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Original article

Synthesis and antimicrobial studies of novel methylene bridged benzisoxazolyl imidazo[2,1-*b*][1,3,4]thiadiazole derivatives

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ABSTRACT

Novel methylene bridged benzisoxazolyl imidazo[2,1-*b*][1,3,4]thiadiazoles (**3a**–**f**) were synthesized from benzisoxazolyl-3-acetic acid and thiosemicarbazide. Reaction of **3** with bromine in glacial acetic acid in the presence of anhydrous sodium acetate yielded the corresponding 5-bromo derivatives (**4a**–**f**). While compound **3** furnished the 5-nitroso derivatives (**5a**–**f**) on refluxing with sodium nitrite solution. Thiocyanato derivatives (**6a**–**f**) were obtained by treating the imidazothiadiazole **3** with bromine and potassium thiocyanate in glacial acetic acid at room temperature. The structures of the newly synthesized compounds were confirmed by IR, NMR, mass and elemental analysis. All the compounds were screened for their antibacterial and antifungal activities. Some of the compounds displayed very good antibacterial and antifungal activity.

1. Introduction

After the discovery of the broad spectrum anthelmintic "tetramisole" [1], the synthesis and biological activities of many condensed imidazo[2,1-*b*][1,3,4]thiazoles [2–5] were reported. In addition, compounds closely related to tetramisole have been investigated. In this regard, many derivatives containing the imidazo[2,1-*b*][1,3,4]thiadiazole ring system, which is bioisosteric with the imidazo[2,1-*b*][1,3,4]thiazole system of tetramisole are being explored. Consequently, a large number of imidazo[2,1-*b*][1,3,4]thiadiazole derivatives have been reported to possess diverse pharmacological properties such as anticancer [6], antitubercular [7], antibacterial [8], antifungal [9], anticonvulsant, analgesic [10] and antisecretory [11] activities. Moreover, much interest has also been focused on the anti-inflammatory [12], cardiotonic [13], diuretic [14] and herbicidal [15] activities displayed by compounds incorporating this heterocyclic system.

Benzisoxazole derivatives are known to possess important biological activities [16] and are useful in different therapies. The 1,2-benzisoxazole moiety is isosteric with indole and can mimic/bind to biologically important enzymes in a manner similar to indole derivatives. Out of many biologically active compounds, zonisamide [17] is widely prescribed as an antiepileptic drug.

In view of the high degree of bioactivity shown by the above two heterocyclic compounds and in continuation of our search for biological active molecules [18–21], it was envisaged to construct a system, which combines both these systems in a single molecular frame work to explore the additive effects towards their biological activities. Hence we report herein the synthesis of hitherto unreported benzisoxazolyl imidazothiadiazoles and their bromo, nitroso and thiocycanato derivatives and evaluation of their antibacterial and antifungal activities.

2. Chemistry

1,2-Benzisoxazole-3-acetic acid required for the present work has been prepared by the reaction of the 4-hydroxycoumarin and hydroxylamine hydrochloride in dry methanol by adopting the well known procedure developed by Gianelli [22]. Reaction of 1,2-benzisoxazole-3-acetic acid with thiosemicarbazide in refluxing phosphorous oxychloride afforded the desired 2-amino-5-benzo[-*d*]isoxazol-3-ylmethyl-[1,3,4]thiadiazole (**2**), which upon condensation with α-haloaryl ketones under reflux in dry ethanol yielded the imidazothiadiazoles **3** in good yields. It is well established that this reaction proceeds via the intermediate iminothiadiazole [8] which under reflux temperature spontaneously undergoes dehydrocyclisation to form the desired fused heterocycle (Scheme 1). The electronic and steric factors at 5th position of 2-amino-5-substituted-1,3,4-thiadiazole are crucial in determining the course of its reaction with substituted α-haloarylketones. The strongly electronegative groups imparts less nucleophilic character to the

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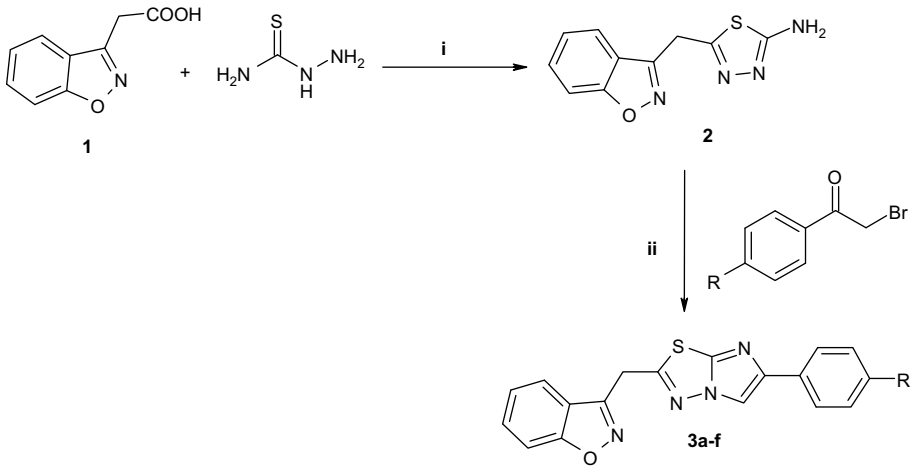


a, R = H; **b,** R = Cl; **c**, R = NO_2; **d,** R = Br; **e,** R = OMe; **f,** R = 3-Coumarinyl

Scheme 1. Reagents and conditions: (i) $POCl_3$, reflux, 45 min, KOH; (ii) dry EtOH, reflux, 18 h, Na_2CO_3.

