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ORIGINAL PAPER

Molecular properties prediction and synthesis of new oxadiazole derivatives possessing 3-fluoro-4-methoxyphenyl moiety as potent anti-inflammatory and analgesic agents

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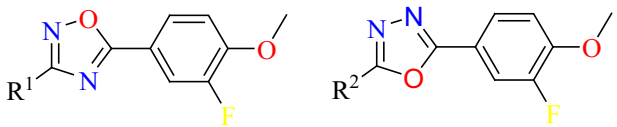
Abstract A new series of 1,2,4- and 1,3,4-oxadiazole derivatives possessing 3-fluoro-4-methoxyphenyl moiety were efficiently synthesized and characterized by spectroscopic methods and elemental analysis. All the compounds were evaluated in vivo for their anti-inflammatory and analgesic properties, and were found to be low lethal as ascertained by the LD_{50} test. The present study suggests that three compounds were found to have good anti-inflammatory activity in the carrageenan-induced rat paw edema test, while a fair number of compounds showed significant analgesic activity in the tail flick test. In silico ADME properties of synthesized compounds were also analysed and showed potential to develop as good oral drug candidates.

Graphical abstract



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Keywords Synthesis · Oxadiazoles · Anti-inflammatory activity · Analgesic activity · Molecular properties prediction

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most widely prescribed therapeutics for the treatment of inflammation and related conditions [1]. The major mechanism by which the NSAIDs elicit their therapeutic effects is by blocking the formation of prostanoids, lipidic mediators derived from fatty acid metabolism, by inhibiting cyclooxygenase (COX) enzymes [2, 3]. But most of the NSAIDs are generally associated with several side effects, especially gastric ulceration [4]. Therefore, the challenge still exists to develop effective NSAIDs with enhanced safety profile.

Oxadiazoles are making a huge impact on multiple drug discovery programmes across a variety of disease areas, including anti-tubercular, antibacterial, antiviral, anti-inflammatory, antifungal, and insecticidal activities [5–13]. Moreover, oxadiazoles are very good bioisosteres for carboxylic acids, esters, and carboxamides, which contribute substantially to increasing pharmacological activity by participating in hydrogen bonding interactions with the receptors [14, 15]. The fluorinated compounds also possess promising pharmacological activities, which originate from their uniquely high thermal stabilities and lipophilicity. The survey of literature reveals that many fluorinated analogues are also available as NSAIDs; examples are diflunisal (Dolobid), flurbiprofen (Froben), deracoxib, lumiracoxib, and flufenamic acid [16, 17]. Thus, the evolution of alternatives to NSAIDs is being attempted all over the world.

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The escalating cost of drug discovery and development is challenging and the chances of success are incredibly low, particularly in recent years [18]. Problems with the drug candidate's absorption, distribution, metabolism, and excretion (ADME), however, have already been identified as important reasons for its failure in the late stages of the drug development process. Therefore, it is important to accurately predict these qualities earlier in the investigation of the lead candidates [19, 20]. Computational methods have emerged as a robust strategy for reducing the number of experimental studies necessary for compound selection and development and for improving the success rate. In this context, in silico approaches are being used today in drug discovery to assess the ADME properties of compounds in the early stages of discovery/development [21]. The first assessment of ADME properties will help pharmaceutical scientists to select the most effective candidates for development, in addition to rejecting individuals with a low likelihood of success. These advantages prompted us to predict the molecular properties that influence many ADME properties.

An appreciation of these findings has driven us to synthesize some new series of oxadiazole derivatives, wherein potent 1,2,4- and 1,3,4-oxadiazole moiety is linked to 3-fluoro-4-methoxyphenyl moiety at the C-5 position to see the additive effect of these rings towards the anti-inflammatory and analgesic activities with higher selectivity and less toxicity.

Results and discussion

Chemistry

Readily available starting materials and simple synthetic procedures are very attractive and convenient for the synthesis of numerous oxadiazoles. The synthetic route used to prepare starting materials and the title compounds is outlined in Scheme 1. The key intermediates, aryl amidoximes **1a**–**1f** and 3-fluoro-4-methoxybenzohydrazide (**4**), are required as starting material for the synthesis of the title compounds **3a**–**3f** and **5a**–**5f**. The aryl amidoximes **1a**–**1f** were synthesized through the condensation of the corresponding aryl nitriles with aqueous hydroxylamine hydrochloride in basic medium by an earlier reported method [23]. Then, it involves the coupling of aryl amidoximes with 3-fluoro-4-methoxybenzoic acid in the presence of HATU (1-[bis(dimethylamino)methylene]-1*H*-[1,2,3]-triazolo[4,5-*b*]pyridinium-3-oxide hexafluorophosphate) and excess of *N*-methylmorpholine (NMM) [24] followed by in situ thermal cyclization afforded 3-(3-fluoro-4-methoxyphenyl)-5-substituted-1,2,4-oxadiazoles **3a**–**3f**. 3-Fluoro-4-methoxybenzohydrazide (**4**) was synthesized according to an earlier reported method [22]. The treatment of hydrazide (**4**) with appropriate aromatic acids in phosphorous oxychloride afforded 2-(3-fluoro-4-methoxyphenyl)-5-substituted-1,3,4-oxadiazoles **5a**–**5f**. The purity of synthesized compounds was checked by TLC, column chromatography, and elemental analysis.

