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Original article

Synthesis and anti-inflammatory evaluation of methylene bridged benzofuranyl imidazo[2,1-*b*][1,3,4]thiadiazoles

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Abstract

A series of 6-substituted and 5,6-disubstituted 2-(6-methyl-benzofuran-3-ylmethyl)-imidazo[2,1-*b*][1,3,4]thiadiazoles have been synthesized. The new compounds have been tested for their *in vivo* analgesic, anti-inflammatory activities. Qualitative SAR studies indicate that the chloro substitution in the imidazole ring and introduction of formyl group at C-5 position of the imidazole ring increased the anti-inflammatory and analgesic activity. All the newly synthesized compounds have been characterized by spectral data and ORTEP diagram of one of the intermediates 6-(4-chlorophenyl)-2-(6-methyl-benzofuran-3-ylmethyl)-5-morpholin-4-ylmethyl imidazo[2,1-*b*][1,3,4]thiadiazole is reported herein.

Keywords: Imidazo[2,1-*b*][1,3,4]thiadiazoles; Anti-inflammatory; Analgesic activity

1. Introduction

Imidazo[2,1-*b*]-1,3,4-thiadiazole derivatives have been of interest to the medicinal chemists for many years because of their anticancer [1], antitubercular [2], antibacterial [3], antifungal [4], anticonvulsant, analgesic [5], and antisecretory [6] activities. Moreover, much interest has also been focused on the anti-inflammatory [7], cardiotonic [8], diuretic [9], and herbicidal [10] activities displayed by compounds incorporating this heterocyclic system. Because the imidazo[2,1-*b*]-1,3,4-thiadiazole system is similar in part to Levamisole, a well known immunomodulator [11] the possibility of reducing the harmful effects of the cytotoxic agents on the immune system also appears to be very attractive. Biheterocycles containing benzofuran with pyridine, thiadiazoles and chromone [12] rings have been found to exhibit antimicrobial, psychotropic and anti-inflammatory activities. For example, Efaroxan (2-imidazolinyl-2,3-dihydrobenzofuran) is a well known antagonist of α-2-adrenoreceptor [13]. Recently we have reported many benzofuran derivatives as good anti-inflammatory agents [14,15].

It was envisaged that these two active pharmacophores, if linked together would generate novel molecular templates which are likely to exhibit interesting biological properties in animal models. The above cited applications prompted us to synthesize a series of new compounds reported in this paper.

2. Chemistry

Benzofuran-3-acetic acid **1** required for the present work has been synthesized by the alkali hydrolysis of 7-methyl-4-bromomethyl coumarins [16], which upon reaction with thiosemicarbazide in presence of $POCl_3$ yielded 5-(6-methyl-benzofuran-3-ylmethyl)-[1,3,4]thiadiazol-2-yl-amine **3**. The imidazo[2,1-*b*][1,3,4]thiadiazole **5** was obtained by the condensation of **3** with α-bromoarylketones **4** under reflux in dry ethanol (Scheme 1). Mannich reaction of **5** with cyclic secondary amine morpholine and formaldehyde in presence of catalytic amount of acetic acid yielded derivative **6**. Vilsmeier–Haack reaction of imidazothiadiazole **5** in DMF and $POCl_3$

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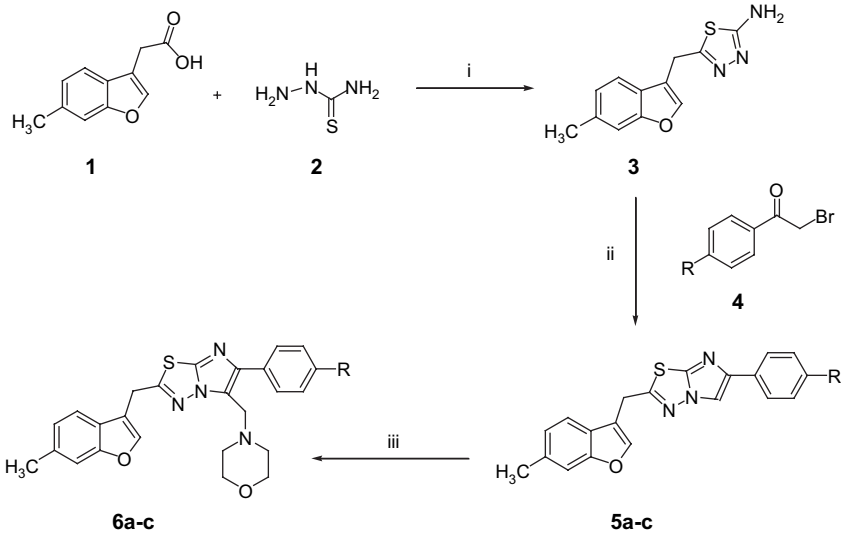
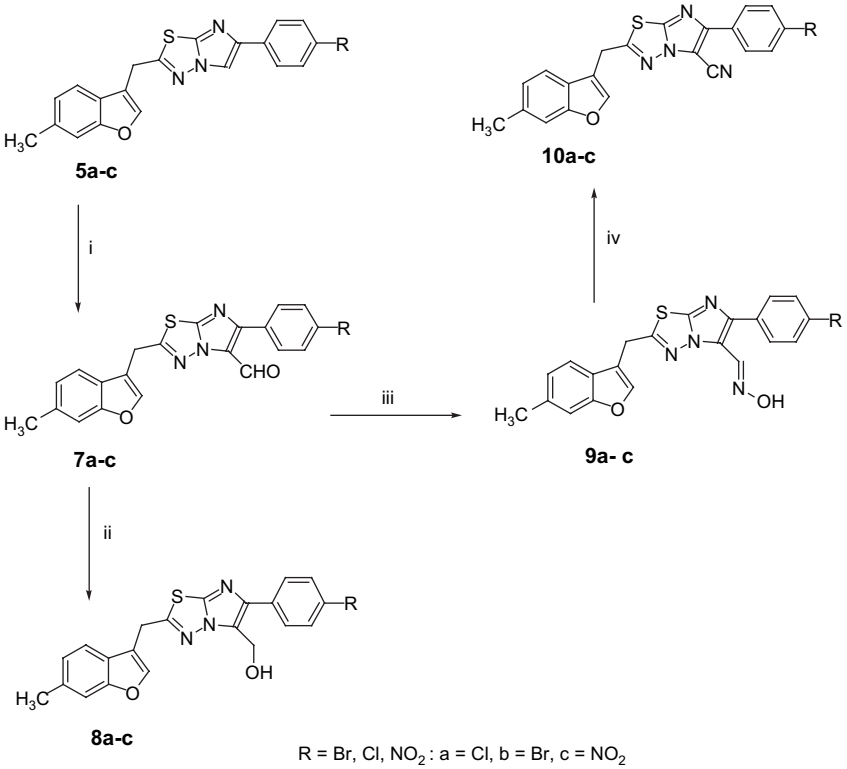


Scheme 1. Synthesis of benzofuranyl thiadiazoles. Reagents and conditions: (i) $POCl_3$, reflux, 30 min, KOH.; (ii) Dry EtOH, reflux, 24 h, Na_2CO_3; (iii) Morpholine, HCHO, AcOH, MeOH, reflux, 8 h.

furnished 5-formyl derivative **7** (Scheme 2). The aldehyde functional group in compound **7** has been utilized to synthesize corresponding alcohols, oxime and nitrile derivatives. The reduction of aldehyde **7** by $NaBH_4$ in methanol at room temperature yielded the respective carbinols **8** in good yields. The condensation of aldehyde **6** with hydroxylamine hydrochloride in pyridine gave corresponding oxime **9**, which on dehydration with acetic anhydride produced the nitrile **10** in moderate yields. Structures of all the newly synthesized compounds are well supported by spectral data such as IR, NMR, Mass and Elemental analysis. Compound **6a** has been confirmed by X-ray diffraction studies.