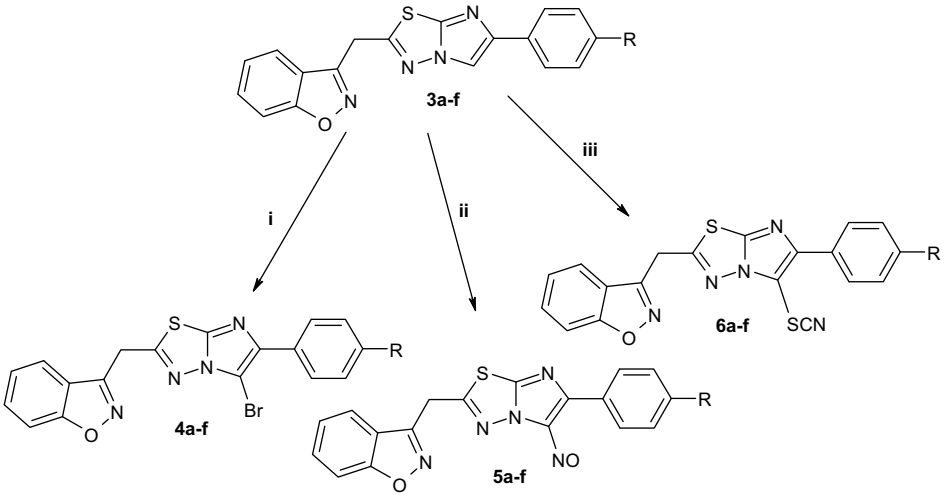
nitrogen at 4th position of the 1,3,4-thiadiazole. Various α-haloaryl ketones were prepared by the bromination of the corresponding ketones.

Thus obtained benzisoxazolyl imidazo[2,1-*b*][1,3,4]thiadiazole derivatives (**3**) were subjected to electrophilic substitution reaction to yield the corresponding 5-substituted derivatives (Scheme 2). Imidazothiadiazoles **3** on treatment with bromine in glacial acetic acid in the presence of anhydrous sodium acetate yielded the corresponding 5-bromo derivatives (**4a**–**f**). Nitroso derivatives (**5a**–**f**) were obtained by refluxing the imidazothiadiazoles **3** with sodium nitrite solution. While imidazothiadiazoles furnished thiocyanato derivatives (**6a**–**f**) on treating with bromine and potassium thiocyanate in glacial acetic acid at room temperature.

3. Results and discussion

The formation of 2-aminothiadiazole (**2**) by the reaction between 1,2-benzisoxazole-3-acetic acid and thiosemicarbazide was confirmed by its IR spectrum, which showed the presence of ν_{N-H} band and the absence of the carbonyl stretching band of the carboxylic acid. Further the ^{1}H NMR spectra displayed a D_2O exchangeable peak due to NH_2 at δ 7.14. Structures of imidazothiadiazole derivatives (**3a**–**f**) were established by the absence of ν_{N-H} band in the IR spectra and appearance of imidazole proton (C_5–H) around δ 8 in the ^{1}H NMR spectra. The ^{13}C NMR and mass spectra of these compounds further confirmed the assigned structures.

All the electrophilic substitution reactions carried out on imidazothiadiazole derivatives (**3a**–**f**) afforded the expected 5-substituted derivatives. The structures of the so obtained 5-bromo derivatives (**4a**–**f**), 5-nitroso derivatives (**5a**–**f**) and 5-thiocyanato derivatives (**6a**–**f**) were confirmed by their analytical and spectral data. All the 5-substituted derivatives showed the absence of C_5–H in their ^{1}H NMR spectra confirming the substitution at 5th position. The IR spectra of compounds **5** displayed the absorption bands at 1529–1540 cm^{-1} for NO while compounds **6** showed bands at 2138–2184 cm^{-1} for SCN. The structures of all the compounds were finally ascertained by the ^{13}C NMR and mass spectral data.



a, R = H; **b,** R = Cl; **c**, R = NO_2; **d,** R = Br; **e,** R = OMe; **f,** R = 3-Coumarinyl

Scheme 2. Reagents and conditions: (i) Br_2/AcOH, sod. acetate; (ii) $NaNO_2$, AcOH, reflux, 2 h; (iii) Br_2 KSCN, AcOH.

4. Antimicrobial activity

The newly synthesized compounds were screened for their antibacterial and antifungal screening using agar well diffusion method [23].

The antibacterial activity of the test compounds was evaluated against two Gram-positive bacteria, *Staphylococcus aureus*-ATCC 25923, *Bacillus subtilis*-ATCC 6633 and Gram-negative bacteria *Pseudomonas aeruginosa*-ATCC 10145, *Escherichia coli*-ATCC 35218. Ampicillin was used as standard drug.

Antifungal activity was screened against two fungal strain, *Candida albicans* and *Aspergilus fumigatus* using Clotrimazole as standard drug. Dimethylformamide was used as solvent control. The bacterial cultures were inoculated on Mueller Hinton Agar (Merck) and fungal culture was inoculated on Potato Dextrose Agar. Media (20 mL) were poured into each sterilized Petri dish (99 mm). Media plates were inoculated with liquid cultures homogeneously by spread plate method.

