

SYNTHESIS AND EVALUATION OF ANTITUBERCULAR AND ANTI FUNGAL ACTIVITY OF SOME NOVEL 6-(4-SUBSTITUTED ARYL)-2-(3,5-DIMETHYL-1H-PYRAZOL-1-YL) IMIDAZO[2,1-B][1,3,4] THIADIAZOLE DERIVATIVES

MANJOOR AHAMAD SYED,^{1*} ALAGWADI KALLANAGOUDA RAMAPPA², SHANKER ALEGAON².

¹Department of Pharma chemistry, RIPER, Anantapur-515721, Andhra Pradesh. ² Department of Pharma chemistry, KLES's college of pharmacy, Belgaum 580010, Karnataka, India. Email: manjooras@gmail.com

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ABSTRACT

The 6-(4-substituted aryl)-2-(3,5-dimethyl-1H-pyrazol-1-yl)imidazo[2,1-b][1,3,4]thiadiazole (**2a-f**) were prepared by treating 2-amino-5-(3,5-dimethyl-H-pyrazole-1-yl)-1,3,4-thiadiazole with appropriate α -haloaryl ketones. The reaction **2** with morpholine using formaldehyde, acetic acid in methanol yielded the corresponding 5- morpholin-4-ylmethyl derivatives (**3a-e**). While compound **2** also produces the 5- pyrrolidine-4-ylmethyl derivatives (**4a-e**) by reaction with pyrrolidine using formaldehyde, acetic acid in methanol. The 5- piperidin-4-ylmethyl derivatives (**5a-e**) were obtained by treating compound **2** with piperidine using formaldehyde, acetic acid in methanol. The structures of the newly synthesized compounds were confirmed by IR, ¹H NMR and Mass spectra. All the compounds were screened for antitubercular and antifungal activity. Some of the compound displayed very good antitubercular and antifungal activity.

Keywords: 2-Amino-5-(3,5-dimethyl-1H-pyrazole-1-yl)-1,3,4-thiadiazole; Imidazo[2,1-b][1,3,4] thiadiazole; Antitubercular activity; Antifungal activity.

INTRODUCTION

Tuberculosis is one of the serious health problems with a wide variety of manifestations caused by *Mycobacterium tuberculosis* and as per the recent report it has been estimated that approximately one-third of the world's population is infected with this microorganism. The treatment of mycobacterial infections especially the tuberculosis, has become an important problem due to the emergence of mono drug and multidrug-resistant strains of *M. tuberculosis*.^[1] Therefore, there is a need for new drugs of new structural classes and with a novel mechanism of action other than isoniazid (INH), rifampicin (RIF), and pyrazinamide (PZA). In this regard, since the last decade search for new antitubercular substances has ranked among the priority areas of chemotherapeutic research. During recent years, there have been intense investigations on thiadiazole and imidazo[2,1-b][1,3,4]thiadiazole compounds, many of which are known to possess interesting biological properties such as antimicrobial,^[2-6] antitubercular,^[7] anti-inflammatory,^[8,9] anticonvulsant,^[10,11] antihypertensive,^[12,13] and anticancer^[14] activities. Recently, these derivatives have attracted the interest of researchers in antitubercular agents. Some members of the imidazo[2,1-b][1,3,4]thiadiazoles family displayed good activity against *M.tuberculosis* H37Rv. The purpose of the present work was to explore and develop the novel molecules with improved potential for treating tuberculosis. In this paper, we report the synthesis and anti fungal evaluation of various imidazo[2,1-b][1,3,4]thiadiazole derivatives.

MATERIALS AND METHODS

Experimental work

The synthesis start from 2-amino-5-mercapto-1,3,4-thiadiazole, the thiol group of compound was readily converted in to hydrazine derivative by heating under reflux with an ethanolic solution of hydrazine hydrate, thus the interaction of hydrazine derivative with acetyl acetone give rise to the formation of 2-amino-5-(3,5-dimethyl-H-pyrazole-1-yl)-1,3,4-thiadiazole. The 6-(4-substituted aryl)-2-(3,5-dimethyl-1H-pyrazol-1-yl)imidazo[2,1-b][1,3,4]thiadiazole (**2a-f**) were prepared by treating 2-amino-5-(3,5-dimethyl-H-pyrazole-1-yl)-1,3,4-thiadiazole with appropriate α -haloaryl ketones (**a-f**). Various α -haloaryl ketones were prepared by bromination of

corresponding ketones using well known Friedel Craft reaction. Mannich reaction of imidazo[2,1-b][1,3,4]thiadiazole (**2a-f**) with different cyclic secondary amines (morpholine, piperidine, and pyrrolidine) and formaldehyde in methanol with catalytic amount of acetic acid yielded corresponding derivatives (**3a-e to 5a-e**).