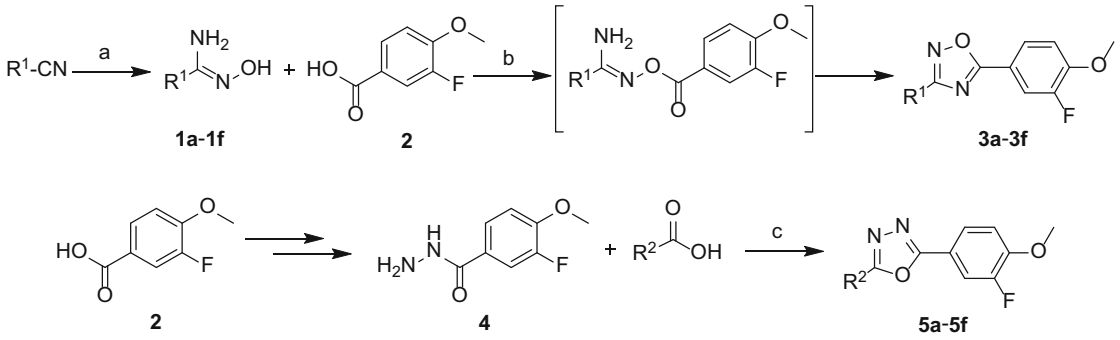
The structures of the synthesized compounds **1a**–**1f**, **3a**–**3f**, and **5a**–**5f** were established on the basis of their single-crystal X-ray diffraction method and spectral data (IR, ^{1}H NMR, ^{13}C NMR, and LC–MS). The structure of O-acylation of amidoxime **1c** was confirmed by single-crystal X-ray diffraction analysis (Fig. 1). The compound was crystallized in orthorhombic $Pna2_1$ space group [24].

The IR spectrum of compound **3e** showed absorption bands at 2985, 1625, 1504, 1288, and 1095 cm^{-1} for its Ar C–H, C=N, C=C, C–O, and C–F groups, respectively. The ^{1}H NMR spectrum of **3e**, which showed multiplet in the region 7.40–8.08 ppm was due to the aromatic protons. Similarly, the three protons of methoxy group appeared as a singlet at 3.96 ppm. The structure of **3e** was confirmed by the appearance of peaks at 169.4 and 174.8 ppm due to oxadiazole C-3 and C-5, respectively, in the ^{13}C NMR spectrum. The LC–mass spectra of compound **3e** showed a molecular ion peak at $m/z = 305$ ($[M+H]^+$, 96 %) and corresponding isotopic peak at $m/z = 307$ ($([M+H] + 2)^+$, 32 %), which was in agreement with the molecular formula $C_{15}H_{10}ClFN_2O_2$. In the IR spectrum, the absence of absorption bands, NH–NH$_2$ in the region of 3448–3437 cm^{-1} and C=O in the region of 1690 cm^{-1}, indicated the formation of compound **5f** from the corresponding acid hydrazide **4**. The absorption bands at 3020, 1610, 1506, 1276 and 1018 cm^{-1} are characteristic of the Ar C–H, C=N, C=C, C–O, and C–F groups, respectively. The ^{1}H NMR spectrum of **5f**, which showed multiplet in the region 6.70–7.90 ppm was due to the aromatic protons. Two singlets, which appeared in the region 3.90 and 2.98 ppm were due to the three protons of the methoxy group and six protons of the *N*,*N*-dimethyl group. The ^{13}C NMR spectra of **5f** revealed two signals at 152.1 and 153.4 ppm corresponding to oxadiazole C-2 and C-5, respectively. Further confirmation of the structure was given by LC–mass spectra, which showed a molecular ion peak at $m/z = 314$ ($[M+H]^+$, 97 %). In the same way, the structures of all the final compounds were confirmed by their characterization data, physical data, and the same is summarized in the Experimental section.

