.95 (d, J = 8.5 Hz, 2H, aryl-H), 2.03 (s, 3H, 4″-SCH_3) ppm; ^{13}C NMR ($CDCl_3$) δ 163.0, 160.3, 141.2, 136.4, 129.6, 129.5, 129.3, 128.8, 126.3, 122.0, 120.9, 114.4, 14.9 ppm; EI-MS *m*/*z* (relative intensity) 392 (M^+, 100%), 345 (40%), 284 (13%), 239 (22%), 213 (67%), 170 (40%), 150 (20%), 101 (55%); HRMS (EI) *m*/*z* calcd for $C_{18}H_{12}F_3N_3S_2$: 391.4332; found: 391.4328.

6.2.2. 2-Trifluoromethyl-5-(4′-methoxyphenyl)-6-(4″-(methylthio)phenyl)imidazo[2,1-*b*]-1,3,4-thiadiazole (15b). This was obtained by reacting 2-amino-5-trifluoromethyl-1,3,4-thiadiazole (**14a**,1.69 g) and α-bromo-1-(4″-(methylthio)phenyl)-2-(4′-methoxy)phenyl-1-ethanone (**13b**, 3.51 g) as described in the general procedure and isolated as cream colored crystals. Yield: 2.56 g (60.90%); mp 142–144 °C; IR (KBr) ν_{max} 3129, 2964, 2841, 1609, 1519, 1495, 1328, 1193, 1146, 745 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.12 (d, J = 8.8 Hz, 2H, aryl-H), 8.08 (d, J = 8.4 Hz, 2H, aryl-H), 7.82 (d, J = 8.9 Hz, 2H, aryl-H), 7.80 (d, J = 8.8 Hz, 2H, aryl-H), 3.78 (s, 3H, 4′-OCH_3), 1.96 (s, 3H, 4″-SCH_3) ppm; ^{13}C NMR ($CDCl_3$) δ 163.7, 159.1, 143.1, 136.4, 129.5, 128.5, 127.7, 126.8, 126.3, 123.2, 120.0, 114.5, 58.5, 14.9 ppm; EI-MS *m*/*z* (relative intensity) 421 (M^+, 40%), 391 (77%), 350 (54%), 288 (12%), 270 (24%), 242 (67%), 169 (100%), 148 (41%), 120 (20%); HRMS (EI) *m*/*z* calcd for $C_{19}H_{14}F_3N_3OS_2$: 421.4592; found: 421.4584.

6.2.3. 2-Trifluoromethyl-5-(4′-methoxyphenyl)-6-(4″-methoxyphenyl)imidazo[2,1-*b*]-1,3,4-thiadiazole (15c). This was obtained by reacting 2-amino-5-trifluoromethyl-1,3,4-thiadiazole (**14a**, 1.69 g) and α-bromo-1-(4″-methoxy)-phenyl-2-(4′-methoxy)phenyl-1-ethanone (**13c**, 3.35 g) as described in the general procedure and isolated as white crystals. Yield: 2.32 g (57.35%); mp 110–112 °C; IR (KBr) ν_{max} 3104, 2969, 2814, 1597, 1442, 1339, 1256, 1169, 755 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.01 (d, J = 8.8 Hz, 2H, aryl-H), 7.98 (d, J = 8.8 Hz, 2H, aryl-H), 7.17 (d, J = 8.5 Hz, 2H, aryl-H), 6.85 (d, J = 8.6 Hz, 2H, aryl-H), 3.88 (s, 3H, 4′-OCH_3), 3.85 (s, 3H, 4″-OCH_3) ppm; ^{13}C NMR ($CDCl_3$) δ 164.5, 159.7, 145.0, 135.3, 129.7, 129.2, 128.8, 127.5, 125.4, 123.6, 120.9, 114.8, 56.5, 57.1 ppm; EI-MS *m*/*z* (relative intensity) 406 (M^+, 45%), 365 (20%), 316 (33%), 276 (14%), 239 (45%), 193 (60%), 165 (62%), 148 (35%), 121 (100%), 104 (77%); HRMS (EI) *m*/*z* calcd for $C_{19}H_{14}F_3N_3O_2S$: 405.3936; found: 405.3931.