Scheme 2. Reagents and conditions: (i) DMF/$POCl_3$, Na_2CO_3; (ii) $NaBH_4$, MeOH, R.T; (iii) NH_2OH, pyridine, reflux; (iv) Ac_2O, reflux, 1 h.

2.1. Spectral characterization

The formation of 2-aminothiadiazole (**3**) was supported by the presence of ν_{N-H} band in the IR spectra and absence of carbonyl stretching band of the carboxyl acid function. The formation of imidazothiadiazole (**5a–c**) was indicated by the absence of ν_{N-H} band in the IR spectra and appearance of imidazole proton (C5–H) around δ 7.90 in ^{1}H NMR spectra. Formation of the Mannich bases (**6a–c**) was corroborated by the absence of imidazole proton and the appearance of a singlet around δ 4.00 ppm in the ^{1}H NMR spectra. Other aliphatic protons of the amine substituents resonated in the expected region as two triplets. Formation of these Mannich bases was further supported by mass spectra and the structure of **6a** has been subsequently confirmed by the X-ray crystal diffraction studies (Fig. 1, CCDC Deposit no. 637482). IR spectra of aldehyde **7** displayed a sharp band for carbonyl stretching frequency ($\nu_{c=o}$) around 1650 cm^{-1} and signal due to C5–H of imidazole was absent and a new signal for aldehydic proton was observed around δ 10 ppm in the ^{1}H NMR spectrum, thus substantiating the formation of imidazo[2,1-*b*][1,3,4]thiadiazoles-5-carbaldehydes (**7a–c**). The reduction products **8a–c** showed the absence of carbonyl stretching frequency ($\nu_{c=o}$) and the presence of broad band in the region 3150 cm^{-1} for ν_{O-H} in IR spectra. The ^{1}H NMR showed the absence of signal due to aldehydic proton, and the methylene protons resonated as singlet at around δ 5 ppm and OH proton was observed as broad singlet. This data confirms the conversion of carbaldehyde **7** to imidazo[2,1-*b*][1,3,4]thiadizole-5-carbinols (**8a–c**). The absence of $\nu_{C=O}$ band and the presence of ν_{O-H} band in the IR spectrum of the product supported the formation of oxime (**9a–c**). ^{1}H NMR spectra also confirmed the formation of aldoximes where the signal for aldehydic proton was absent, the azomethine and the –OH proton (D_2O exchangeable) resonated around δ 8.3 and 11.6 ppm, respectively. These compounds on treatment with acetic anhydride readily converted in to nitriles (**10a–c**) and these are characterized by a sharp band around 2200 cm^{-1} in the IR spectrum assigned for ν_{CN} and there was no band due to $\nu_{O-H.}$ The ^{1}H NMR of the compound was the useful probe to check the formation of the product as it reveals the absence of both the –OH and azomethine protons.

3. Biological activity

3.1. Acute toxicity studies

For testing the acute toxicity potential of the test compounds, albino rats of either sex weighing 25–30 g were selected, separated into groups each containing six rats. The dosage was varied from 100 up to 3000 mg kg^{-1} body weight.

The rats were continuously observed for 8 h for any signs of acute toxicity such as increased–decreased motor activity, ataxia, tremors, convulsions, sedation, lacrimation, etc. After 24 h the rats were sacrificed, stomach, intestine, and liver were inspected under the magnifying lenses for any ulcer-haemorrhagic spots.

3.2. Anti-inflammatory activity

Fourteen of all the newly synthesized compounds were screened for their *in vivo* inhibition of formalin induced paw oedema in rats, which is one of the most acceptable test procedures to evaluate anti-inflammatory agents [17]. Anti-inflammatory activity exhibited by the compounds may be attributed to the inhibition of cyclo oxygenase enzyme, which plays a vital role in inflammation process [18]. The standard used for the present anti-inflammatory activity testing is Ibuprofen. The test compounds were found to be significantly active.

3.3. Analgesic activity

Analgesic activity was measured by rat tail-flick method used by D'Amour and Smith [19]. The reaction time was

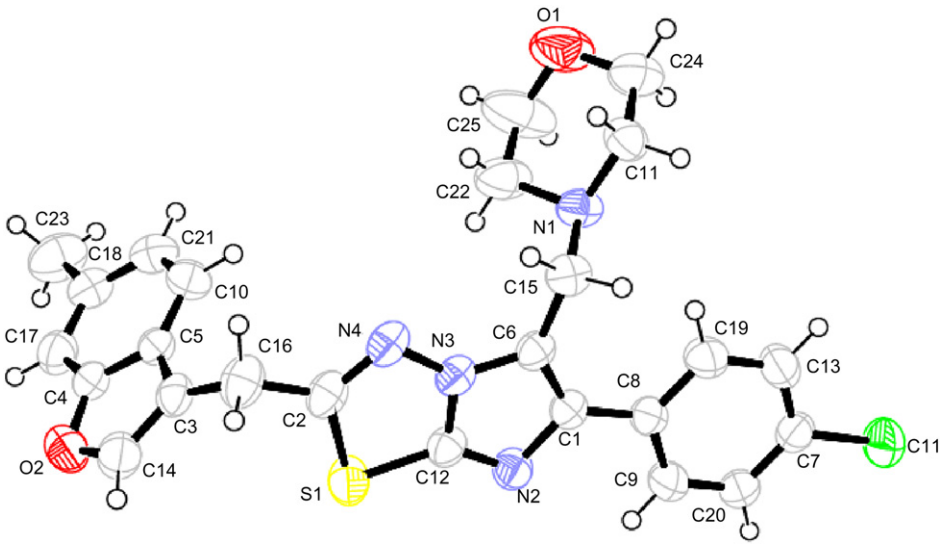


Fig. 1. ORTEP diagram with 50% thermal ellipsoidal probability of compound **6a**.