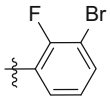
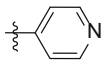
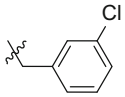
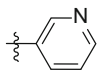
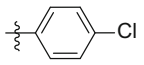
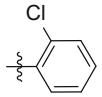
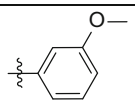
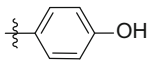
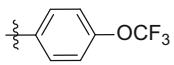
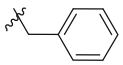
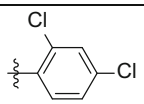
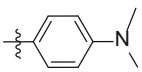
Anti-inflammatory activity

Anti-inflammatory activity of all the synthesized compounds was evaluated by carrageenan-induced rat paw edema model (Table 1). The results revealed that the compounds exhibited moderate to excellent anti-inflammatory activity ranging from 39 to 73 % inhibition and the

Scheme 1



(a) aq. $NH_2OH.HCl$, $NaHCO_3$, EtOH, reflux, 6 h; **(b)** HATU, DMF, NMM, reflux, 3 h; **(c)** $POCl_3$, reflux, 5 h

Compound	3a	3b	3c	3d	3e	3f
R^1						
Compound	5a	5b	5c	5d	5e	5f
R^2						

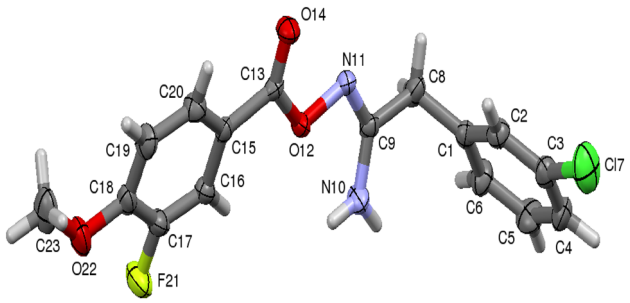


Fig. 1 Molecular structure of *O*-acylation of amidoxime **1c** with the atom numbering scheme. Thermal ellipsoids for non-hydrogen atoms are drawn at the 30 % probability level

standard drug diclofenac Na showed 75 % inhibition at 3 h post-drug administration. Compounds **3a**, **3f**, and **5a** turned out to be the most active with significant paw edema inhibition. Interestingly, most of the new compounds showed remarkable activity in the first 1 h of administration (>50 % inhibition), particularly compounds **3a** and **3f** exhibited higher inhibition than that of diclofenac Na in the initial 1 h. Compound **3a** demonstrated a high degree of activity with (73 %) inhibition followed by **3f** (71 %) and **5a** (69 %). Compounds **3a** and **3f** with a halogen group at C-2 and compound **5a** having an electron donating group at C-3 of the phenyl ring were correlated with higher inhibition values. The compounds **5f**, **3c**, and **3e** also showed moderate anti-inflammatory activity with 58, 56, and 51 %, respectively. Compounds **5e** (39 %) and **5b** (41 %) had the lowest effect and were poor inhibitors; presumably as a result of unfavourable electronic character and polarity. However, halogens can consequently increase the polarity and can also serve to block metabolism at particularly reactive sites and reduce metabolism of the aromatic group, by decreasing its electron density [30].

Analgesic activity

The results of the analgesic activity indicated that the test compounds exhibited moderate analgesic activity at 30 min of reaction time; the activity increased at 60 min, further, it reached peak level at 90 min, while decline in activity was observed at 120 min (Table 2). Particularly, samples **3a** (7.54 ± 0.12), **3f** (7.10 ± 0.06), and **5a** (7.32 ± 0.18) displayed excellent activity in 90 min duration and their results were comparable with standard drug pentazocine. Compounds **5f**, **3c**, **5e**, and **3e** showed moderate activity with rapid onset of action when compared with the standard. This suggests that electron diminishing and electron releasing groups are responsible for the activity. Moreover, compounds which showed best anti-inflammatory activity also exhibited significant analgesic activity.

Table 1 Anti-inflammatory activity of oxadiazole derivatives **3a–3f** and **5a–5f** by the carrageenan-induced rat paw edema assay

Treatments[a]	Mean paw volume/cm^3 ± SEM[b]			% Inhibition of edema		
	1/h	2/h	3/h	1/h	2/h	3/h
Control	1.13 ± 0.01	1.45 ± 0.001	1.42 ± 0.001	–	–	–
Diclofenac Na	0.42 ± 0.02***	0.39 ± 0.001***	0.36 ± 0.01***	63	73	75
3a	0.40 ± 0.05***	0.43 ± 0.003***	0.39 ± 0.002***	65	70	73
3b	0.51 ± 0.01*	0.70 ± 0.004**	0.78 ± 0.006	55	52	45
3c	0.46 ± 0.04**	0.57 ± 0.005*	0.63 ± 0.001**	59	61	56
3d	0.52 ± 0.02*	0.72 ± 0.006*	0.81 ± 0.003	54	50	43
3e	0.48 ± 0.06**	0.67 ± 0.005**	0.70 ± 0.006**	58	54	51
3f	0.38 ± 0.01***	0.45 ± 0.006***	0.41 ± 0.004**	66	69	71
5a	0.43 ± 0.04***	0.48 ± 0.005***	0.44 ± 0.002***	62	67	69
5b	0.57 ± 0.03*	0.76 ± 0.003	0.84 ± 0.004	50	48	41
5c	0.54 ± 0.05*	0.68 ± 0.002*	0.75 ± 0.002*	52	53	47
5d	0.49 ± 0.06	0.70 ± 0.004	0.79 ± 0.003	57	52	44
5e	0.58 ± 0.02*	0.56 ± 0.003	0.87 ± 0.001*	49	62	39
5f	0.45 ± 0.03***	0.54 ± 0.002**	0.60 ± 0.002**	60	63	58

[a] Dose levels: test compounds (50 mg/kg b.w.), diclofenac Na (10 mg/kg b.w.)