All the compounds were dissolved in dimethylsulfoxide (DMSO) to get a concentration of 100 μg. Each sample (100 μL) was loaded into the wells of agar plates directly. Plates inoculated with the bacteria were incubated at 37 °C for 24 h and the fungal culture was incubated at 25 °C for 72 h. All determinations were done in triplicates. The standard antibiotic, Ampicillin (100 μg/ml) for bacteria and Clotrimazole (100 μg/ml) for fungal were used as positive controls and 100 μl of DMSO used as a negative control, zone of inhibitions were recorded in mm.

Priliminary screening was conducted for all compounds at 100 μg/mL concentration, against the above-mentioned microorganisms. Different series of dilutions of compounds were made (0.5, 1.0, …, 5.0 μg/mL) to determine the MIC. The results are given in Table 1.

The investigation of antibacterial screening revealed that some of the tested compounds showed moderate to good bacterial inhibition. Particularly compounds **3b**, **3d**, **4b**, **4e** and **6b** have shown very good activity against *Bacillus subtilis*-ATCC 6633 and *Escherichia coli*-ATCC 35218. Compound **6b** has exhibited very good activity against all the bacterial strains, however compounds **3b** and **3d** are highly active against *Escherichia coli*-ATCC 35218 when compared to Ampicilin. The high activity is attributed to the presence of electron withdrawing chloro and bromo functional groups.

Antifungal results indicated that compounds **3d**, **3f** and **6e** have shown good activity against *Candida albicans*. Compound **3d** showed very good activity comparable to that of standard while compounds **3a**, **3e** and **6f** have shown good activity against *Aspergilus fumigates*.

5. Conclusion

We have synthesized novel methylene bridged benzisoxazolyl imidazothiadiazoles and their bromo, nitroso and thiocyanato derivatives. The results of antibacterial screening reveals that among all the compounds screened five compounds showed good bacterial inhibition while six of the newly synthesized compounds displayed good antifungal activity.

6. Experimental protocols

Melting points were determined in open capillaries and are uncorrected. The IR spectra were recorded on Nicolet Impact 410 FT IR spectrophotometer using KBr pellets. ^{1}H and ^{13}C NMR were recorded on Bruker 300-MHz FT NMR spectrometer in $CDCl_3$ and DMSO-d_6 with TMS as internal standard. Mass spectrum was recorded on Finnigan MAT (Model MAT8200) spectrometer and elemental analyses were carried out using Heraus CHN rapid analyzer. The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel plate using hexane and ethyl acetate.

6.1. Preparation of Benzo[d]isoxazol-3-yl-acetic acid (*1*)

1,2-Benzisoxazole-3-acetic acid was prepared according to the literature method [22]. Colorless crystalline solid. Yield 73%, m.p 123–125 °C (lit. m.p. 126 °C).

Table 1
Antibacterial and antifungal activities of the compounds (**2**, **3a–f**, **4a–f**, **5a–f** and **6a–f**) [zone of inhibition in mm and MIC values (mean of triplicates)].

Compd. No.	*B. substilis*	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. fumigatus*
2	15 (1)	15 (1.5)	10 (3.5)	14 (2.5)	14 (3)	11 (3.5)
3a	11 (2)	16 (1)	09 (3.5)	09 (3.5)	12 (3.5)	19 (2)
3b	17 (0.5)	12 (2)	18 (1.5)	12 (3)	11 (3.5)	16 (1.5)
3c	11 (2.5)	14 (1.5)	13 (3)	08 (3.5)	10 (3.5)	11 (4)
3d	18 (0.5)	15 (2.5)	20 (1)	14 (2.5)	22 (2)	13 (3.5)
3e	14 (2.5)	10 (3.5)	12 (3)	11 (3)	15 (3)	19 (2)
3f	15 (1)	13 (3)	10 (3.5)	10 (3)	20 (2.5)	13 (3.5)
4a	12 (2.5)	18 (1)	11 (3)	16 (2)	14 (3)	12 (3.5)
4b	18 (0.5)	17 (1.5)	15 (2)	12 (2.5)	15 (3)	10 (4)
4c	12 (2.5)	11 (2)	10 (3.5)	11 (3)	09 (4)	08 (4.5)
4d	12 (2.5)	13 (2)	14 (2)	16 (2)	16 (2.5)	16 (2.5)
4e	18 (0.5)	11 (1.5)	16 (2)	17 (2)	10 (4)	15 (2.5)
4f	10 (3)	15 (2)	13 (2.5)	14 (2.5)	14 (3)	17 (2.5)
5a	13 (2)	18 (1)	11 (3)	10 (3)	13 (3.5)	16 (2.5)
5b	14 (1.5)	10 (2)	13 (3)	15 (2.5)	15 (3)	17 (2.5)
5c	14 (1.5)	12 (1.5)	09 (3.5)	11 (3)	17 (2.5)	11 (4)
5d	12 (2.5)	16 (1)	14 (2)	12 (3)	17 (2.5)	15 (2.5)
5e	11 (3)	13 (1.5)	16 (2)	08 (3.5)	14 (3)	10 (4)
5f	15 (1.5)	14 (1.5)	12 (3)	14 (2.5)	15 (3)	12 (3.5)
6a	14 (1.5)	16 (1.5)	12 (3)	17 (2)	12 (3.5)	15 (2.5)
6b	17 (1)	18 (1)	16 (2)	19 (1.5)	15 (3)	11 (4)
6c	08 (2.5)	09 (2)	7 (4)	10 (3)	11 (3.5)	09 (3.5)
6d	14 (2)	12 (1.5)	15 (2)	18 (2)	14 (3)	12 (4)
6e	15 (1)	09 (2)	13 (3)	19 (1.5)	20 (2.5)	16 (2.5)
6f	15 (1)	14 (1.5)	14 (3.5)	16 (2)	12 (3.5)	19 (2)
Ampicilin	18 (0.5)	19 (1)	16 (2)	17 (2)	–	–
Clotrimazole	–	–	–	–	22 (2)	20 (2)
Control	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO

MIC values are given in brackets.