Table 1a
Comparison of oedema volume at different time intervals of compounds **5a–10a**

Compound	Oedema volume at different time intervals** ± S.E						
	0 h	0.5 h	1 h	2 h	3 h	4 h	5 h
Control	0.5333 ± 0.061	1.383 ± 0.060	1.617 ± 0.047	1.783 ± 0.054	2.00 ± 0.051	2.117 ± 0.070	2.150 ± 0.056
Standard	0.583 ± 0.006	0.800 ± 0.044	0.933 ± 0.071	1.017 ± 0.047**	0.800 ± 0.036**	0.800 ± 0.036**	0.583 ± 0.060**
5a	0.916 ± 0.060	1.267 ± 0.049	1.400 ± 0.057*	1.033 ± 0.033**	0.966 ± 0.066**	0.933 ± 0.061**	0.900 ± 0.051**
5b	1.117 ± 0.047	1.300 ± 0.044	1.450 ± 0.034	1.567 ± 0.055*	1.783 ± 0.054*	1.883 ± 0.074*	1.667 ± 0.143**
5c	1.083 ± 0.060	1.383 ± 0.030	1.400 ± 0.025*	1.350 ± 0.056**	1.183 ± 0.030**	1.150 ± 0.056**	1.080 ± 0.030**
6a	1.083 ± 0.047	1.317 ± 0.047	1.367 ± 0.033**	1.267 ± 0.042**	1.133 ± 0.042**	1.100 ± 0.025**	1.067 ± 0.033**
6b	1.033 ± 0.088	1.267 ± 0.080	1.417 ± 0.079*	1.217 ± 0.030**	1.133 ± 0.033**	1.033 ± 0.042**	1.017 ± 0.074**
6c	1.017 ± 0.030	1.333 ± 0.042	1.533 ± 0.033	1.350 ± 0.022**	1.167 ± 0.033**	1.100 ± 0.036**	1.050 ± 0.034**
7a	1.083 ± 0.070	1.333 ± 0.066	1.433 ± 0.033	1.350 ± 0.034**	1.150 ± 0.042**	1.117 ± 0.030**	1.100 ± 0.057**
7b	1.083 ± 0.060	1.367 ± 0.049	1.417 ± 0.030*	1.583 ± 0.054*	1.783 ± 0.060*	1.900 ± 0.057*	1.883 ± 0.060*
8a	0.916 ± 0.007	1.250 ± 0.067	1.383 ± 0.040**	1.267 ± 0.049**	1.083 ± 0.030**	0.966 ± 0.021**	0.883 ± 0.047**
8b	1.117 ± 0.060	1.350 ± 0.042	1.500 ± 0.036	1.550 ± 0.022**	1.267 ± 0.042**	1.167 ± 0.049**	1.133 ± 0.049**
9a	0.983 ± 0.060	1.300 ± 0.057	1.533 ± 0.033	1.583 ± 0.054*	1.800 ± 0.036*	1.833 ± 0.060*	1.483 ± 0.065**
10a	1.000 ± 0.051	1.317 ± 0.060	1.400 ± 0.063*	1.283 ± 0.047**	1.133 ± 0.049**	1.033 ± 0.042**	1.000 ± 0.025**

Model: acute inflammation; method: carragennan induced oedema; test animal: albino rats; number of animals per group: 6; route of administration: oral; standard: Ibuprofen (100 mg kg^{-1}); test compounds: 100 mg kg^{-1}. **$P < 0.01$, *$P < 0.05$. Statistical analysis: the statistical analysis was performed by one-way ANOVA followed by Dunnet's '*t*' test.

measured at the end of 60, 90, 120 and 300 min after the administration of the compound and the standard employed was Nimesulide.

4. Results and discussion

4.1. Acute toxicity studies

All the compounds have shown good safety profile till the highest dose. No adverse effect or mortality was detected in albino rats up to 3 g kg^{-1}, *p.o.* of SPA during the 24 h observation period.

There was no sedation, convulsions and tremors upon inspection, no ulceration and no haemorrhagic spots were observed. Postmortem examination of the stomach, and intestine did not reveal any ulcer-haemorrhagic spots.

4.2. Anti-inflammatory activity

4.2.1. Carragennan induced rat paw oedema method

The present study reports the anti-inflammatory activity of benzofuranyl imidazo[2,1-*b*][1,3,4]thiadiazoles derivatives (**5–10**). When compared with the control all the compounds showed reduction in oedema volume. Compounds **5** with no substituents at C-5 in the imidazole ring showed similar range of inhibition at the end of the 5 h except for the bromo substituted compound **5b**. The nitro derivative **5c** showed a delayed onset of action but the inhibition was pronounced at the end of third hour.

Amongst the various substituents at C-5 the best effects were observed with formyl and hydroxymethyl groups **7** and **8**. In these compounds also the bromo substituents has reduced the activity to the considerable extent (12.41%) as observed in **7b**. The conversion of the aldehyde at C-5 into oxime lead to the compound **9**, which also did not significantly inhibit the inflammation even at the end of 5 h. However, the corresponding nitrile **10a** was significantly active (Tables 1a and 1b).

These results obtained in these experiments are well supported by the analgesic activity data, which was evaluated by the reaction time (in seconds) at various time intervals.

4.3. Analgesic activity

The maximum reaction time observed in the case of the standard was 9.683 s and many compounds synthesized during the present investigation showed reaction times in the similar range.

Amongst the substituted imidazothiadiazoles the chloro and nitro compounds **5a** and **5c** showed reaction time of 9.633 and 9.900 s. The bromo compound **5b** also did not exhibit any significant activity.

Amongst the substitution at C-5 the morpholinomethyl derivative **6c** and the C-5 formyl derivative were quite promising. The nitrile **10a** showed considerable protection.

Table 1b
Anti-inflammatory activities of compounds **5a–10a** (% inhibition)

Compound	% Inhibition of inflammation at various time intervals					
	0.5 h	1 h	2 h	3 h	4 h	5 h
Control	0.0	0.0	0.0	0.0	0.0	0.0
Standard	42.15	42.30	42.90	60.00	66.14	72.86
5a	8.38	13.44	42.06	51.66	55.91	58.13
5b	6.00	10.32	12.00	10.85	11.05	22.46
5c	0.00	13.41	24.28	40.85	45.67	49.76
6a	4.77	15.46	28.93	43.35	48.03	50.37
6b	8.38	12.36	31.74	43.35	51.20	52.69
6c	3.6	5.19	24.28	41.65	48.03	51.16
7a	3.6	11.37	24.28	42.50	47.23	48.83
7b	1.15	12.36	11.21	10.85	10.25	12.41
8a	9.61	14.47	28.93	45.85	54.33	58.93
8b	2.38	7.20	13.06	36.65	44.87	47.30
9a	6.00	5.19	11.21	10.00	11.05	31.00
10a	4.77	13.41	28.04	43.35	51.20	53.48

It can be seen that there is a good correlation between the anti-inflammatory and the analgesic activity of the compounds (Table 2).

5. Experimental

5.1. Chemistry

The melting points were determined by open capillaries on a Buchi-apparatus and are uncorrected. The IR spectra were recorded on a Nicolet-Impact-410 FT-IR spectrometer, using KBR pellets. ^{1}H NMR and ^{13}C NMR spectra were recorded, respectively, on a Bruker 300 MHz and 75 MHz spectrometer, in $CDCl_3$ and DMSO-d_6 using TMS, as an internal standard and the values are expressed in δ ppm.The mass spectra were recorded using Agilent-single quartz LC–MS. The elemental analysis was carried out using Heraus CHN rapid analyzer. All the chemicals purchased were of analytical reagent grade, and were used without further purification unless otherwise stated.