[b] *SEM* standard error mean; $n = 6$, ANOVA followed by Dunnett's test, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ significant from control

Table 2 Analgesic activity of oxadiazole derivatives **3a–3f** and **5a–5f** by the tail flick method

Treatments[a]	Tail flick latency/s (mean ± SEM)[b]				
	0/min	30/min	60/min	90/min	120/min
Control	2.50 ± 0.58	2.63 ± 0.17	2.58 ± 0.26	2.56 ± 0.30	2.52 ± 0.24
Pentazocine	2.60 ± 0.50	6.67 ± 0.35***	7.35 ± 0.24***	7.79 ± 0.18***	7.66 ± 0.16***
3a	2.00 ± 0.11	5.75 ± 0.26***	7.00 ± 0.14**	7.54 ± 0.12***	6.92 ± 0.16**
3b	2.50 ± 0.02	3.90 ± 0.19	5.31 ± 0.11*	4.87 ± 0.08*	4.45 ± 0.10
3c	2.38 ± 0.08	6.08 ± 0.35*	6.24 ± 0.33**	6.75 ± 0.05**	6.32 ± 0.14**
3d	2.11 ± 0.24	4.80 ± 0.12	5.22 ± 0.18*	5.42 ± 0.30*	5.11 ± 0.24*
3e	2.42 ± 0.20	5.50 ± 0.15*	5.92 ± 0.32*	6.24 ± 0.11**	5.83 ± 0.23*
3f	2.11 ± 0.01	5.45 ± 0.16**	6.53 ± 0.11**	7.10 ± 0.06***	6.86 ± 0.17**
5a	2.50 ± 0.10	5.83 ± 0.26***	6.77 ± 0.16**	7.32 ± 0.18***	6.81 ± 0.22**
5b	2.24 ± 0.22	5.31 ± 0.18*	5.50 ± 0.12*	5.42 ± 0.33**	5.10 ± 0.16
5c	2.10 ± 0.02	4.04 ± 0.04*	4.88 ± 0.08*	5.36 ± 0.22	4.72 ± 0.02
5d	2.60 ± 0.30	3.60 ± 0.06	4.30 ± 0.09	5.50 ± 0.15*	4.80 ± 0.12
5e	2.35 ± 0.04	5.42 ± 0.10**	6.43 ± 0.15***	6.86 ± 0.08*	2.30 ± 0.11**
5f	2.43 ± 0.07	6.00 ± 0.18***	6.86 ± 0.06**	6.60 ± 0.24**	6.40 ± 0.12*

[a] Dose levels: test compounds (50 mg/kg b.w.), pentazocine (10 mg/kg b.w.)

[b] *SEM* standard error mean; $n = 6$, ANOVA followed by Dunnett's test, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ significant from control

ADME profiling

From the varieties of pharmacological activities of the oxadiazole scaffold, it is important to explore the drug-like properties of this series of compounds. The computational study of synthesized compounds was assessed using ADME (absorption, distribution, metabolism, and elimination; http://www.molinspiration.com/cgi-bin/properties) prediction method and is presented in Table 3. The number of rotatable bonds (n-ROTB) and Lipinski's rule of five were calculated [32]. The rule states that most molecules with good membrane permeability have a molecular weight ≤500, a log $P \leq 5$, hydrogen bond donor sites ≤5, and hydrogen bond acceptor sites (N and O atoms) ≤10. This approach has been widely used as a filter for substances that were likely to be further developed in drug design

Table 3 Pharmacokinetic parameters important for good oral bioavailability of oxadiazole derivatives **3a–3f** and **5a–5f**

Compound	%ABS	Volume/Å^3	TPSA/Å^2	NROTB	HBA	HBD	Log *P*	MW	Lipinski's violations
3a	92	253.41	48.16	3	4	0	4.75	367.14	0
3b	88	226.43	61.05	3	5	0	2.58	271.25	0
3c	92	260.93	48.16	4	4	0	4.48	318.73	0
3d	88	226.43	61.05	3	5	0	2.80	271.25	0
3e	92	244.13	48.16	3	4	0	4.55	304.70	0
3f	92	244.13	48.16	3	4	0	4.50	304.70	0
5a	89	256.14	57.39	4	5	0	3.91	300.29	0
5b	85	238.61	68.39	3	5	1	3.39	286.26	0
5c	89	270.87	57.39	5	5	0	4.84	354.26	0
5d	92	247.39	48.16	4	4	0	3.47	284.29	0
5e	92	257.66	48.16	3	4	0	5.16	339.15	1
5f	91	276.50	51.40	4	5	0	3.98	313.33	0