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

General scheme of the present work

Melting points were determined in open capillaries and are uncorrected. The IR spectra were recorded on a Nicolet Impact 410 FT IR spectrophotometer using KBr pellets. ¹H NMR recorded on a Bruker 300-MHz FT NMR spectrometer in CDCl₃ and DMSO-d₆ with TMS as internal standard. Mass spectra were recorded on a Quattro II Micromass Walter instrument (UK Ltd.) by electron impact technique.

General procedure for Preparation of 2-alkyl/aryl-6-arylimidazo(2,1-b)-1,3,4-thiadiazoles. (**2a-f**)^[19]

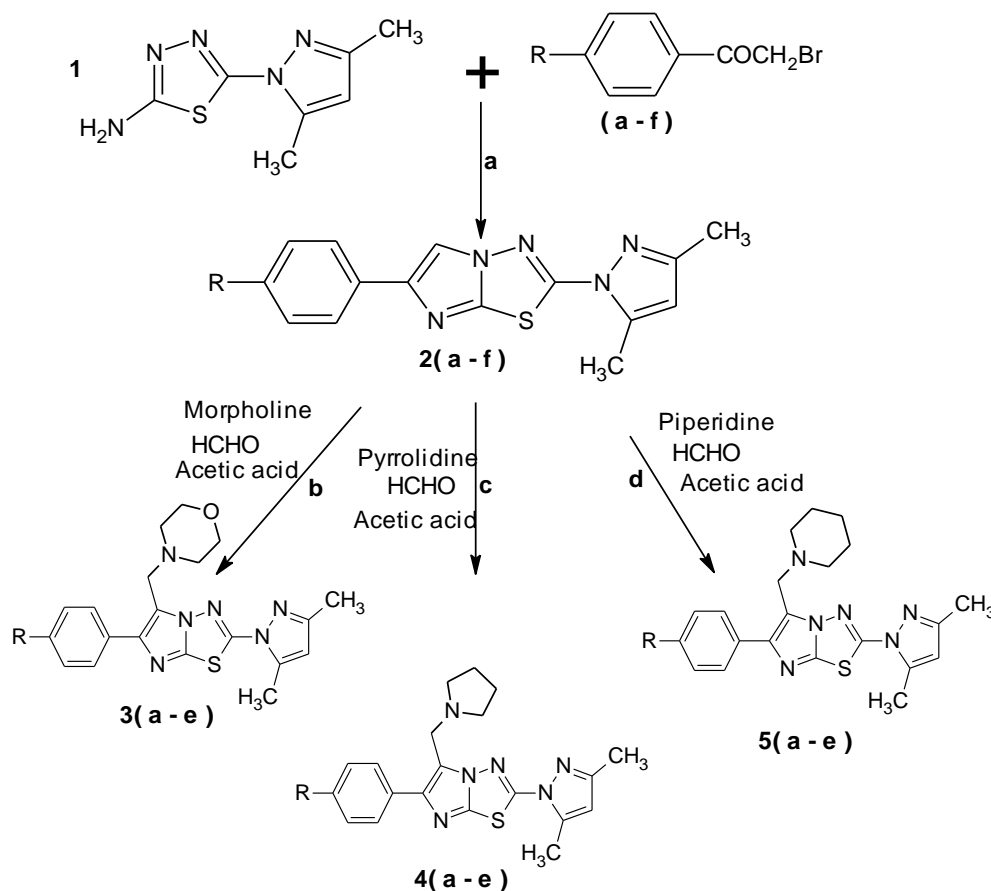
A mixture of equimolar quantities of 2-amino-5-alkyl/aryl-1,3,4-thiadiazoles, (0.01 mol) and bromoacetyl compound (0.01 mol) was refluxed in dry ethanol for 8 hrs. The excess of solvent was distilled off and the solid hydrobromide that separated was collected by filtration, suspended in water, and neutralized by aqueous sodium carbonate solution to get free base it was filtered, washed with water, dried, and recrystallized from suitable solvent.