5.1.1. Synthesis of 5-(6-methyl-benzofuran-3-ylmethyl)-[1,3,4]thiadiazol-2-yl-amine (3)

6-Methyl-benzofuran-3-acetic acid (19.2 g, 0.1 mol) and thiosemicarbazide (9.1 g, 0.1 mol) in phosphorous oxychloride (30 ml) were refluxed gently for 30 min. The solution was cooled; water (90 ml) was added carefully. The separated solid was filtered and suspended in water, and basified with aqueous potassium hydroxide. The solid was filtered, washed with water, dried and crystallized from mixture of DMF and ethanol (9:1) to obtain a colorless solid in 65% yield. IR (KBr) cm^{-1} 3281, 3102, 2960, 1635, 1521; ^{1}H NMR ($CDCl_3$, 300 MHz, TMS): δ 2.39 (s, 3H, C6–CH_3 of benzofuran), 4.21 (s, 2H, C3–CH_2 of benzofuran), 7.00 (br s, 2H, NH_2, D_2O exchangeable), 7.06 (d, 1H, $J = 5.9$ Hz, C5–H of benzofuran), 7.36 (s, 1H, C7–H of benzofuran), 7.40 (d, 1H, $J = 5.9$ Hz, C4–H of benzofuran), 7.84 (s, 1H, C2–H of benzofuran); LC–MS 246 [M + H]. Anal. Calcd for $C_{12}H_{11}N_3OS$: C, 58.76; H, 4.52; N, 17.13. Found: C, 58.84; H, 4.58; N, 17.16. M.p. 224–226 °C (aq. DMF).

5.1.2. Synthesis of 2-(6-methyl-benzofuran-3-ylmethyl)-6-phenyl-imidazo[2,1-b][1,3,4]thiadiazole (5): general procedure

A mixture of equimolar quantities of 5-(6-methyl-benzofuran-3-ylmethyl)-[1,3,4]thiadiazol-2-ylamine (0.01 mol) and appropriately substituted bromoacetyl compound (0.01 mol) was refluxed in dry ethanol for 24 h. The excess of solvent distilled off and solid hydrobromide that separated was collected by filtration, suspended in water and neutralized by aqueous sodium carbonate solution to get free base (**2**). It was filtered, washed with water, dried and recrystallized from suitable solvent.

*5.1.2.1. 6-(4-Chlorophenyl)-2-(6-methyl-benzofuran-3-ylmethyl)-imidazo[2,1-b][1,3,4]thiadiazole (**5a**).* Colorless solid (chloroform); yield 60%; m.p. 162–164 °C; IR (KBr) cm^{-1} 3112, 3022, 2915, 1631, 1527, 1471; ^{1}H NMR ($CDCl_3$, 300 MHz, TMS): δ 2.44 (s, 3H, C6–CH_3 of

Table 2
Analgesic activity of compounds **5a–10a**

Compound	Reaction time (in seconds)							
	0	30	60	120	180	% Increase	300	% Increase
Control	2.683 ± 0.132	2.600 ± 0.150	2.650 ± 0.154	2.667 ± 0.189	2.683 ± 0.151	0.00	2.683 ± 0.132	0.00
Standard	2.433 ± 0.120	5.100 ± 0.216**	7.683 ± 0.291**	8.650 ± 0.325**	9.683 ± 0.115**	260.90	8.550 ± 0.117**	218.67
5a	2.567 ± 0.135	3.483 ± 0.181*	4.033 ± 0.128**	8.750 ± 0.501**	9.633 ± 0.271**	259.03	7.950 ± 0.172**	196.31
5b	2.600 ± 0.112	2.650 ± 0.117	2.667 ± 0.111	2.583 ± 0.149	2.700 ± 0.77	0.633	2.683 ± 0.087	0.0
5c	2.550 ± 0.170	4.650 ± 0.299**	7.400 ± 0.498**	9.567 ± 0.240**	9.900 ± 0.068**	268.98	8.350 ± 0.147**	211.21
6a	2.983 ± 0.147	3.767 ± 0.190**	5.450 ± 0.275**	6.767 ± 0.391**	8.200 ± 0.538**	205.62	6.950 ± 0.498	159.03
6b	2.483 ± 0.124	3.500 ± 0.139*	4.067 ± 0.130**	6.450 ± 0.256**	8.150 ± 0.326**	203.76	7.300 ± 0.275**	172.08
6c	2.667 ± 0.158	3.600 ± 0.308**	5.933 ± 0.375**	8.433 ± 0.334**	9.300 ± 0.272**	246.62	8.033 ± 0.091**	199.40
7a	2.800 ± 0.123	3.550 ± 0.140**	5.350 ± 0.117**	6.667 ± 0.143**	7.867 ± 0.201**	193.21	6.517 ± 0.107**	142.89
7b	2.767 ± 0.143	3.983 ± 0.155**	4.433 ± 0.140**	6.500 ± 0.036**	9.083 ± 0.199**	238.53	7.550 ± 0.286**	181.40
8a	2.717 ± 0.199	3.650 ± 0.254**	4.350 ± 0.310**	5.467 ± 0.265**	7.133 ± 0.276**	165.85	5.433 ± 0.120**	102.49
8b	2.383 ± 0.175	3.667 ± 0.135**	4.583 ± 0.210**	5.917 ± 0.179**	7.400 ± 0.203**	177.30	6.067 ± 0.277**	126.12
9a	2.350 ± 0.117	2.483 ± 0.083	2.667 ± 0.098	2.633 ± 0.066	2.717 ± 0.060	1.26	2.600 ± 0.096	−3.09
10a	2.500 ± 0.129	3.700 ± 0.141**	4.067 ± 0.166**	6.617 ± 0.188**	8.250 ± 0.386**	207.49	7.167 ± 0.397**	167.12

Method: tail-flick method; test animal: albino rats; number of animals per group: 6; route of administration: oral; standard: Nimesulide (100 mg kg^{-1}); test compounds: 100 mg kg^{-1}.**$P < 0.01$, *$P < 0.05$.
Statistical analysis: the statistical analysis was performed by one-way ANOVA followed by Dunnet's '*t*' test.

benzofuran), 4.38 (s, 2H, C3–CH_2 of benzofuran), 7.16 (d, 1H, $J = 8.1$ Hz, C5–H of benzofuran), 7.31 (s, 1H, C7–H of benzofuran), 7.40 (d, 1H, $J = 8.1$ Hz, C4–H of benzofuran), 7.37 (d, 2H, $J = 7.8$ Hz, C2, C6–H phenyl), 7.66 (s, 1H, C2–H of benzofuran), 7.65 (d, 2H, $J = 7.8$ Hz, C3, C5–H phenyl), 7.99 (s, 1H, C5–H imidazole); ^{13}C NMR ($CDCl_3$, 75 MHz, TMS): δ 22.1, 27.3, 109.9, 112.4, 114.5, 120.0, 121.7, 124.9, 125.0, 130.0, 130.01, 133.2, 135.9, 136.2, 136.2, 142.8, 145.6, 146.2, 156.5, 163.9; LC–MS 380 [M + H]. Anal. Calcd for $C_{20}H_{14}ClN_3OS$ (%): C, 63.24; H, 3.71; N, 11.06. Found: C, 63.29; H, 3.75; N, 11.10.