%ABS percentage of absorption, *TPSA* topological polar surface area, *NROTB* number of rotatable bonds, *MW* molecular weight, *log P* logarithm of compound partition coefficient between *n*-octanol and water, *HBA* number of hydrogen bond donors, *HBD* number of hydrogen bond acceptors

programmes. In addition, the topological polar surface area (TPSA) was calculated, since it is a very useful parameter for the prediction of transport properties of drugs in the intestines and blood–brain barrier crossing [33]. TPSA is a sum of the surfaces of polar atoms (usually oxygen, nitrogen, and attached hydrogen) in a molecule. TPSA and volume are inversely proportional to %ABS. TPSA was used to calculate the percentage of absorption (%ABS) according to the equation: $\%ABS = 109 \pm 0.345 \times TPSA$. Molecules violating more than one of these rules may have problems with bioavailability [34, 35].

The synthesized compounds in general possess sufficient number of rotatable bonds (3-5) and, therefore, exhibit good conformational flexibility. From all ADME parameters, it can be observed that all the synthesized compounds exhibited excellent %ABS (85–92 %). The most active compounds showed **3a** (92 %), **3f** (92 %), and **5a** (89 %) absorption, respectively. Furthermore, none of the compounds violated Lipinski's rule of five, except **5e**, which violated only one rule of five as represented by the log *P* value. Thus, the results showed the most possible utility of the series for developing compounds with good drug-like properties. Theoretically, these compounds should present good passive oral absorption and differences in their bioactivity cannot be attributed to this property.

Conclusion

To summarize, two new sets of oxadiazole hybrids having 3-fluoro-4-methoxyphenyl group were successfully synthesized. The structures of the new derivatives were confirmed by numerous spectral studies such as IR, ^{1}H NMR, ^{13}C NMR, and LC–MS followed by single-crystal X-ray diffraction method and elemental analysis. These oxadiazole hybrids were tested for their in vivo anti-inflammatory and analgesic potential. Some of the compounds, exclusively **3a**, **3f**, and **5a** revealed potent anti-inflammatory and analgesic activities. The presence of the electron donor, acceptor or halo-substituted groups in the phenyl ring at positions C-2 and C-3, in addition, to the 3-fluoro-4-methoxyphenyl ring at position C-5 of the oxadiazole ring, causes a significant escalation in the activities. In silico ADME prediction of the synthesized compounds indicated that they had potential to develop as good oral drug candidates. These results prompted us to establish structure–activity relationships in the listing of synthesized compounds. These results further confirmed that suitable incorporation of different structural elements into a new single chemical entity enables achievement of higher inhibitory potency and selectivity. Besides, the active compounds in the present study make for interesting compounds when compared to the current therapeutic agents.

Experimental

All solvents and reagents were purchased from Sigma-Aldrich Chemicals Pvt. Ltd. (Bangalore, India). Melting point was taken in an open capillary tube. The purity of the compound was confirmed by thin-layer chromatography using Merck silica gel 60 F_{254}-coated aluminium plates. IR spectrum was recorded on Shimadzu-FTIR Infrared spectrometer in KBr. ^{1}H NMR and ^{13}C NMR spectra were recorded on 400 MHz and 100 MHz Bruker AMX-400 and Agilent-NMR spectrometers, with 5-mm PABBO BB-1H TUBES with TMS as internal standard in DMSO-d_6/

$CDCl_3$. LC–MS was obtained using Agilent 1200 series LC and Micromass zQ spectrometer. Elemental analysis was carried out using VARIO EL-III (Elementar Analysensysteme GmbH).

General procedure for the synthesis of aryl amidoximes 1a–1f

Sodium bicarbonate (5.88 g, 70 mmol) was added in portions to a solution of 4.79 g hydroxylamine hydrochloride (70 mmol) in 20 cm^3 of water. A solution of aryl nitriles (35 mmol) in 50 cm^3 of ethanol was then added, and the mixture stirred under reflux for 6 h. The precipitate formed was filtered off and purified by crystallization from ethanol. Melting points of compounds **1b–1f** were in agreement with the literature values [23, 25–28].

*3-Bromo-2-fluorobenzamidoxime (**1a**, $C_7H_6BrFN_2O$)*
Pale brown solid; yield 72 %; m.p.: 145–147 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.82 (s, 2H, NH_2), 7.20–8.05 (m, 3H, Ar–H), 10.20 (s, 1H, OH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 110.5, 122.3, 127.2, 127.4, 136.1, 158.4, 165.2 ppm; IR (KBr): $\bar{\nu}$ = 3445, 3358 (NH_2), 3190 (OH), 3061 (Ar C–H), 1665 (C=N), 962 (N–O) cm^{-1}; LC–MS: *m/z* (%) = 233 ($[M+H]^+$, 98), 235 ($([M+H] + 2)^+$, 86).