6-(4-chlorophenyl)-2-(3,5-dimethyl-1H-pyrazol-1-yl)imidazo[2,1-b][1,3,4]thiadiazole. (**2a**)

yellow solid crystals (90%), m.p. 185-188°C, IR (KBr) ν cm⁻¹ (Ar-CH)3054, (C=N)1674, (C-N)1100, (C-H)2900,679(C-Cl). ¹H NMR (400MHz, DMSO) δ 8.10 (s, 1H, C5-Imidazole), δ 7.91 (d, 2H, Ar-H,

C2, C6), δ 7.52 (d, 2H, Ar-H, C3, C5), δ 7.14 (d, 2H, C2, C5), δ 2.8,2.9 (s, 3H, CH₃), δ 5.99 (s, =CH,pyrazole); Anal. Calcd for C₁₅H₁₂ClN₅S: C,

54.63; H, 3.67; N, 21.23. Found: C, 54.75; H, 3.67; N, 21.28. Mass, m/z (%), 330.1 (M+1, 100%).



R = H, Cl, Br, OCH₃, NO₂, CH₃.

Scheme . Reagent and conditions: dry ethanol, reflux, 12 h, NaCO_3 ; (b) morpholine, HCHO, AcOH, methanol reflux 8 h; (c) pyrrolidine, HCHO, AcOH, methanol, reflux 8 h; (d) piperidine, HCHO, AcOH, methanol, reflux

6-(4-bromophenyl)-2-(3,5-dimethyl-1H-pyrazol-1-yl)imidazo[2,1-b][1,3,4]thiadiazole.(2b)

yellow solid crystals (81%), m.p. 135-140°C, IR (KBr) ν cm⁻¹ (Ar-CH)3064, (C=N)1619, (C-N)1197, (C-H)2900,500(C-Br). ¹H NMR (400MHz, DMSO) δ 8.08 (s, 1H, C5-Imidazole), δ 7.85 (d, 2H, Ar-H, C2, C6), δ 7.68 (d, 2H, Ar-H, C3, C5), δ 2.6,2.7 (s, 3H, CH₃), δ 5.57 (s, =CH,pyrazole); Anal. Calcd for C₁₅H₁₂BrN₅S: C, 48.14; H, 3.23; N, 18.71. Found: C, 48.13; H, 3.23; N, 18.72.

2-(3,5-dimethyl-1H-pyrazol-1-yl)-6-(4-methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazole.(2c)

yellow solid crystals (86%), m.p. 164-167°C, IR (KBr) ν cm⁻¹ (Ar-CH)3064, (C=N)1619, (C-N)1197, (C-H)2900,500(C-Br). ¹H NMR (400MHz, DMSO) δ 8.13 (s, 1H, C5-Imidazole), δ 7.96 (d, 2H, Ar-H, C2, C6), δ 7.00 (d, 2H, Ar-H, C3, C5), δ 2.5,2.8 (s, 3H, CH₃), δ 3.89 (s, 3H, Ar-OCH₃), δ 6.19 (s, =CH,pyrazole); Anal. Calcd for C₁₆H₁₅N₅O: C, 59.06; H, 4.65; N, 21.52. Found: C, 59.06; H, 4.65; N, 21.52.

2-(3,5-dimethyl-1H-pyrazol-1-yl)-6-(4-nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazole.(2d)

yellow solid crystals (85%), m.p. 125-130°C, IR (KBr) ν cm⁻¹ (Ar-CH)3064, (C=N)1601, (C-N)1197, (C-H)2855. ¹H NMR (400MHz, DMSO) δ 8.13 (s, 1H, C5-Imidazole), δ 7.96 (d, 2H, Ar-H, C2, C6), δ 7.00 (d, 2H, Ar-H, C3, C5), δ 2.5, 2.8 (s, 3H, CH₃), δ 6.19 (s, =CH,pyrazole); Anal. Calcd for C₁₅H₁₂N₆O₂S: C, 52.93; H, 3.55; N, 24.69. Found: C, 52.94; H, 3.55; N, 24.71.