*6.2. Preparation of 2-amino-5-benzo[*d*]isoxazol-3-ylmethyl-[1,3,4] thiadiazole (**2**)*

1,2-Benzisoxazole-3-acetic acid (10 g, 0.06 mol) and thiosemicarbazide (5.14 g, 0.6 mol) in phosphorous oxychloride (30 ml) was refluxed gently for 45 min. The reaction mixture was cooled and quenched (highly exothermic) with cold water (90 ml). The resulting solution was refluxed for additional 4 h and filtered hot. The filtrate was cooled and basified with aqueous potassium hydroxide solution. The solid that separated was filtered, washed with water, dried and recrystallized from ethanol and DMF. Brown solid, yield 56%, m.p. 210–212 °C, IR (KBr) ν, cm^{-1}: 3283, 3112, 2767, 2679, 1622, 1518; ^1H NMR (300 MHz, DMSO-d_6) δ: 4.66 (s, 2H, CH_2), 7.14 (s, 1H, D_2O exchangeable, NH), 7.39–7.76 (m, 4H, Ar-H). GC–MS 233. Anal. calcd. for $C_{10}H_8N_4OS$: C, 51.71; H, 3.47; N, 24.12. Found: C, 51.79; H, 3.42; N, 24.19%.

*6.3. Preparation of 3-(6-arylimidazo[2,1-*b*][1,3,4]thiadiazol-2-ylmethyl)benzo[*d*]isoxazole (**3a–f**)*

General method. A mixture of equimolar quantities of 2-amino-5-benzo[*d*]isoxazol-3-ylmethyl-[1,3,4]thiadiazole **2** (2.32 g, 0.01 mol) and bromoacetyl compound (0.01 mol) was refluxed in dry ethanol for 18 h. The excess of solvent was distilled off and the solid hydrobromide salt that separated was collected by filtration, suspended in water and neutralized by aqueous sodium carbonate solution to get free base (**3a–f**). It was filtered, washed with water, dried and recrystallized from suitable solvent.

*6.3.1. 3-(6-Phenylimidazo[2,1-*b*][1,3,4]thiadiazol-2-ylmethyl) benzo[*d*]isoxazole (**3a**)*

Brown crystalline solid (ethanol), yield 54%, m.p. 164–166 °C; IR (KBr) ν, cm^{-1}: 2922, 2851, 1599, 1501, 1486; ^1H NMR (300 MHz, $CDCl_3$) δ: 4.74 (s, 2H, CH_2), 7.30–7.63 (m, 5H, Ar-H), 7.73 (d, $J = 7.83$ Hz, 2H, Ar-H), 7.82 (d, $J = 7.39$ Hz, 2H, Ar-H), 8.02 (s, 1H, C_5–H, imidazole); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 28.9, 109.7, 110.6, 120.9, 121.5, 124.4, 125.5, 128.0, 129.1, 130.9, 134.0, 146.0, 146.9, 153.5, 158.8, 164.0. Anal. calcd. for $C_{18}H_{12}ON_4S$: C, 65.04; H, 3.64; N, 16.86. Found: C, 65.10; H, 3.71; N, 16.92%.

*6.3.2. 3-[6-(4-Chlorophenyl)imidazo[2,1-*b*][1,3,4]thiadiazol-2-ylmethyl]benzo[*d*]isoxazole (**3b**)*

White crystalline solid (ethanol + DMF), yield 51%, m.p. 152–154 °C; IR (KBr) ν, cm^{-1}: 2924, 2854, 1597, 1504; ^1H NMR (300 MHz, $CDCl_3$) δ: 4.74 (s, 2H, CH_2), 7.39 (d, $J = 8.4$ Hz, 2H, Ar-H), 7.61–7.69 (m, 4H, Ar-H), 7.75(d, $J = 8.4$ Hz, 2H, Ar-H), 7.99 (s, 1H, C_5–H, imidazole). Anal. calcd. for $C_{18}H_{11}ClN_4OS$: C, 58.94; H, 3.02; N, 15.27. Found: C, 59.00; H, 3.10; N, 15.32%.

*6.3.3. 3-[6-(4-Nitrophenyl)imidazo[2,1-*b*][1,3,4]thiadiazol-2-ylmethyl]benzo[*d*]isoxazole (**3c**)*

Yellow crystalline solid (ethanol + DMF), yield 50%, m.p. 168–170 °C; IR (KBr) ν, cm^{-1}: 2924, 2853, 1599, 1501; ^1H NMR (300 MHz, $CDCl_3$) δ: 4.77 (s, 2H, CH_2), 7.35–7.73 (m, 4H, Ar-H), 7.98 (d, $J = 8.61$ Hz, 2H, Ar-H), 8.16 (s, 1H, C_5–H, imidazole), 8.29 (d, $J = 8.58$ Hz, 2H, Ar-H). Anal. calcd. for $C_{18}H_{11}N_5O_3S$: C, 57.29; H, 2.94; N, 18.56. Found: C, 57.35; H, 3.01; N, 18.62%.