General procedure for the synthesis of 3,5-disubstituted 1,2,4-oxadiazoles 3a–3f

To a solution of 0.51 g 3-fluoro-4-methoxybenzoic acid (**2**, 3 mmol) in 10 cm^3 of DMF 1.15 g 1-[bis(dimethylamino)methylene]-1*H*-[1,2,3]-triazolo[4,5-*b*]pyridinium-3-oxide hexafluorophosphate (HATU, 3 mmol) was added followed by 1 cm^3 *N*-methylmorpholine (9 mmol) and lastly substituted aryl amidoximes **1a–1f** in DMF. The reaction mixture was stirred and refluxed for 3 h. After removal of solvent under vacuum, the reaction mixture was purified on a silica gel column eluting with 1–5 % methanol in dichloromethane. The desired 3,5-disubstituted 1,2,4-oxadiazoles were isolated and crystallized from ethanol.

3-(3-Bromo-2-fluorophenyl)-5-(3-fluoro-4-methoxyphenyl)-1,2,4-oxadiazole (**3a**, $C_{15}H_9BrF_2N_2O_2$)
Pale yellow solid; yield 80 %; m.p.: 178–180 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.91 (s, 3H, OCH_3), 7.00–8.02 (m, 6H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 56.3, 110.7, 113.4, 115.8, 116.0, 116.5, 116.6, 117.0, 150.9, 151.7, 153.5, 155.8, 158.3, 165.1, 174.4 ppm; IR (KBr): $\bar{\nu}$ = 3078 (Ar C-H), 1622 (C=N), 1514 (C=C), 1280 (C–O), 1128 (C–F) cm^{-1}; LC–MS: *m/z* (%) = 367 ($[M+H]^+$, 95), 369 ($([M+H] + 2)^+$, 99).

4-[5-(3-Fluoro-4-methoxyphenyl)-1,2,4-oxadiazol-3-yl]pyridine (**3b**, $C_{14}H_{10}FN_3O_2$)
White solid; yield 91 %; m.p.: 135–137 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.95 (s, 3H, OCH_3), 7.42–8.82 (m, 7H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 56.3, 111.3, 115.5, 115.7, 116.0, 117.2, 119.6, 121.8, 142.5, 162.7, 164.9, 172.3 ppm; IR (KBr): $\bar{\nu}$ = 2958 (Ar C–H), 1614 (C=N), 1522 (C=C), 1270 (C–O), 1072 (C–F) cm^{-1}; LC–MS: *m/z* (%) = 272 ($[M + H]^+$, 68).

3-(3-Chlorobenzyl)-5-(3-fluoro-4-methoxyphenyl)-1,2,4-oxadiazole (**3c**, $C_{16}H_{12}ClFN_2O_2$)
Pale yellow solid; yield 85 %; m.p.: 140–142 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.65 (s, 2H, CH_2), 3.95 (s, 3H, OCH_3), 7.22–7.85 (m, 7H, Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 32.0, 56.3, 119.0, 123.4, 127.2, 127.5, 128.6, 131.0, 133.6, 139.5, 139.7, 140.7, 147.6, 149.2, 162.6, 167.8 ppm; IR (KBr): $\bar{\nu}$ = 2941 (Ar C–H), 1631 (C=N), 1535 (C=C), 1290 (C–O), 1105 (C–F) cm^{-1}; LC–MS: *m/z* (%) = 319 ($[M+H]^+$, 95), 321 ($([M+H] + 2)^+$, 32).

3-[5-(3-Fluoro-4-methoxyphenyl)-1,2,4-oxadiazol-3-yl]pyridine (**3d**, $C_{14}H_{10}FN_3O_2$)
White solid; yield 86 %; m.p.: 151–153 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.96 (s, 3H, OCH_3), 7.90–8.92 (m, 7H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 56.4, 110.7, 114.1, 114.3, 116.2, 119.7, 120.5, 122.6, 142.5, 156.7, 162.7, 169.0 ppm; IR (KBr): $\bar{\nu}$ = 2972 (Ar C–H), 1610 (C=N), 1515 (C=C), 1282 (C–O), 1065 (C–F) cm^{-1}; LC–MS: *m/z* (%) = 272 ($[M+H]^+$, 72).

3-(4-Chlorophenyl)-5-(3-fluoro-4-methoxyphenyl)-1,2,4-oxadiazole (**3e**, $C_{15}H_{10}ClFN_2O_2$)
White solid; yield 82 %; m.p.: 123–125 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.96 (s, 3H, OCH_3), 7.40–8.08 (m, 7H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 56.3, 113.4, 116.1, 116.8, 117.0, 127.2, 127.4, 129.1, 129.9, 137.4, 150.9, 151.5, 153.4, 169.4, 174.8 ppm; IR (KBr): $\bar{\nu}$ = 2985 (Ar C–H), 1624 (C=N), 1504 (C=C), 1288 (C–O), 1095 (C–F) cm^{-1}; LC–MS: *m/z* (%) = 305 ($[M+H]^+$, 96), 307 ($([M+H] + 2)^+$, 32).