2-(3,5-dimethyl-1H-pyrazol-1-yl)-6-phenylimidazo[2,1-b][1,3,4]thiadiazole.(2e)

yellow solid crystals (84%), m.p. 170-172°C, IR (KBr) ν cm⁻¹ (Ar-CH)3095, (C=N)1692, (C-N)1194, (C-H)2900,500(C-Br). ¹H NMR (400MHz, DMSO) δ 8.13 (s, 1H, C5-Imidazole), δ 7.96 (d, 2H, Ar-H, C2, C6), δ 7.00 (d, 2H, Ar-H, C3, C5), δ 2.5,2.8 (s, 3H, CH₃), δ 6.19 (s, =CH,pyrazole); Anal. Calcd for C₁₅H₁₃N₅S: C, 61.00; H, 4.44; N, 23.71. Found: C, 61.02; H, 4.44; N, 23.74.

2-(3,5-dimethyl-1H-pyrazol-1-yl)-6-(4-methylphenyl)imidazo[2,1-b][1,3,4]thiadiazole.(2f)

yellow solid crystals (81%), m.p. 155-158°C, IR (KBr) ν cm⁻¹ (Ar-CH)3080, (C=N)1686, (C-N)1185, (C-H)2913. ¹H NMR (400MHz, DMSO) δ 7.98 (s, 1H, C5-Imidazole), δ 7.83 (d, 2H, Ar-H, C2, C6), δ 7.29 (d, 2H, Ar-H, C3, C5), δ 2.3,2.5 (s, 3H, CH₃), δ 3.67 (s, 3H, Ar-CH₃), δ 5.92 (s, =CH,pyrazole); Anal. Calcd for C₁₆H₁₅N₅S: C, 62.11; H, 4.89; N, 22.64. Found: C, 62.14; H, 4.89; N, 22.66.

General procedure for Preparation of 2-alkyl/aryl-5-(morpholin-1-ylmethyl)-6-arylimidazo (2, 1b)-1,3,4-thiadiazoles. (3a-3e)[20]

A mixture of 2-alkyl/aryl-6-arylimidazo[2,1-b][1,3,4]-thiadiazole (0.005 mol), morpholine (0.52 g, 0.006 mol), formalin (1 mL), and acetic acid (1 mL) in methanol (20 mL) was refluxed for 8 h (monitored by TLC). The solution was diluted with water, extracted with chloroform, the combined chloroform extract was washed with water and dried over anhydrous sodium sulfate. The solution was

evaporated to dryness in vacuum and the residue was recrystallized from appropriate solvent.

6-(4-bromophenyl)-2-(3,5-dimethyl-1H-pyrazol-1-yl)-5-(morpholin-4-ylmethyl)imidazo[2,1-b][1,3,4]thiadiazole. (3a)

Brown solid crystals (60%), m.p. 95-100°C, IR (KBr) ν cm⁻¹ (C=N)1673, (C-N)1107, (C-H)2900,500(C-Br). ¹H NMR (400MHz, CDCl₃) δ 3.98 (s, 2H, CH₂) δ 7.90 (d, 2H, Ar-H, C2, C6), δ 7.68(d, 2H, Ar-H, C3, C5), δ 3.2 (t, 4H, C3, C5-H), δ 3.95 (t, 4H, C2, C6-H), δ 2.5,2.8 (s, 3H, CH₃), δ 5.67 (s, =CH,pyrazole) ; Anal. Calcd for C₂₀H₂₁BrN₆O: C, 50.74; H, 4.47; N, 17.75. Found: C, 50.74; H, 4.47; N, 17.76.