*6.3.4. 3-[6-(4-Bromophenyl)imidazo[2,1-*b*][1,3,4]thiadiazol-2-ylmethyl]benzo[*d*]isoxazole (**3d**)*

Brown color crystalline solid (ethanol + dioxan), yield 51%, m.p. 157–159 °C; IR (KBr) ν, cm^{-1}: 2924, 2854, 1511, 1474; ^1H NMR (300 MHz, $CDCl_3$) δ: 4.74 (s, 2H, CH_2), 7.38 (d, $J = 6.8$ Hz, 2H, Ar-H), 7.54 (d, $J = 7.9$ Hz, 2H, Ar-H), 7.61–7.72 (m, 4H, Ar-H), 8.01 (s, 1H, C_5–H, imidazole). Anal. calcd. for $C_{18}H_{11}BrN_4OS$: C, 52.57; H, 2.70; N, 13.62. Found: C, 52.49; H, 2.76; N, 13.58%.

*6.3.5. 3-[6-(4-Methoxyphenyl)imidazo[2,1-*b*][1,3,4]thiadiazol-2-ylmethyl]benzo[*d*]isoxazole (**3e**)*

Yellow solid (ethanol + dioxan), yield 55%, m.p. 161–163 °C; IR (KBr) ν, cm^{-1}: 2922, 2852, 1595, 1501; ^1H NMR (300 MHz, $CDCl_3$) δ: 3.80 (s, 3H, OCH_3), 4.73 (s, 2H, CH_2), 7.30–7.65 (m, 6H, Ar-H), 7.72 (d, $J = 7.21$ Hz, 2H, Ar-H), 8.02 (s, 1H, C_5–H, imidazole), ^{13}C NMR (75 MHz, $CDCl_3$) δ: 30.1, 56, 110, 113, 116.3, 119.9, 120, 122, 125.5, 126, 130.1, 133.6, 140.4, 144.2, 156.8, 159, 163.6. Anal. calcd. for $C_{19}H_{14}N_4O_2S$: C, 62.97; H, 3.89; N, 15.46. Found: C, 63.02; H, 3.82; N, 15.51%.

*6.3.6. 3-(2-Benzo[*d*]isoxazol-3-ylmethylimidazo[2,1-*b*][1,3,4] thiadiazol-6-yl)-chromen-2-one (**3f**)*

Pale yellow silky solid (ethanol + DMF), yield 69%, m.p. 177–179 °C; IR (KBr) ν, cm^{-1}: 2926, 2858, 1710, 1591, 1524, 1501; ^1H NMR (300 MHz, $CDCl_3$ + TFA) δ: 5.06 (s, 2H, CH_2), 7.27–8.01 (m, 8H, Ar-H), 8.63 (s, 1H, C_4–H, coumarin), 8.77 (s, 1H, C_5–H, imidazole), GC–MS: 400.9 (m/z), Anal. calcd. for $C_{21}H_{12}N_4O_3S$: C, 62.99; H, 3.02; N, 13.99. Found: C, 63.06; H, 3.10; N, 13.91%.

*6.4. Preparation of 3-(5-bromo-6-arylimidazo[2,1-*b*][1,3,4] thiadiazol-2-ylmethyl)benzo[*d*]isoxazole (**4a–f**)*

General method. To a well stirred solution of 3-(6-Arylimidazo[2,1-*b*][1,3,4]thiadiazol-2-ylmethyl)-benzo[*d*]isoxazoles **3a–f** (0.01 mol) in glacial acetic acid (5 mL) and anhydrous sodium acetate (0.02 mol) was added bromine (0.01 mol) dropwise with stirring at room temperature. After the addition, stirring was continued for 2 h. The reaction mixture was poured on to ice cold water and basified with ammonia solution. The separated solid was collected, washed with water, dried and purified by column chromatography using hexane ethyl acetate mixture (7:3, v/v) as eluent.

*6.4.1. 3-(5-Bromo-6-phenylimidazo[2,1-*b*][1,3,4]thiadiazol-2-ylmethyl)benzo[*d*]isoxazole (**4a**)*

Pale yellow amorphous solid, yield 80%, m.p. 129–131 °C; IR (KBr) ν, cm^{-1}: 2923, 2851, 1603, 1597, 1506, 786; ^1H NMR (300 MHz, $CDCl_3$) δ: 4.80 (s, 2H, CH_2), 7.52–7.81 (m, 7H, Ar-H), 7.92 (d, $J = 7.71$ Hz, 2H, Ar-H). Anal. calcd. for $C_{18}H_{11}BrN_4OS$: C, 52.57; H, 2.70; N, 13.62. Found: C, 52.62; H, 2.74; N, 13.58%.

*6.4.2. 3-[5-Bromo-6-(4-chlorophenyl)imidazo[2,1-*b*][1,3,4] thiadiazol-2-ylmethyl]benzo[*d*]isoxazole (**4b**)*

Yellow crystalline solid, yield 75%, m.p. 141–143 °C; IR (KBr) ν, cm^{-1}: 2922, 2854, 1597, 1508, 855; ^1H NMR (300 MHz, $CDCl_3$) δ: 4.79 (s, 2H, CH_2), 7.59–7.76 (m, 4H, Ar-H), 7.80 (d, $J = 8.1$ Hz, 2H, Ar-H), 7.89 (d, $J = 8.3$ Hz, 2H, Ar-H). Anal. calcd. for $C_{18}H_{10}$ $BrClN_4OS$: C, 48.51; H, 2.26; N, 12.57. Found: C, 48.47; H, 2.31; N, 12.61%.