*5.1.2.2. 6-(4-Bromophenyl)-2-(6-methyl-benzofuran-3-ylmethyl)-imidazo[2,1-b][1,3,4]thiadiazole (**5b**).* Colorless solid (ethanol); yield 50%; m.p. 178–180 °C; IR (KBr) cm^{-1} 3116, 2914, 1621, 1473, 1095; ^{1}H NMR ($CDCl_3$, 300 MHz, TMS): δ 2.48 (s, 3H, C6–CH_3 of benzofuran), 4.37 (s, 2H, C3–CH_2 of benzofuran), 7.09 (d, 1H, $J = 7.8$ H, C5–H of benzofuran), 7.34 (s, 1H, C7–H of benzofuran), 7.40 (d, 1H, $J = 7.80$ Hz, C4–H of benzofuran), 7.52 (d, 2H, $J = 8.1$ Hz, C2, C6–H phenyl), 7.62 (s, 1H, C2–H of benzofuran), 7.67 (d, 2H, $J = 8.1$ Hz, C3, C5–H phenyl), 7.97 (s, 1H, C5–H imidazole); ^{13}C NMR ($CDCl_3$, 75 MHz, TMS): δ 22.0, 27.3, 109.7, 112.4, 114.7, 119.2, 121.7, 124.6, 124.9, 127.0, 127.01, 132.2, 132.2, 133.2, 135.9, 142.8, 145.4, 146.2, 156.3, 163.4; LC–MS 425 [M + H]. Anal. Calcd for $C_{20}H_{14}BrN_3OS$ (%): C, 56.61; H, 3.33; N, 9.90. Found: C, 56.65; H, 3.30; N, 9.94.

*5.1.2.3. 6-(4-Nitrophenyl)-2-(6-methyl-benzofuran-3-ylmethyl)-imidazo[2,1-b][1,3,4]thiadiazole (**5c**).* Colorless solid (chloroform); yield 60%; m.p. 162–164 °C; IR (KBr) cm^{-1} 2921, 1602, 1523, 1307; ^{1}H NMR ($CDCl_3$, 300 MHz, TMS): δ 2.49 (s, 3H, C6–CH_3 of benzofuran), 4.41 (s, 2H, C3–CH_2 of benzofuran), 7.10 (d, 1H, $J = 7.80$ Hz, C5–H of benzofuran), 7.35 (s, 1H, C7–H of benzofuran), 7.41 (d, 1H, $J = 7.80$ Hz, C4–H of benzofuran), 7.95 (d, 2H, $J = 8.70$ Hz, C2, C6–H phenyl), 7.64 (s, 1H, C2–H of benzofuran), 8.26 (d, 2H, $J = 7.80$ Hz, C3, C5–H phenyl), 8.13 (s, 1H, C5–H imidazole); ^{13}C NMR ($CDCl_3$, 75 MHz, TMS): δ 22.1, 27.3, 109.9, 112.4, 114.5, 120.0, 121.7, 124.9, 125.0, 130.0, 130.0, 133.2, 135.9, 136.2, 136.2, 142.8, 145.6, 146.2, 156.5, 163.9; LC–MS 391 [M + H]. Anal. Calcd for $C_{20}H_{14}ClN_3OS$ (%): C, 63.24; H, 3.71; N, 11.06. Found: C, 63.29; H, 3.75; N, 11.10.

*5.1.3. Synthesis of [2-(6-methyl-benzofuran-3-ylmethyl)-5-morpholine-4-ylmethyl-6-phenyl-imidazo[2,1-b][1,3,4]thiadiazole (**6**): general procedure*

2-(6-Methyl-benzofuran-3-ylmethyl)-6-phenyl-imidazo[2,1-*b*][1,3,4]thiadiazole **5** (0.005 mol), morpholine (0.006 mol), formaldehyde (1 ml) and acetic acid catalytic amount in methanol (20 ml) was refluxed for 8 h (monitored by TLC). The solution was diluted with water, extracted with chloroform (3 × 30 ml), the combined chloroform extract was washed with water (3 × 30 ml), and dried over anhydrous sodium sulfate. The solution was evaporated to dryness in vacuum and the residue was crystallized from appropriate solvent.

*5.1.3.1. 6-(4-Chlorophenyl)-2-(6-methyl-benzofuran-3-ylmethyl)-5-morpholin-4-ylmethyl-imidazo[2,1-b][1,3,4]thiadiazole (**6a**).* Colorless solid (chloroform + petroleum ether); yield 65%; m.p. 158–161 °C; IR (KBr) cm^{-1} 3080, 2965, 1626; ^{1}H NMR ($CDCl_3$, 300 MHz, TMS): δ 2.46 (s, 3H, C6–CH_3 of benzofuran), 2.56 (t, 4H, $J = 4.0$ Hz, C3, C5–H of morpholine), 3.71 (t, 4H, $J = 4.0$ Hz, C2, C6–H of morpholine), 3.89 (s, 2H, CH_2), 4.39 (s, 2H, C3–CH_2 of benzofuran), 7.06 (d, 1H, $J = 5.9$ Hz, C5–H of benzofuran), 7.32 (s, 1H, C7–H of benzofuran), 7.38–7.41 (m, 3H, C4–H of benzofuran, C2, C6–H of phenyl), 7.61 (s, 1H, C2–H of benzofuran), 7.89 (d, 2H, $J = 6.0$ Hz, C3, C5–H of phenyl); LC–MS 480 [M + H]. Anal. Calcd for $C_{25}H_{23}ClN_4O_2S$ (%): C, 62.69; H, 4.84; N, 11.70. Found: C, 62.78; H, 4.97; N, 11.82.

*5.1.3.2. 6-(4-Bromophenyl)-2-(6-methyl-benzofuran-3-ylmethyl)-5-morpholin-4-ylmethyl-imidazo[2,1-b][1,3,4]thiadiazole (**6b**).* Colorless solid (chloroform + petroleum ether); yield 65%; m.p. 128–131 °C; IR (KBr) cm^{-1} 3091, 2930, 1646, 1455; ^{1}H NMR ($CDCl_3$, 300 MHz, TMS): δ 2.48 (s, 3H, C6–CH_3 of benzofuran), 2.57 (t, 4H, $J = 4.2$ Hz, C3, C5–H of morpholine), 3.70 (t, 4H, $J = 4.2$ Hz, C2, C6–H of morpholine), 3.89 (s, 2H, CH_2), 4.40 (s, 2H, C3–CH_2 of benzofuran), 7.09 (d, 1H, $J = 6.0$ Hz, C5–H of benzofuran), 7.33 (s, 1H, C7–H of benzofuran), 7.40 (d, 1H, $J = 6.0$ Hz, C4–H of benzofuran), 7.57 (d, 2H, $J = 6.3$ Hz, C2, C6–H of phenyl), 7.63 (s, 1H, C2–H of benzofuran), 7.84 (d, 2H, $J = 6.2$ Hz, C3, C5–H of phenyl). Anal. Calcd for $C_{25}H_{23}BrN_4O_2S$ (%): C, 57.36; H, 4.43; N, 10.70. Found: C, 57.42; H, 4.50; N, 10.78.

*5.1.3.3. 6-(4-Nitrophenyl)-2-(6-methyl-benzofuran-3-ylmethyl)-5-morpholin-4-ylmethyl-imidazo[2,1-b][1,3,4]thiadiazole (**6c**).* Colorless solid (chloroform + hexane); yield 59%; m.p. 104–106 °C; IR (KBr) cm^{-1} 2925, 1608, 1527, 1307; ^{1}H NMR ($CDCl_3$, 300 MHz, TMS): δ 2.48 (s, 3H, C6–CH_3 of benzofuran), 2.60 (t, 4H, $J = 4.5$ Hz, C3, C5–H of morpholine), 3.75 (t, 4H, $J = 4.4$ Hz, C2, C6–H of morpholine), 5.11 (s, 2H, CH_2), 4.42 (s, 2H, C3–CH_2 of benzofuran), 7.12 (d, 1H, $J = 6.2$ Hz, C5–H of benzofuran), 7.38 (s, 1H, C7–H of benzofuran), 7.41 (d, 1H, $J = 6.2$ Hz, C4–H of benzofuran), 7.98 (d, 2H, $J = 7.0$ Hz, C2, C6–H phenyl) 7.62 (s, 1H, C2–H of benzofuran), 8.23 (d, 2H, $J = 7.0$ Hz, C3, C5–H of phenyl). Anal. Calcd for $C_{25}H_{23}N_5O_4S$ (%): C, 61.34; H, 4.74; N, 14.31. Found: C, 61.43; H, 4.83; N, 14.40.