2-(3,5-dimethyl-1H-pyrazol-1-yl)-6-(4-methoxyphenyl)-5-(morpholin-4-ylmethyl)imidazo [2,1-b][1,3,4]thiadiazole.(3b)

Brown solid crystals (63%), m.p. 73-77°C, IR (KBr) ν cm⁻¹ (C=N)1664, (C-N)1171, (C-H)2927. ¹H NMR (400MHz, CDCl₃) δ 3.95 (s, 2H, CH₂) δ 7.9 (d, 2H, Ar-H, C2, C6), δ 7.2 (d, 2H, Ar-H, C3, C5), δ 3.2 (t, 4H, C3, C5-H), δ 3.5 (t, 4H, C2, C6-H), δ 2.1,2.5 (s, 3H, CH₃), δ 3.89 (s, 3H,Ar- OCH₃), δ 4.9 (s, =CH,pyrazole) ; Anal. Calcd for C₂₁H₂₄N₆O₂S: C, 59.41; H, 5.70; N, 19.80. Found: C, 59.43; H, 5.70; N, 19.82. Mass, m/z (%), 424.2 (M+1, 100%).

2-(3,5-dimethyl-1H-pyrazol-1-yl)-5-(morpholin-4-ylmethyl)-6-(4-nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazole.(3c)

Dark brown solid crystals (65%), m.p. 87-92°C, IR (KBr) ν cm⁻¹ (C=N)1688, (C-N)1105, (C-H)2923. ¹H NMR (400MHz, CDCl₃) δ 4.01 (s, 2H, CH₂) δ 7.96 (d, 2H, Ar-H, C2, C6), δ 7.5 (d, 2H, Ar-H, C3, C5), δ 2.59 (t, 4H, C3, C5-H), δ 3.22 (t, 4H, C2, C6-H), δ 2.1,2.2 (s, 3H, CH₃), δ 5.5 (s, =CH,pyrazole) ; Anal. Calcd for C₂₀H₂₁N₇O₃S: C, 54.66; H, 4.82; N, 22.31. Found: C, 54.67; H, 4.82; N, 22.31.

2-(3,5-dimethyl-1H-pyrazol-1-yl)-5-(morpholin-4-ylmethyl)-6-phenylimidazo[2,1-b][1,3,4]thiadiazole.(3d)

Brownish yellow solid crystals (73%), m.p. 78-80°C, IR (KBr) ν cm⁻¹ (C=N)1619, (C-N)1197, (C-H)2900,500(C-Cl). ¹H NMR (400MHz, CDCl₃) δ 3.7 (s, 2H, CH₂) δ 7.96 (d, 2H, Ar-H, C2, C6), δ 7.5 (d, 2H, Ar-H, C3, C5), δ 2.59 (t, 4H, C3, C5-H), δ 3.22 (t, 4H, C2, C6-H), δ 2.1,2.2 (s, 3H, CH₃), δ 6.00 (s, =CH,pyrazole) ; Anal. Calcd for C₂₀H₂₂N₆O: C, 60.89; H, 5.62; N, 21.30. Found: C, 60.91; H, 5.62; N, 21.33.

2-(3,5-dimethyl-1H-pyrazol-1-yl)-6-(4-methylphenyl)-5-(morpholin-4-ylmethyl)imidazo[2,1-b][1,3,4]thiadiazole.(3e)

Brownish yellow solid crystals (68%), m.p. 95-97°C, IR (KBr) ν cm⁻¹ (C=N)1670, (C-N)1110, (C-H)2997. ¹H NMR (400MHz, CDCl₃) δ 3.77 (s, 2H, CH₂) δ 7.96 (d, 2H, Ar-H, C2, C6), δ 7.34(d, 2H, Ar-H, C3, C5), δ 2.49 (t, 4H, C3, C5-H), δ 3.33 (t, 4H, C2, C6-H), δ 2.1,2.2 (s, 3H, CH₃), δ 3.55 (s, 3H,Ar- OCH₃), δ 4.61 (s, =CH,pyrazole) ; Anal. Calcd for C₂₁H₂₄N₆O: C, 61.74; H, 5.92; N, 20.57. Found: C, 61.76; H, 5.92; N, 20.59. Mass, m/z (%), 408.3 (M+1, 100%).

General procedure for Preparation of 2-alkyl/aryl-5-(pyrrolidine-1-ylmethyl)-6-arylimidazo(2,1b)-1,3,4-thiadiazoles. (4a-4e)[20]

A mixture of 2-alkyl/aryl-6-arylimidazo[2,1-b][1,3,4]-thiadiazole (0.005 mol), pyrrolidine (0.52 g, 0.006 mol), formalin (1 mL), and acetic acid (1 mL) in methanol(20 mL) was refluxed for 8 h (monitored by TLC).The solution was diluted with water, extracted withchloroform (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 recrystallized from appropriate solvent.