*6.4.3. 3-[5-Bromo-6-(4-nitrophenyl)imidazo[2,1-*b*][1,3,4] thiadiazol-2-ylmethyl]benzo[*d*]isoxazole (**4c**)*

Yellow color solid yield 75%, m.p. 136–138 °C; IR (KBr) ν, cm^{-1}: 2924, 2852, 1596, 1506, 856; ^1H NMR (300 MHz, $CDCl_3$) δ: 4.80 (s, 2H, CH_2), 7.52–8.18 (m, 6H, Ar-H), 8.31(d, $J = 8.1$ Hz, 2H, Ar-H). Anal. calcd. for $C_{18}H_{10}BrN_5O_3S$: C, 47.38; H, 2.21; N, 15.35. Found: C, 47.42; H, 2.18; N, 15.40%.

*6.4.4. 3-[5-Bromo-6-(4-bromophenyl)imidazo[2,1-*b*][1,3,4] thiadiazol-2-ylmethyl]benzo[*d*]isoxazole (**4d**)*

Yellow color solid, yield 85%, m.p. 127–129 °C; IR (KBr) ν, cm^{-1}: 2924, 2854, 1597, 1509, 856; ^1H NMR (300 MHz, $CDCl_3$) δ: 4.80 (s, 2H, CH_2), 7.38–7.65 (m, 4H, Ar-H), 7.76 (d, $J = 7.87$ Hz, 2H, Ar-H),

7.91 (d, $J = 8.31$ Hz, 2H, Ar-H), GC–MS: 490.7 (*m/z*). Anal. calcd. for $C_{18}H_{10}Br_2N_4OS$: C, 44.11; H, 2.06; N, 11.43. Found: C, 44.09; H, 2.10; N, 11.40%.

*6.4.5. 3-[5-Bromo-6-(4-methoxyphenyl)imidazo[2,1-*b*][1,3,4] thiadiazol-2-ylmethyl]benzo[*d*]isoxazole (**4e**)*

Yellow color solid, yield 79%, m.p. 145–147 °C; IR (KBr) ν, cm^{-1}: 2924, 2853, 1597, 1501, 856; ^1H NMR (300 MHz, $CDCl_3$) δ: 3.81 (s, 3H, OCH_3), 4.80 (s, 2H, CH_2), 7.73–7.79 (m, 6H, Ar-H), 7.81 (d, $J = 7.5$ Hz, 2H, Ar-H), ^{13}C NMR (75 MHz, $CDCl_3$) δ: 29.6, 110.6, 122.9, 123, 124.9, 125, 129.0, 129.9, 132.6, 137.0, 145.0, 147.1, 154.5, 157.8, 164.0. Anal. calcd. for $C_{19}H_{13}BrN_4O_2S$: C, 51.71; H, 2.97; N, 12.70. Found: C, 51.69; H, 3.02; N, 12.75%.

*6.4.6. 3-(2-Benzo[*d*]isoxazol-3-ylmethyl-5-bromo-imidazo[2,1-*b*] [1,3,4]thiadiazol-6-yl)chromen-2-one (**4f**)*

Bright yellow color solid yield 78%, m.p. 151–153 °C; IR (KBr) ν, cm^{-1}: 2923, 2854, 1714, 1596, 1504, 854; ^1H NMR (300 MHz, $CDCl_3$) δ: 4.79 (s, 2H, CH_2), 7.61–8.10 (m, 8H, Ar-H), 8.47 (s, 1H, C_4–H, coumarin), Anal. calcd. for $C_{21}H_{11}BrN_4O_3S$: C, 52.62; H, 2.31; N, 11.69. Found: C, 52.58; H, 2.36; N, 11.73%.

*6.5. Preparation of 3-(5-nitroso-6-arylimidazo[2,1-*b*][1,3,4] thiadiazol-2-ylmethyl)benzo[*d*]isoxazole (**5a–f**)*

General method. To a well stirred solution of 3-(6-arylimidazo[2,1-*b*][1,3,4]thiadiazol-2-ylmethyl) benzo[*d*]isoxazoles, **3a–f** (0.01 mol) in acetic acid (10 mL) was added sodium nitrite solution (0.021 mol, in 5 mL water) dropwise at room temperature. After the addition, stirring was continued for 30 min and the mixture was refluxed for 2 h. The reaction mixture was poured in to ice cold water, separated solid was collected, washed with water, dried and recrystallized from suitable solvent.

*6.5.1. 3-(5-Nitroso-6-phenylimidazo[2,1-*b*][1,3,4]thiadiazol-2-ylmethyl)benzo[*d*]isoxazole (**5a**)*

Brown crystalline solid (chloroform + hexane), yield 40%, m.p. 199–201 °C; IR (KBr) ν, cm^{-1}: 2956, 2850, 1603, 1529, 1501; ^1H NMR (300 MHz, $CDCl_3$) δ: 4.79 (s, 2H, CH_2), 7.59–7.71 (m, 5H, Ar-H), 7.77 (d, $J = 7.2$ Hz, 2H, Ar-H), 7.82 (d, $J = 7.6$ Hz, 2H, Ar-H). Anal. calcd. for $C_{18}H_{11}N_5O_2S$: C, 59.83; H, 3.07; N, 19.38. Found: C, 59.87; H, 3.11; N, 19.34%.