6.2.4. 2-Trifluoromethyl-5-(4′-methoxyphenyl)-6-phenylimidazo[2,1-*b*]-1,3,4-thiadiazole (15d). This was obtained by reacting 2-amino-5-trifluoromethyl-1,3,4-thiadiazole (**14a**, 1.69 g) and α-bromo-1-phenyl-2-(4′-methoxy)phenyl-1-ethanone (**13d**, 3.05 g) as described in the general procedure and isolated as yellow crystals. Yield: 2.38 g (63.50%); mp 138–140 °C; IR (KBr) ν_{max} 3045, 2932, 2823, 1604, 1519, 1418, 1292, 1251, 1175, 736 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.58 (d, J = 8.8 Hz, 2H, aryl-H), 7.45 (d, J = 8.1 Hz, 2H, aryl-H), 7.25–7.18 (m, 3H, aryl-H), 6.85 (d, J = 8.8 Hz, 2H, aryl-H), 3.82 (s, 3H, 4′-OCH_3) ppm; ^{13}C NMR ($CDCl_3$) δ 163.6, 161.9, 144.2, 137.8, 129.6, 129.4, 129.1, 126.3, 122.0, 120.9, 114.4, 55.6 ppm; EI-MS *m*/*z* (relative intensity) 375 (M^+, 100%), 279(61%), 237 (32%), 190 (59%), 177 (40%), 147 (20%), 133 (90%), 103 (55%); HRMS (EI) *m*/*z* calcd for $C_{18}H_{12}F_3N_3OS$: 375.0653; found: 375.0650.

6.2.5. 2-Trifluoromethyl-5-phenyl-6-*p*-tolylimidazo[2,1-*b*]-1,3,4-thiadiazole (15e). This was obtained by reacting 2-amino-5-trifluoromethyl-1,3,4-thiadiazole (**14a**, 1.69 g) and α-bromo-1-*p*-tolyl-2-phenyl-1-ethanone (**13e**, 2.89 g)

as described in the general procedure and isolated as dark yellow crystals. Yield: 1.66 g (46.52%); mp 178–180 °C; IR (KBr) ν_{max} 3050, 2936, 2845, 1609, 1510, 1414, 1275, 1156, 748 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.51–7.45 (m, 5H, aryl-H), 7.09 (d, J = 8.1 Hz, 2H, aryl-H), 6.93 (d, J = 8.2 Hz, 2H, aryl-H), 1.22 (s, 3H, 4′-CH_3) ppm; ^{13}C NMR ($CDCl_3$) δ 163.0, 158.9, 142.1, 133.2, 130.1, 129.5, 128.8, 127.5, 121.5, 119.8, 115.2, 24.3 ppm.

6.2.6. 2-Trifluoromethyl-5-phenyl-6-(4″-(methylsulfonyl)-phenyl)imidazo[2,1-*b*]-1,3,4-thiadiazole (15f). This was obtained by reacting 2-amino-5-trifluoromethyl-1,3,4-thiadiazole (**14a**, 1.69 g) and α-bromo-1-(4″-(methylsulfonyl)phenyl)-2-phenyl-1-ethanone (**13f**, 3.53 g) as described in the general procedure and isolated as white crystals. Yield: 2.88 g (68.28%); mp 146–148 °C; IR (KBr) ν_{max} 3019, 2922, 2832, 1592, 1492, 1402, 1296, 1149, 743 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.26 (d, J = 8.4 Hz, 2H, aryl-H), 8.21 (d, J = 8.5 Hz, 2H, aryl-H), 7.67 (d, J = 8.8 Hz, 2H, aryl-H), 7.45-7.25 (m, 3H, aryl-H), 3.15 (s, 3H, 4″-SO_2CH_3) ppm; ^{13}C NMR ($CDCl_3$) δ 166.6, 147.2, 138.8, 136.3, 132.8, 129.5, 129.1, 128.8, 126.7, 121.1, 112.5, 46.3 ppm; EI-MS *m*/*z* (relative intensity) 424 (M^+, 100%), 392 (83%), 345 (55%), 316 (18%), 259 (35%), 240 (47%), 208 (40%), 166 (35%), 152 (20%), 105 (15%); HRMS (EI) *m*/*z* calcd for $C_{18}H_{12}F_3N_3O_2S_2$: 423.7320; found: 423.7313.