6-(4-chlorophenyl)-2-(3,5-dimethyl-1H-pyrazol-1-yl)-5-(pyrrolidin-1-ylmethyl)imidazo[2,1-b][1,3,4]thiadiazole.(4a)

Brownish yellow solid crystals (62%), m.p. 96-101°C, IR (KBr) ν cm⁻¹ (C=N)1619, (C-N)1197, (C-H)2900,500(C-Cl). ¹H NMR (400MHz, CDCl₃) δ 3.7 (s, 2H, CH₂) δ 7.96 (d, 2H, Ar-H, C2, C6), δ 7.5 (d, 2H, Ar-H, C3, C5), δ 2.59 (m, 4H, C3, C5-H), δ 3.22 (m, 4H, C2, C6-H), δ 2.1,2.2 (s, 3H, CH₃), δ 6.13 (s, =CH,pyrazole) ; Anal. Calcd for

C₂₀H₂₁ClN₆S: C, 58.17; H, 5.13; N, 20.35. Found: C, 58.25; H, 5.13; N, 20.39. Mass, m/z (%), 418.2 (M+1, 100%).

2-(3,5-dimethyl-1H-pyrazol-1-yl)-6-(4-methoxyphenyl)-5-(pyrrolidin-1-ylmethyl)imidazo [2,1-b][1,3,4]thiadiazole.(4b)

Yellowish brown solid crystals (54%), m.p. 88-93°C, IR (KBr) ν cm⁻¹ (C=N)1665, (C-N)1172, (C-H)2928. ¹H NMR (400MHz, CDCl₃) δ 3.7 (s, 2H, CH₂) δ 7.99 (d, 2H, Ar-H, C2, C6), δ 6.95 (d, 2H, Ar-H, C3, C5), δ 3.32 (m, 4H, C3, C5-H), δ 3.38 (m, 4H, C2, C6-H), δ 2.1,2.5 (s, 3H, CH₃), δ 3.8 (s, 3H,Ar- CH₃), δ 5.72 (s, =CH,pyrazole) ; Anal. Calcd for C₂₁H₂₄N₆O: C, 61.74; H, 5.92; N, 20.57. Found: C, 61.76; H, 5.92; N, 20.59.

2-(3,5-dimethyl-1H-pyrazol-1-yl)-6-(4-nitrophenyl)-5-(pyrrolidin-1-ylmethyl)imidazo[2,1-b][1,3,4]thiadiazole.(4c)

Yellowish brown solid crystals (68%), m.p. 82-86°C, IR (KBr) ν cm⁻¹ (C=N)1686, (C-N)1105, (C-H)2926. ¹H NMR (400MHz, CDCl₃) δ 3.7 (s, 2H, CH₂) δ 7.99 (d, 2H, Ar-H, C2, C6), δ 6.95 (d, 2H, Ar-H, C3, C5), δ 3.32 (m, 4H, C3, C5-H), δ 3.38 (m, 4H, C2, C6-H), δ 2.1,2.5 (s, 3H, CH₃), δ 5.72 (s, =CH,pyrazole) ; Anal. Calcd for C₂₁H₂₁N₇O₂S: C, 56.72; H, 5.00; N, 23.15. Found: C, 56.72; H, 5.00; N, 23.17.

2-(3,5-dimethyl-1H-pyrazol-1-yl)-6-phenyl-5-(pyrrolidin-1-ylmethyl)imidazo[2,1-b][1,3,4]thiadiazole.(4d)

Brown solid crystals (61%), m.p. 85-90°C, IR (KBr) ν cm⁻¹ (C=N)1673, (C-N)1101, (C-H)2927. ¹H NMR (400MHz, CDCl₃) δ 3.8 (s, 2H, CH₂) δ 7.99 (d, 2H, Ar-H, C2, C6), δ 6.95 (d, 2H, Ar-H, C3, C5), δ 2.51 (m, 4H, C3, C5-H), δ 3.41 (m, 4H, C2, C6-H), δ 2.09,2.51 (s, 3H, CH₃), δ 5.80 (s, =CH,pyrazole) ; Anal. Calcd for C₂₀H₂₂N₆S: C, 63.47; H, 5.86; N, 22.20. Found: C, 63.49; H, 5.86; N, 22.23.