*6.5.2. 3-[6-(4-Chlorophenyl)-5-nitroso-imidazo[2,1-*b*][1,3,4] thiadiazol-2-ylmethyl]benzo[*d*]isoxazole (**5b**)*

Brown color solid (chloroform + hexane), yield 35%, m.p. 215–217 °C; IR (KBr) ν, cm^{-1}: 2957, 2850, 1606, 1531, 1504; ^1H NMR (300 MHz, $CDCl_3$) δ: 4.80 (s, 2H, CH_2), 7.68–7.75 (m, 6H, Ar-H), 7.82 (d, $J = 7.6$ Hz, 2H, Ar-H). Anal. calcd. for $C_{18}H_{10}ClN_5O_2S$: C, 54.62; H, 2.55; N, 17.69. Found: C, 54.50; H, 2.58; N, 17.72%.

*6.5.3. 3-[6-(4-Nitrophenyl)-5-nitroso-imidazo[2,1-*b*][1,3,4] thiadiazol-2-ylmethyl]benzo[*d*]-isoxazole (**5c**)*

Brown color solid (ethanol + dioxan), yield 35%, m.p. 222–224 °C; IR (KBr) ν, cm^{-1}: 2959, 2853, 1606, 1539, 1501; ^1H NMR (300 MHz, $CDCl_3$) δ: 4.80 (s, 2H, CH_2), 7.50–7.66 (m, 2H, Ar-H), 7.77 (d, $J = 8.04$ Hz, 2H, Ar-H), 8.25 (d, $J = 8.8$ Hz, 2H, Ar-H), 8.33 (d, $J = 9$ Hz, 2H, Ar-H), GC–MS: 406 (*m/z*). Anal. calcd. for $C_{18}H_{10}N_6O_4S$: C, 53.20; H, 2.48; N, 20.68. Found: C, 53.25; H, 2.44; N, 20.72%.

*6.5.4. 3-[6-(4-Bromophenyl)-5-nitroso-imidazo[2,1-*b*][1,3,4] thiadiazol-2-ylmethyl]benzo[*d*]isoxazole (**5d**)*

Brown crystalline solid (ethanol + dioxan), yield 47%, m.p. 216–218 °C; IR (KBr) ν, cm^{-1}: 2959, 2851, 1606, 1531, 1501; ^1H NMR (300 MHz, $CDCl_3$) δ: 4.81 (s, 2H, CH_2), 7.51–7.78 (m, 4H, Ar-H), 7.80 (d, $J = 7.8$ Hz, 2H, Ar-H), 7.86 (d, $J = 7.71$ Hz, 2H, Ar-H), ^{13}C NMR (75 MHz, $CDCl_3$) δ: 31.8, 106.1, 117.4, 119.4, 120.6, 122, 124.2, 124.7, 126.3, 128.1, 128.5, 130.4, 133.8, 142.3, 159.1, 163.8. Anal. calcd. for $C_{18}H_{10}BrN_5O_2S$: C, 49.11; H, 2.29; N, 15.91. Found: C, 49.08; H, 2.33; N, 15.87%.

*6.5.5. 3-[6-(4-Methoxyphenyl)-5-nitroso-imidazo[2,1-*b*][1,3,4] thiadiazol-2-ylmethyl]benzo[*d*]isoxazole (**5e**)*

Brown crystalline solid (ethanol + dioxan), yield 46%, m.p. 229–231 °C; IR (KBr) ν, cm^{-1}: 2958, 2854, 1604, 1536, 1500; ^1H NMR (300 MHz, $CDCl_3$) δ: 3.81 (s, 3H, OCH_3), 4.80 (s, 2H, CH_2), 7.49–7.71 (m, 4H, Ar-H), 7.75 (d, $J = 7.41$ Hz, 2H, Ar-H), 7.87 (d, $J = 7.39$ Hz, 2H, Ar-H). Anal. calcd. for $C_{19}H_{13}N_5O_3S$: C, 58.30; H, 3.35; N, 17.89. Found: C, 58.26; H, 3.33; N, 17.85%.

*6.5.6. 3-(2-Benzo[*d*]isoxazol-3-ylmethyl-5-nitroso-imidazo[2,1-*b*] [1,3,4]thiadiazol-6-yl)chromen-2-one (**5f**)*

Pale yellow solid (ethanol + dioxan), yield 42%, m.p. 253–255 °C; IR (KBr) ν, cm^{-1}: 2959, 2854, 1715, 1603, 1524, 1501; ^1H NMR (300 MHz, $CDCl_3$) δ: 4.80 (s, 2H, CH_2), 7.49 (d, $J = 7.4$ Hz, 1H, Ar-H), 7.54–8.03 (m, 7H, Ar-H), 8.41 (s, 1H, C_4–H, coumarin). Anal. calcd. for $C_{19}H_{11}N_5O_4S$: C, 58.74; H, 2.58; N, 16.31. Found: C, 58.70; H, 2.62; N, 16.34%.

*6.6. Preparation of 3-(6-aryl-5-thiocyanato-imidazo[2,1-*b*][1,3,4] thiadiazol-2-ylmethyl)benzo[*d*]isoxazole (**6a–f**)*

General method. To a well stirred solution of 3-(6-arylimidazo[2,1-*b*][1,3,4]thiadiazol-2-ylmethyl)benzo[*d*]isoxazoles, **3a–f** (0.01 mol) in glacial acetic acid (5 mL) and potassium thiocyanate (0.02 mol) was added bromine (0.01 mol) in glacial acetic acid, dropwise with stirring at room temperature. Then stirring was continued for 1 h at 20–25 °C and then at room temperature for 30 min. The reaction mixture was poured in to ice water the separated solid was collected, washed with water, dried and recrystallized from suitable solvent.