*5.1.4. Synthesis of 2-(6-methyl-benzofuran-3-ylmethyl)-6-phenyl-imidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde (**7**). Vilsmeir–Haack reaction: general procedure*

Vilsmeir–Haack reagent was prepared by adding phosphoryl-chloride (3 ml) in dimethyl formamide (20 ml) at 0 °C with stirring. Then appropriately substituted 2-(6-methyl-benzofuran-3-ylmethyl)-6-phenyl-imidazo[2,1-b][1,3,4]thiadiazole (**5a–c**, 0.01 mol) was added to the reagent and stirred at 0 °C for 30 min. The mixture was further stirred for 2 h at room

temperature and at 60 °C for additional 2 h. The reaction mixture was then poured in sodium carbonate solution and stirred at 90 °C for 2 h. After cooling, the mixture was diluted with water, and extracted with chloroform. Chloroform layer was dried over anhydrous sodium sulfate. The residue obtained after removal of chloroform was recrystallized from suitable solvent to get the crystalline solid.

*5.1.4.1. 6-(4-Chlorophenyl)-2-(6-methyl-benzofuran-3-ylmethyl)-imidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde (**7a**).* Brown solid (benzene + petroleum ether); yield 60%; m.p. 184–186 °C; IR (KBr) cm^{-1} 3105, 2917, 2837, 1648; ^1H NMR ($CDCl_3$, 300 MHz, TMS): δ 2.43 (s, 3H, C6–CH_3 of benzofuran), 4.53 (s, 2H, C3–CH_2 of benzofuran), 7.51 (d, 1H, $J = 8.4$ Hz, C5–H of benzofuran), 7.41 (d, 1H, $J = 8.4$ Hz, C4–H of benzofuran), 7.32 (s, 1H, C7–H of benzofuran), 7.70 (s, 1H, C2–H of benzofuran), 7.47 (d, 2H, $J = 8.0$ Hz, C2, C6–H of phenyl), 7.82 (d, 2H, $J = 8.4$ Hz, C3, C5–H of phenyl), 10.0 (s, 1H, CHO); ^{13}C NMR ($CDCl_3$, 75 MHz, TMS): δ 21.7, 27.2, 111.8, 114.3, 119.4, 124.3, 126.8, 127.0, 128.7, 129.4, 129.4, 130.6, 130.6, 131.0, 133.2, 136.5, 143.8, 154.4, 154.9, 166.9, 177.5; LC–MS 408 [M + H]. Anal. Calcd for $C_{21}H_{14}ClN_3O_2S$ (%): C, 61.84; H, 3.46; N, 10.30. Found: C, 61.97; H, 3.58; N, 10.45.

*5.1.4.2. 6-(4-Bromophenyl)-2-(6-methyl-benzofuran-3-ylmethyl)-imidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde (**7b**).* Brown solid (benzene); yield 60%; m.p. 208–210 °C; IR (KBr) cm^{-1} 3105, 3030, 2915, 2837, 1647; ^1H NMR ($CDCl_3$, 300 MHz, TMS): δ 2.48 (s, 3H, C6–CH_3 of benzofuran), 4.54 (s, 2H, C3–CH_2 of benzofuran), 7.10 (d, 1H, $J = 8.0$ Hz, C5–H of benzofuran), 7.41 (d, 1H, $J = 8.0$ Hz, C4–H of benzofuran), 7.35 (s, 1H, C7–H of benzofuran), 7.67 (s, 1H, C2–H of benzofuran), 7.64 (d, 2H, $J = 8.4$ Hz, C2, C6–H of phenyl), 7.75 (d, 2H, $J = 8.4$ Hz, C3, C5–H of phenyl), 10.07 (s, 1H, CHO); LC–MS 453 [M + H]. Anal. Calcd for $C_{21}H_{14}BrN_3O_2S$ (%): C, 55.76; H, 3.12; N, 9.29. Found: C, 55.85; H, 3.22; N, 9.18.

*5.1.4.3. 6-(4-Nitrophenyl)-2-(6-methyl-benzofuran-3-ylmethyl)-imidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde (**7c**).* Brown solid (benzene); yield 65%; m.p. 190–192 °C; IR (KBr) cm^{-1} 2922, 1654, 1603, 1524, 1307; ^1H NMR ($CDCl_3$, 300 MHz, TMS): δ 2.49 (s, 3H, C6–CH_3 of benzofuran), 4.40 (s, 2H, C3–CH_2 of benzofuran), 7.11 (d, 1H, $J = 8.0$ Hz, C5–H of benzofuran), 7.40 (d, 1H, $J = 8.0$ Hz, C4–H of benzofuran), 7.33 (s, 1H, C7–H of benzofuran), 7.64 (s, 1H, C2–H of benzofuran), 7.98 (d, 2H, $J = 8.5$ Hz, C2, C6–H of phenyl), 8.28 (d, 2H, $J = 8.5$ Hz, C3, C5–H of phenyl), 10.12 (s, 1H, CHO); LC–MS 419 [M + H]. Anal. Calcd for $C_{21}H_{14}N_4O_4S$ (%): C, 60.28; H, 3.37; N, 13.39. Found: C, 60.40; H, 3.45; N, 13.44.

*5.1.5. Synthesis of [2-(6-methyl-benzofuran-3-ylmethyl)-6-phenyl-imidazo[2,1-b][1,3,4]thiadiazole-5-yl]-methanol (**8**): general procedure*

To a stirred solution of 2-(6-methyl-benzofuran-3-ylmethyl)-6-phenyl-imidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde **7** (0.001 mol) in dry methanol (20 ml) was added in small portions sodium borohydride (0.0015 mol). The mixture was stirred at room temperature for 2 h (monitored by TLC), poured over ice water. The solid separated was collected by filtration, washed with cold methanol, dried and crystallized from appropriate solvent.

*5.1.5.1. [6-(4-Chlorophenyl)-2-(6-methyl-benzofuran-3-ylmethyl)-imidazo[2,1-b][1,3,4]thiadiazol-5-yl]-methanol (**8a**).* Colorless solid (ethanol); yield 70%; m.p. 184–186 °C; IR (KBr) cm^{-1} 3181, 2921, 1649, 1496, 1476; ^1H NMR ($CDCl_3$, 300 MHz, TMS): δ 2.44 (s, 3H, C6–CH_3 of benzofuran), 2.50 (br s, 1H, OH, D_2O exchangeable), 4.38 (s, 2H, C3–CH_2 of benzofuran), 5.06 (s, 2H, C5–CH_2–O of imidazole), 7.17 (d, 1H, $J = 8.0$ Hz, C5–H of benzofuran), 7.31 (s, 1H, C7–H of benzofuran), 7.40 (d, 1H, $J = 8.0$ Hz, C4–H of benzofuran), 7.50 (d, 2H, $J = 7.9$ Hz, C2, C6–H of phenyl), 7.65 (s, 1H, C2–H of benzofuran), 7.70 (d, 2H, $J = 7.9$ Hz, C3, C5–H of phenyl); LC–MS 410 [M + H]. Anal. Calcd for $C_{21}H_{16}ClN_3O_2S$ (%): C, 61.53; H, 3.93; N, 10.25. Found: C, 61.62; H, 4.01; N, 10.32.