3-(2-Chlorophenyl)-5-(3-fluoro-4-methoxyphenyl)-1,2,4-oxadiazole (**3f**, $C_{15}H_{10}ClFN_2O_2$)
White solid; yield 77 %; m.p.: 163–165 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.93 (s, 3H, OCH_3), 7.20–8.00 (m, 7H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 56.4, 112.8, 115.9, 117.2, 119.7, 121.1, 124.4, 128.7, 131.0, 139.7, 147.6, 149.4, 157.7, 167.9, 172.8 ppm; IR (KBr): $\bar{\nu}$ = 3030 (Ar C–H), 1620 (C=N), 1520 (C=C), 1275 (C–O), 1080 (C–F) cm^{-1}; LC–MS: *m/z* (%) = 305 ($[M+H]^+$, 92), 307 ($([M+H] + 2)^+$, 35).

General procedure for the synthesis of 2,5-disubstituted 1,3,4-oxadiazoles 5a–5f

A mixture of 0.92 g 3-fluoro-4-methoxybenzohydrazide (**4**, 5 mmol) with different aromatic carboxylic acid (5 mmol) was refluxed with 10 cm^3 phosphorous oxychloride for 5 h. Reaction mixture was concentrated through rotovap, the residue was quenched with ice water and the solid separated was filtered off, washed with water, and purified by crystallization from ethanol to afford 2,5-disubstituted 1,3,4-oxadiazoles.

2-(3-Fluoro-4-methoxyphenyl)-5-(3-methoxyphenyl)-1,3,4-oxadiazole (**5a**, $C_{16}H_{13}FN_2O_3$)
Pale brown solid; yield 70 %; m.p.: 154–156 °C; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 3.81$ (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 6.70–7.98 (m, 7H, Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 56.6$, 59.1, 110.6, 114.9, 121.1, 127.2, 127.3, 128.6, 130.8, 132.3, 139.7, 139.9, 147.6, 149.3, 153.6, 155.0 ppm; IR (KBr): $\bar{\nu} = 3055$ (Ar C–H), 1614 (C=N), 1535 (C=C), 1262 (C–O), 1089 (C–F) cm^{-1}; LC–MS: *m/z* (%) = 301 ($[M + H]^+$, 95).

4-[5-(3-Fluoro-4-methoxyphenyl)-1,3,4-oxadiazol-2-yl]phenol (**5b**, $C_{15}H_{11}FN_2O_3$)
Pale red solid; yield 59 %; m.p.: 120–122 °C; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 3.97$ (s, 3H, OCH_3), 6.82–7.89 (m, 7H, Ar–H), 9.38 (s, 1H, OH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 57.3$, 116.1, 116.2, 121.3, 128.1, 128.7, 128.9, 129.7, 146.3, 149.7, 150.5, 153.7, 157.0 ppm; IR (KBr): $\bar{\nu} = 3366$ (OH), 3063 (Ar C–H), 1616 (C=N), 1526 (C=C), 1270 (C–O), 1050 (C–F) cm^{-1}; LC–MS: *m/z* (%) = 287 ($[M+H]^+$, 70).

2-(3-Fluoro-4-methoxyphenyl)-5-[4-(trifluoromethoxy)phenyl]-1,3,4-oxadiazole (**5c**, $C_{16}H_{10}F_4N_2O_3$)
White solid; yield 55 %; m.p.: 145–147 °C; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 3.90$ (s, 3H, OCH_3), 6.94–8.05 (m, 7H, Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 56.4$, 110.7, 119.6, 121.0, 122.2, 127.2, 127.4, 128.6, 139.6, 144.1, 147.6, 149.3, 152.7, 157.6 ppm; IR (KBr): $\bar{\nu} = 3032$ (Ar C–H), 1621 (C=N), 1538 (C=C), 1259 (C–O), 1025 (C–F) cm^{-1}; LC–MS: *m/z* (%) = 355 ($[M+H]^+$, 86).

2-Benzyl-5-(3-fluoro-4-methoxyphenyl)-1,3,4-oxadiazole (**5d**, $C_{16}H_{13}FN_2O_2$)
Light cream solid; yield 84 %; m.p.: 172–174 °C; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 3.90$ (s, 3H, OCH_3), 4.32 (s, 2H, CH_2), 7.28–7.75 (m, 8H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 31.9$, 56.3, 113.4, 114.8, 116.6, 116.7, 123.5, 123.8, 127.6, 128.8, 129.0, 150.5, 150.9, 153.4 ppm; IR (KBr): $\bar{\nu} = 3016$ (Ar C–H), 1622 (C=N), 1517 (C=C), 1282 (C–O), 1018 (C–F) cm^{-1}; LC–MS: *m/z* (%) = 285 ($[M+H]^+$, 99).