*6.6.1. 3-(6-Phenyl-5-thiocyanato-imidazo[2,1-*b*][1,3,4]thiadiazol-2-ylmethyl)benzo[*d*]isoxazole (**6a**)*

Colorless solid (ethanol), yield 45%, m.p. 230–232 °C; IR (KBr) ν, cm^{-1}: 2916, 2851, 2138, 1569; ^1H NMR (300 MHz, DMSO-d_6) δ: 4.80 (s, 2H, CH_2), 7.46–7.79 (m, 5H, Ar-H), 7.84 (d, $J = 7.8$ Hz, 2H, Ar-H), 7.87 (d, $J = 7.69$ Hz, 2H, Ar-H). Anal. calcd. for $C_{19}H_{11}N_5OS_2$: C, 65.88; H, 4.07; N, 16.17. Found: C, 65.92; H, 4.10; N, 16.13%.

*6.6.2. 3-[6-(4-Chlorophenyl)-5-thiocyanato-imidazo[2,1-*b*][1,3,4] thiadiazol-2-ylmethyl]benzo[*d*]isoxazole (**6b**)*

Colorless crystalline solid (ethanol), yield 34%, m.p. 231–233 °C; IR (KBr) ν, cm^{-1}: 2922, 2827, 2146, 1548; ^1H NMR (300 MHz, DMSO-d_6) δ: 4.80 (s, 2H, CH_2), 7.49–7.69 (m, 4H, Ar-H), 7.75 (d, $J = 7.62$ Hz, 2H, Ar-H), 7.79 (d, $J = 7.41$ Hz, 2H, Ar-H). Anal. calcd. for $C_{19}H_{10}ClN_5OS_2$: C, 59.92; H, 3.44; N, 14.71. Found: C, 59.98; H, 3.39; N, 14.75%.

*6.6.3. 3-[6-(4-Nitrophenyl)-5-thiocyanato-imidazo[2,1-*b*][1,3,4] thiadiazol-2-ylmethyl]benzo[*d*]isoxazole (**6c**)*

Colorless solid (ethanol + dioxan), yield 33%, m.p. 256–258 °C; IR (KBr) ν, cm^{-1}: 2924, 2854, 2164, 1563; ^1H NMR (300 MHz, DMSO, d_6) δ: 4.80 (s, 2H, CH_2), 7.52–7.71 (m, 6H, Ar-H), 8.0 (d, $J = 8.1$ Hz, 2H, Ar-H). Anal. calcd. for $C_{19}H_{10}N_6O_3S_2$: C, 59.92; H, 3.44; N, 14.71. Found: C, 59.98; H, 3.39; N, 14.75%.

*6.6.4. 3-[6-(4-Bromophenyl)-5-thiocyanato-imidazo[2,1-*b*][1,3,4] thiadiazol-2-ylmethyl]benzo[*d*]isoxazole (**6d**)*

Pale yellow crystalline solid (ethanol), yield 39%, m.p. 179–182 °C; IR (KBr) ν, cm^{-1}: 2927, 2854, 2154, 1534; ^1H NMR (300 MHz,

DMSO-d_6) δ: 4.80 (s, 2H, CH_2), 7.55–7.68 (m, 6H, Ar-H), 7.73 (d, $J = 6.8$ Hz, 2H, Ar-H), 7.80 (d, $J = 6.8$ Hz, 2H, Ar-H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 30.2, 108.7, 110.2, 110.8, 122.9, 124.5, 124.8, 125.9, 128.1, 130.1, 130.9, 133.0, 145.0, 146.6, 152.5, 159.8, 163.9. Anal. calcd. for $C_{19}H_{10}BrN_5OS_2$: C, 53.66; H, 3.08; N, 13.17. Found: C, 53.7; H, 3.12; N, 13.21%.

*6.6.5. 3-[6-(4-Methoxyphenyl)-5-thiocyanato-imidazo[2,1-*b*][1,3,4]thiadiazol-2-ylmethyl]benzo[*d*]isoxazole (**6e**)*

Yellow crystalline solid (ethanol), yield 48%, m.p. 172–174 °C; IR (KBr) ν, cm^{-1}: 2924, 2854, 2184, 1541; ^1H NMR (300 MHz, DMSO-d_6) δ: 3.79 (s, 3H, OCH_3), 4.80 (s, 2H, CH_2), 7.52–7.69 (m, 4H, Ar-H), 7.72 (d, $J = 7.4$ Hz, 2H, Ar-H), 7.79 (d, $J = 7.6$ Hz, 2H, Ar-H). GC–MS: 419 (m/z). Anal. calcd. for $C_{20}H_{13}N_5O_2S_2$: C, 63.81; H, 4.28; N, 14.88. Found: C, 63.84; H, 4.32; N, 14.91%.

*6.6.6. 3-(2-Benzo[*d*]isoxazol-3-ylmethyl-5-thiocyanato-imidazo[2,1-*b*][1,3,4]thiadiazol-6-yl)chromen-2-one (**6f**)*

Pale yellow silky solid (dioxan), yield 55%, m.p. 191–193 °C; IR (KBr) ν, cm^{-1}: 2924, 2856, 2161, 1720, 1569; ^1H NMR (300 MHz, DMSO-d_6) δ: 4.80 (s, 2H, CH_2), 7.47–7.98 (m, 8H, Ar-H), 8.40 (s, 1H, C_4–H, coumarin). Anal. calcd. for $C_{22}H_{11}N_5O_3S_2$: C, 63.76; H, 3.40; N, 13.52. Found: C, 63.81; H, 3.37; N, 14.49%.

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