*5.1.5.2. [6-(4-Bromophenyl)-2-(6-methyl-benzofuran-3-ylmethyl)-imidazo[2,1-b][1,3,4]thiadiazol-5-yl]-methanol (**8b**).* Colorless solid (ethanol); yield 75%; m.p. 188–190 °C; IR (KBr) cm^{-1} 3165, 2919, 1625, 1493; ^1H NMR ($CDCl_3$, 300 MHz, TMS): δ 2.48 (s, 3H, C6–CH_3 of benzofuran), 2.69 (br s, 1H, OH, D_2O exchangeable), 4.37 (s, 2H, C3–CH_2 of benzofuran), 5.04 (s, 2H, C5–CH_2–O of imidazole), 7.08 (d, 1H, $J = 8.0$ Hz, C5–H of benzofuran), 7.33 (s, 1H, C7–H of benzofuran), 7.39 (d, 1H, $J = 8.0$ Hz, C4–H of benzofuran), 7.54 (d, 2H, $J = 8.1$ Hz, C2, C6–H of phenyl), 7.62 (s, 1H, C2–H of benzofuran), 7.64 (d, 2H, $J = 8.1$ Hz, C3, C5–H of phenyl); ^{13}C NMR ($CDCl_3$, 75 MHz, TMS): δ 22, 27.3, 54.4, 112.4, 114.7, 119.2, 122.2, 122.7, 124.5, 124.9, 129.3, 129.3, 132.2, 132.2, 133.2, 135.9, 142.8, 143.6, 145.1, 156.3, 164.1; LC–MS 455 [M + H]. Anal. Calcd for $C_{21}H_{16}BrN_3O_2S$ (%): C, 55.51; H, 3.55; N, 9.25. Found: C, 55.60; H, 3.67; N, 9.29. M.p. (alcohol), yield 75%.

*5.1.5.3. [6-(4-Nitrophenyl)-2-(6-methyl-benzofuran-3-ylmethyl)-imidazo[2,1-b][1,3,4]thiadiazol-5-yl]-methanol (**8c**).* Colorless solid (ethanol); yield 72%; m.p. 172–174 °C; IR (KBr) cm^{-1} 3179, 2921, 1620, 1523, 1307; ^1H NMR ($CDCl_3$, 300 MHz, TMS): δ 2.45 (s, 3H, C6–CH_3 of benzofuran), 2.75 (br s, 1H, OH, D_2O exchangeable), 4.36 (s, 2H, C3–CH_2 of benzofuran), 5.20 (s, 2H, C5–CH_2–O of imidazole), 7.10 (d, 1H, $J = 7.8$ Hz, C5–H of benzofuran), 7.36 (s, 1H, C7–H of benzofuran), 7.40 (d, 1H, $J = 7.8$ Hz, C4–H of benzofuran), 7.98 (d, 2H, $J = 8.6$ Hz, C2, C6–H of phenyl), 7.60 (s, 1H, C2–H of benzofuran), 8.30 (d, 2H, $J = 8.6$ Hz, C3, C5–H of phenyl). Anal. Calcd for $C_{21}H_{16}N_4O_4S$ (%): C, 59.99; H, 3.84; N, 13.33. Found: C, 60.10; H, 3.92; N, 13.25.

*5.1.6. Synthesis of 2-(6-methyl-benzofuran-3-ylmethyl)-6-phenyl-imidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde oxime (**9**): general procedure*

A mixture of 2-(6-methyl-benzofuran-3-ylmethyl)-6-phenyl-imidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde **7** (0.001 mol)

and hydroxyl amine hydrochloride (0.0012 mol) was refluxed in pyridine (10 ml) for 6 h (monitored by TLC), the cooled mixture was then poured over ice and the precipitate was collected by filtration, washed with water, aqueous ethanol, dried and recrystallized from appropriate solvent to yield the corresponding aldoximes.

*5.1.6.1. 6-(4-Chlorophenyl)-2-(6-methyl-benzofuran-3-ylmethyl)-imidazo[2,1-b][1,3,4] thaidiazole-5-carbaldehyde oxime (**9a**).* Yellow solid (ethanol); yield 58%; m.p. 246–248 °C; IR (KBr) cm^{-1} 3258, 3098, 2921, 1621, 1535, 1498; ^1H NMR (DMSO-d_6, 300 MHz, TMS): δ 2.39 (s, 3H, C6–CH_3 of benzofuran), 4.55 (s, 2H, C3–CH_2 of benzofuran), 7.08–8.10 (m, 8H, C5, C4, C7, C2–H of benzofuran, C2, C6, C3, C5–H of phenyl), 8.33 (s, 1H, CHN), 11.60 (br s, 1H, OH, D_2O exchangeable). Anal. Calcd for $C_{21}H_{15}ClN_4O_2S$ (%): C, 59.64; H, 3.58; N, 13.25. Found: C, 59.74; H, 3.70; N, 13.30.

*5.1.6.2. 6-(4-Bromophenyl)-2-(6-methyl-benzofuran-3-ylmethyl)-imidazo[2,1-b][1,3,4] thaidiazole-5-carbaldehyde oxime (**9b**).* Colorless solid (ethanol); yield 61%; m.p. 240–242 °C; IR (KBr) cm^{-1} 3265, 3098, 2922, 1620, 1535, 1495; ^1H NMR (DMSO-d_6, 300 MHz, TMS): δ 2.40 (s, 3H, C6–CH_3 of benzofuran), 4.58 (s, 2H, C3–CH_2 of benzofuran), 7.09–8.00 (m, 8H, C5, C4, C7, C2–H of benzofuran, C2, C6, C3, C5–H of phenyl), 8.30 (s, 1H, CHN), 11.60 (br s, 1H, OH, D_2O exchangeable); LC–MS 468 [M + H]. Anal. Calcd for $C_{21}H_{15}BrN_4O_2S$ (%): C, 53.97; H, 3.24; N, 11.99. Found: C, 54.10; H, 3.35; N, 12.08.

*5.1.6.3. 6-(4-Nitrophenyl)-2-(6-methyl-benzofuran-3-ylmethyl)-imidazo[2,1-b][1,3,4] thaidiazole-5-carbaldehyde oxime (**9c**).* Colorless solid (ethanol); yield 60%; m.p. 224–226 °C; IR (KBr) cm^{-1} 3265, 3100, 2918, 1618, 1525, 1310; ^1H NMR (DMSO-d_6, 300 MHz, TMS): δ 2.42 (s, 3H, C6–CH_3 of benzofuran), 4.48 (s, 2H, C3–CH_2 of benzofuran), 7.12–8.40 (m, 4H, C5, C4, C7, C2–H of benzofuran), 7.98 (d, 2H, $J = 8.2$ Hz, C2, C6–H of phenyl), 8.25 (d, 2H, $J = 8.2$ Hz, C3, C5–H of phenyl) 8.35 (s, 1H, CHN), 11.62 (br s, 1H, OH, D_2O exchangeable). Anal. Calcd for $C_{21}H_{15}N_5O_4S$ (%): C, 58.19; H, 3.49; N, 16.16. Found: C, 58.27; H, 3.57; N, 16.24.