6.2.7. 2-Sulfonamido-5-(4′-methoxyphenyl)-6-(4″-methoxyphenyl)imidazo[2,1-*b*]-1,3,4-thiadiazole (15g). This was obtained by reacting 2-amino-5-sulfonamido-1,3,4-thiadiazole (**14b**, 1.80 g) and α-bromo-1-(4″-methoxy)-phenyl-2-(4′-methoxy)phenyl-1-ethanone (**13c**, 3.35 g) as described in the general procedure and isolated as white crystals. Yield: 1.66 g (39.90%); mp 298–300 °C; IR (KBr) ν_{max} 3397, 3164, 2932, 2831, 1610, 1493, 1352, 1255, 1176, 833 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.63 (s, 2H, SO_2NH_2), 7.52 (d, J = 8.9 Hz, 2H, aryl-H), 7.46 (d, J = 8.8 Hz, 2H, aryl-H), 6.94 (d, J = 8.8 Hz, 2H, aryl-H), 6.83 (d, J = 8.8 Hz, 2H, aryl-H), 3.86 (s, 3H, 4′-OCH_3), 3.82 (s, 3H, 4″-OCH_3) ppm; ^{13}C NMR (DMSO-d_6) δ 163.0, 159.8, 141.0, 134.3, 130.2, 128.2, 127.3, 122.3, 119.2, 113.7, 55.7, 55.2 ppm; EI-MS *m*/*z* (relative intensity) 416 (M^+, 80%) 386 (50%), 336 (26%), 278 (21%), 203 (34%), 177 (44%), 160 (51%), 133 (100%), 103 (60%); HRMS (EI) *m*/*z* calcd for $C_{18}H_{16}N_4O_4S_2$: 416.2740; found: 416.2735.

6.2.8. 2-Sulfonamido-5-(4′-methoxyphenyl)-6-phenylimidazo[2,1-*b*]-1,3,4-thiadiazole (15h). This was obtained by reacting 2-amino-5-sulfonamido-1,3,4-thiadiazole (**14 b**, 1.80 g) and α-bromo-1-phenyl-2-(4′-methoxy)-phenyl-1-ethanone (**13d**, 3.05 g) as described in the general procedure and isolated as light yellow crystals. Yield: 2.65 g (68.91%); mp 302–304 °C; IR (KBr) ν_{max} 3402, 3069, 2959, 2820, 1604, 1554, 1496, 1413, 1349, 1109, 918 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.71 (s, 2H, SO_2NH_2), 8.08–7.83 (m, 3H, aryl-H), 7.52 (d, J = 8.0 Hz, 2H, aryl-H), 7.50 (d, J = 8.0 Hz, 2H, aryl-H), 6.92 (d, J = 8.5 Hz, 2H, aryl-H), 3.78 (s, 3H, 4′-OCH_3) ppm; ^{13}C NMR (DMSO-d_6) δ 164.3, 159.3, 142.8, 131.6, 130.2, 129.8, 129.3, 129.1, 126.9, 114.8, 56.0 ppm; EI-MS *m*/*z* (relative intensity) 386 (M^+, 63%), 375 (68%), 306 (31%), 279 (34%), 248 (27%), 213 (33%), 177 (52%), 147 (60%), 133 (100%), 103 (64%); HRMS (EI) *m*/*z* calcd for $C_{17}H_{14}N_4O_3S_2$: 386.0507; found: 386.0504.