2-(2,4-Dichlorophenyl)-5-(3-fluoro-4-methoxyphenyl)-1,3,4-oxadiazole (**5e**, $C_{15}H_9Cl_2FN_2O_2$)
White solid; yield 72 %; m.p.: 116–118 °C; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 3.95$ (s, 3H, OCH_3), 7.39–8.19 (m, 6H, Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 56.3$, 110.7, 117.4, 118.2, 118.6, 125.5, 125.6, 127.2, 127.4, 128.6, 136.1, 139.7, 147.6, 153.2, 155.6 ppm; IR (KBr): $\bar{\nu} = 3025$ (Ar C–H), 1608 (C=N), 1542 (C=C), 1241 (C–O), 1030 (C–F) cm^{-1}; LC–MS: *m/z* (%) = 339 ($[M+H]^+$, 94), 341 ($([M+H] + 2)^+$, 65), 343 ($([M+H] + 4)^+$, 10).

4-[5-(3-Fluoro-4-methoxyphenyl)-1,3,4-oxadiazol-2-yl]-N,N-dimethylaniline (**5f**, $C_{17}H_{16}FN_3O_2$)
Cream solid; yield 80 %; m.p.: 160–162 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.98$ (s, 6H, 2 CH_3), 3.90 (s, 3H, OCH_3), 6.70–7.90 (m, 7H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 40.2$, 56.3, 111.2, 111.3, 113.4, 114.6, 117.2, 123.3, 128.3, 150.2, 150.3, 151.0, 152.1, 153.4 ppm; IR (KBr): $\bar{\nu} = 3020$ (Ar C–H), 1610 (C=N), 1506 (C=C), 1276 (C–O), 1018 (C–F) cm^{-1}; LC–MS: *m/z* (%) = 314 ($[M+H]^+$, 97).

Animals

Healthy male Wistar rats (150–200 g) and male Swiss albino mice (Mus musculus) were used for pharmacological screenings. The animals were procured from Venkateshwara Enterprises, Bangalore, India, (245/CPCSEA) and housed individually in polypropylene cages, maintained under standard conditions of alternating 12-h light and dark cycles at a constant temperature (25 ± 2 °C and 35–60 % relative humidity). The pharmacological evaluations were conducted after obtaining ethical clearance from the Institutional Animal Ethical Committee of K.S. Hegde Medical Academy, Deralakatte, Mangalore (India), Reg. no. 115/1999/CPCSER. The animals were fed standard mice and rat pellets procured from Hindustan Lever Ltd., Mumbai, India, and water ad libitum.

Acute toxicity

The test compounds used in the pharmacological study were evaluated for their acute toxicity in mice. Symptoms of intoxications were not observed in the animals (disorientation, hyperactivity, piloerection, and hyperventilation). Median lethal dose (LD_{50}) in male Swiss albino mice (Mus musculus) was determined by employing the standard methods [36, 37]. From the experiment, oral LD_{50} of test compounds were found to be 500 mg/kg body weight at

48 h duration. Hence, 50 mg/kg, i.e., 1/10 of cutoff value was taken as the screening dose for the evaluation of anti-inflammatory and analgesic activities.

Anti-inflammatory activity

The in vivo anti-inflammatory activity was evaluated using the carrageenan-induced rat paw edema assay model [29]. Male Wistar rats (150–200 g) were fasted with free access to water at least 12 h prior to the experiments and were divided randomly into 14 groups of six each. The control group received 1 cm^3 of 0.5 % sodium CMC, while the standard group received 10 mg/kg b.w. of diclofenac Na and test groups received 50 mg/kg b.w. of synthesized compounds **3a–3f** and **5a–5f**. The rats were dosed orally, 1 h later a subplantar injection of 0.05 cm^3 of 1 % solution of carrageenan in sterile distilled water was administered to the left hind footpad of each animal. The paw edema volume was measured with a digital plethysmometer at 1, 2, and 3 h after the carrageenan injection. The paw edema volume was compared with the vehicle control group and the percentage inhibition of edema was calculated using the formula:

$$\% \text{ Inhibition} = (1 - V_t/V_c) \times 100$$

where V_t is the mean paw volume of the test drug-treated rats and V_c is the mean paw volume of the control group.

Analgesic activity

The analgesic activity was determined by the tail flick method [31]. Male Wistar rats (150–200 g) in groups of six animals each were selected by the random sampling technique. Pentazocine at a dose level of 10 mg/kg was administered as a reference drug for comparison. The test compounds, at dose level of 50 mg/kg, were administered orally using the intragastric tube. The animals were held in position by a suitable restrain with the tail extending out and the tail (up to 5 cm) was then dipped in a beaker of water maintained at 55 ± 1 °C. The time taken to withdraw the tail clear out of water was taken as the reaction time and recorded in seconds. The readings were recorded at 0, 30, 60, 90, and 120 min after administration of compounds. A cutoff point of 10 s was observed to prevent tail damage.

Statistical analysis

In the present study, data values were expressed as mean ± SEM, by one-way ANOVA with Dunnett's t test to compare the difference between the groups. $P < 0.05$ was considered as significant difference.

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