*5.1.7. Synthesis of 2-(6-methyl-benzofuran-3-ylmethyl)-6-phenyl-imidazo[2,1-b][1,3,4]thiadiazole-5-carbonitrile (**10**): general procedure*

The 2-(6-methyl-benzofuran-3-ylmethyl)-6-phenyl-imidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde oxime **9** (0.001 mol) was suspended in acetic anhydride (10 ml) and refluxed for 1 h. Poured over cold water, neutralized by sodium carbonate solution, the solid that separated was collected by filtration, washed repeatedly with water, dried and recrystallized from appropriate solvent.

*5.1.7.1. 6-(4-Chlorophenyl)-2-(6-methyl-benzofuran-3-ylmethyl)-imidazo[2,1-b][1,3,4]thiadiazole-5-carbonitrile (**10a**).* Yellow solid (chloroform + petroleum ether); yield 61%; m.p. 154–156 °C; IR (KBr) cm^{-1} 3068, 2917, 2212; ^1H NMR (DMSO-d_6, 300 MHz, TMS): δ 2.43 (s, 3H, C6–CH_3 of benzofuran), 4.65 (s, 2H, C3–CH_2 of benzofuran), 7.12 (d, 1H, $J = 5.8$ Hz, C5–H of benzofuran), 7.43 (s, 1H, C7–H of benzofuran), 7.55 (d, 1H, $J = 5.8$ Hz, C4–H of benzofuran), 7.64 (d, 2H, $J = 6.4$ Hz, C2, C6–H of phenyl), 7.97 (d, 2H, $J = 6.4$ Hz, C3, C5–H of phenyl), 8.03 (s, 1H, C2–H of benzofuran); LC–MS 405 [M + H]. Anal. Calcd for $C_{21}H_{13}ClN_4OS$ (%): C, 62.30; H, 3.24; N, 13.84. Found: C, 62.40; H, 3.35; N, 13.97.

*5.1.7.2. 6-(4-Bromophenyl)-2-(6-methyl-benzofuran-3-ylmethyl)-imidazo [2,1-b][1,3,4]thiadiazole-5-carbonitrile (**10b**).* Yellow solid (chloroform); yield 68%; m.p. 165–167 °C; IR (KBr) cm^{-1} 3068, 2917, 2225, 1630, 1602; ^1H NMR (DMSO-d_6, 300 MHz, TMS): δ 2.40 (s, 3H, C6–CH_3 of benzofuran), 4.62 (s, 2H, C3–CH_2 of benzofuran), 7.15 (d, 1H, $J = 6.0$ Hz, C5–H of benzofuran), 7.42 (s, 1H, C7–H of benzofuran), 7.53 (d, 1H, $J = 6.0$ Hz, C4–H of benzofuran), 7.62 (d, 2H, $J = 6.5$ Hz, C2, C6–H of phenyl), 7.95 (d, 2H, $J = 6.5$ Hz, C3, C5–H of phenyl), 7.99 (s, 1H, C2–H of benzofuran); LC–MS 450 [M + H]. Anal. Calcd for $C_{21}H_{13}BrN_4OS$ (%): C, 56.13; H, 2.92; N, 12.47. Found: C, 56.21; H, 3.00; N, 12.54.

*5.1.7.3. 6-(4-Nitrophenyl)-2-(6-methyl-benzofuran-3-ylmethyl)-imidazo [2,1-b][1,3,4]thiadiazole-5-carbonitrile (**10c**).* Yellow solid (chloroform); yield 63%; m.p. 174–174 °C; IR (KBr) cm^{-1} 3118, 2917, 2225, 1630, 1602, 1530, 1317; ^1H NMR (DMSO-d_6, 300 MHz, TMS): δ 2.44 (s, 3H, C6–CH_3 of benzofuran), 4.58 (s, 2H, C3–CH_2 of benzofuran), 7.12 (d, 1H, $J = 6.80$ Hz, C5–H of benzofuran), 7.45 (s, 1H, C7–H of benzofuran), 7.52 (d, 1H, $J = 6.80$ Hz, C4–H of benzofuran), 7.98 (d, 2H, $J = 8.0$ Hz, C2, C6–H of phenyl), 8.25 (d, 2H, $J = 8.0$ Hz, C3, C5–H of phenyl), 8.00 (s, 1H, C2–H of benzofuran). Anal. Calcd for $C_{21}H_{13}N_5O_3S$ (%): C, 60.71; H, 3.15; N, 16.86. Found: C, 60.80; H, 3.23; N, 16.93.

5.2. Pharmacology

5.2.1. Animals

Albino rats of Wister strain (150–200 g) and Swiss albino mice (25–30 g) of either sex were procured from the central animal house of the institute. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 2 °C; relative humidity 60–70%) in a 12 h light–dark cycle. The rats were given a standard laboratory diet and water *ad libitum*. Food was withdrawn 12 h before and during the experimental hours. All experimental protocols were approved by the institutional animal ethics committee.

5.2.2. Acute toxicity studies

For testing the acute toxicity potential of the test compounds, albino rats of either sex weighing 25–300 g were selected, separated into groups each containing six rats. The dosage was varied from 100 up to 3000 mg kg^{-1} body weight.

The rats were continuously observed for 8 h for any signs of acute toxicity such as increased–decreased motor activity, ataxia, tremors, convulsions, sedation, lacrimation, etc. After

24 h the rats were sacrificed, stomach, intestine, and liver were inspected under the magnifying lenses for any ulcer-haemorrhagic spots.

5.2.3. Anti-inflammatory activity

For the antiinflammatory activity the animals were divided into different groups as shown in Tables 1a and b. Acute inflammation was produced by subplantar injection of 0.1 ml of 1% suspension of carrageenan with 2% gum acacia in normal saline, in the right hind paw of the rats, 1 h after oral administration of the drugs. The paw volume was measured plethysmometrically (Ugo Basile, Italy) at 0 and 3 h after the carrageenan injection. The difference between the two readings was taken as the volume of oedema and the percentage anti-inflammatory activity was calculated. Aspirin 100 mg kg^{-1}, *p.o.* suspended in 2% gum acacia was used as the standard drug.

5.2.4. Analgesic activity

The animals (reaction time: 3–4 s) were divided into groups as shown in Table 2. Nimesulide is used as the standard drug. The drugs were administered orally. The tail-flick latency was assessed by the time taken by the rat to withdraw its tail from the organ bath containing hot water (temperature 55 ± 0.5°C). The tail-flick latency of treated animals was compared with control and standard.

5.2.5. Statistical analysis

The results were analyzed for statistical significance using one-way ANOVA followed by Dunnet's test. A P *value* < 0.05 was considered significant.

6. Conclusion

It can be seen that amongst the test compounds chloro substitution in the phenyl ring at position 6 of the imidazo[2,1-*b*][1,3,4]thiadiazoles ring increases both analgesic and anti-inflammatory activity and corresponding hydroxymethyl and morpolinomethyl substitution at position 5 also favoured significant activity. In general, test compounds are non-toxic up to 3000 mg kg^{-1} body weights of the animals and show significant *in vivo* anti-inflammation and analgesic activity. Further studies are required to establish their exact mechanism of action.

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