6.2.9. 2-Sulfonamido-5-(4′-methoxyphenyl)-6-(4″-fluorophenyl)imidazo[2,1-*b*]-1,3,4-thiadiazole (15i). This was obtained by reacting 2-amino-5-sulfonamido-1,3,4-thiadiazole (**14b**,1.80 g) and α-bromo-1-(4″-fluorophenyl)-2-(4′-methoxyphenyl)-1-ethanone (**13g**, 3.23 g) as described in the general procedure and isolated as white fluffy crystals. Yield: 2.08 g (51.69%); mp 186–188 °C; IR (KBr) ν_{max} 3447, 3030, 2920, 2845, 1615, 1586, 1433, 1225, 1187, 834 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.8 (s, 2H, SO_2NH_2), 7.93 (d, J = 8.5 Hz, 2H, aryl-H), 7.77 (d, J = 8.5 Hz, 2H, aryl-H), 7.59 (d, J = 8.8 Hz, 2H, aryl-H), 7.53 (d, J = 8.8 Hz, 2H, aryl-H), 3.8 (s, 3H, 4′-OCH_3) ppm; ^{13}C NMR (DMSO-d_6) δ 162.9, 158.2, 147.1, 134.3, 131.0, 129.5, 128.7, 127.1, 124.8, 121.0, 113.7, 57.6 ppm; EI-MS *m*/*z* (relative intensity) 403 (M^+-1, 20%) 316 (32%), 288 (23%), 241 (26%), 212 (100%), 169 (80%), 148 (37%), 105 (25%); HRMS (EI) *m*/*z* calcd for $C_{17}H_{13}FN_4O_3S_2$: 404.0413; found: 404.0409.

6.2.10. 2-Sulfonamido-5-(4′-methoxyphenyl)-6-(4″-(methylsulfonyl)phenyl)imidazo[2,1-*b*]-1,3,4-thiadiazole (15j). This was obtained by reacting 2-amino-5-sulfonamido-1,3,4-thiadiazole (**14b**, 1.80 g) and α-bromo-1-(4″-(methylsulfonyl)phenyl)-2-(4′-methoxyphenyl)-1-ethanone (**13h**, 3.84 g) as described in the general procedure and isolated as white crystals. Yield: 2.46 g (53.17%); mp 160–162 °C; IR (KBr) ν_{max} 3452, 3080, 2961, 2873, 1622, 1575, 1489, 1402, 1256, 1152, 861 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.71 (s, 2H, SO_2NH_2), 8.26 (d, J = 8.3 Hz, 2H, aryl-H), 8.07 (d, J = 8.3 Hz, 2H, aryl-H), 7.53 (d, J = 8.5 Hz, 2H, aryl-H), 7.23 (d, J = 8.5 Hz, 2H, aryl-H), 3.81 (s, 3H, 4′-OCH_3), 3.08 (s, 3H, 4″-SO_2CH_3) ppm; ^{13}C NMR (DMSO-d_6) δ 163.0, 159.4, 144.5, 135.3, 132.8, 130.6, 130.1, 127.9, 127.3, 126.2, 121.7, 114.3, 56.5, 46.4 ppm.

6.3. Biological evaluation

6.3.1. In vitro cylcooxygenase inhibition studies. The selected compounds listed in Table 1 were tested for their ability to inhibit in vitro COX-1 and COX-2 using a colorimetric COX (ovine) inhibitor screening kit (Catalog No. 760 111, Cayman Chemicals Inc., Ann Arbor, MI, USA) using the previously established method.[31]

6.3.2. In vivo anti-inflammatory activity. The preliminary in vivo anti-inflammatory activity was evaluated using carrageenan-induced rat paw edema assay model of inflammation by adopting the method of Winter et al.[32] for the selected compounds listed in Table 1. Male albino rats (170–220 g) were fasted with free access to water at least 12 h prior to experiments and divided randomly into nine groups of six each. Control group received 1 mL of vehicle (0.5% methyl cellulose and

0.025% Tween 20), standard group received 10 mg/kg of celecoxib, and test groups received 10 mg/kg of synthesized compounds. The rats were dosed orally, 1 h later, a subplantar injection of 0.05 mL of 1% solution of carrageenan in 0.9% sterile solution was administered to the left hind foot pad of each animal. The paw edema volume was measured with a digital plethysmometer (Ugo-Basile, Italy) at 0, 2, 4 h after carrageenan injection. Paw edema volume was compared with vehicle control group and percent reduction was calculated as 1 – (edema volume in the drug treated group/edema volume in the control group) × 100.

Acknowledgments

We express our sincere thanks to Shri. Prabhakar B. Kore, Chairman, K.L.E. Society, and Dr. F.V. Manvi, Principal, College of Pharmacy, J. N. Medical College, Belgaum (India), for providing the necessary facilities.

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