2-(3,5-dimethyl-1H-pyrazol-1-yl)-6-(4-methylphenyl)-5-(pyrrolidin-1-ylmethyl)imidazo[2,1-b][1,3,4]thiadiazole.(4e)

Brown solid crystals (66%), m.p. 115-120°C, IR (KBr) ν cm⁻¹ (C=N)1603, (C-N)1181, (C-H)2978. ¹H NMR (400MHz, CDCl₃) δ 4.01 (s, 2H, CH₂) δ 7.99 (d, 2H, Ar-H, C2, C6), δ 7.80 (d, 2H, Ar-H, C3, C5), δ 2.01 (m, 4H, C3, C5-H), δ 3.33 (m, 4H, C2, C6-H), δ 2.09,2.51 (s, 3H, CH₃), δ 2.38(s, 3H, Ar-CH₃), δ 5.80 (s, =CH,pyrazole) ; Anal. Calcd for C₂₁H₂₄N₆S: C, 64.26; H, 6.16; N, 21.41. Found: C, 64.29; H, 6.17; N, 21.43.

General procedure for Preparation of 2-alkyl/aryl-5-(piperidine-1-ylmethyl)-6- arylimidazo(2,1b)-1,3,4-thiadiazoles. (5a-5e)[20]

A mixture of 2-alkyl/aryl-6-arylimidazo[2,1-b][1,3,4]-thiadiazole (0.005 mol), piperidine (0.52 g, 0.006 mol), formalin (1 mL), and acetic acid (1 mL) 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 recrystallized from appropriate solvent.

6-(4-bromophenyl)-2-(3,5-dimethyl-1H-pyrazol-1-yl)-5-(piperidin-1-ylmethyl)imidazo[2,1-b][1,3,4]thiadiazole.(5a)

Yellowish brown solid crystals (54%), m.p. 102-105°C, IR (KBr) ν cm⁻¹ (C=N)1678, (C-N)1100, (C-H)2932, 600(C-Br). ¹H NMR (400MHz, CDCl₃) δ 4.01 (s, 2H, CH₂) δ 7.97 (d, 2H, Ar-H, C2, C6), δ 7.52 (d, 2H, Ar-H, C3, C5), δ 1.25&1.58 (m, 6H, C3,C4,C5-H), δ 2.59 (m, 4H, C2, C6-H), δ 2.10,2.51 (s, 3H, CH₃), δ 5.99 (s, =CH,pyrazole) ; Anal. Calcd for C₂₁H₂₃BrN₆S: C, 53.50; H, 4.92; N, 17.83. Found: C, 53.50; H, 4.92; N, 17.84.

2-(3,5-dimethyl-1H-pyrazol-1-yl)-6-(4-methoxyphenyl)-5-(piperidin-1-ylmethyl)imidazo [2,1-b][1,3,4]thiadiazole.(5b)

Yellowish brown solid crystals (51%), m.p. 132-135°C, IR (KBr) ν cm⁻¹ (C=N)1665, (C-N)1170, (C-H)2932. ¹H NMR (400MHz, CDCl₃) δ 3.85 (s, 2H, CH₂) δ 7.98 (d, 2H, Ar-H, C2, C6), δ 7.28 (d, 2H, Ar-H, C3, C5), δ 1.25 (m, 6H, C3,C4,C5-H), δ 3.22 (m, 4H, C2, C6-H), δ 2.10,2.51 (s, 3H, CH₃), δ 3.88 (s, 3H,Ar- OCH₃), δ 5.33 (s, =CH,pyrazole) ; Anal. Calcd for C₂₂H₂₆N₆O: C, 62.53; H, 6.20; N, 19.89. Found: C, 62.56; H, 6.21; N, 19.91.

2-(3,5-dimethyl-1H-pyrazol-1-yl)-6-(4-nitrophenyl)-5-(piperidin-1-ylmethyl)imidazo[2,1-b][1,3,4]thiadiazole.(5c)

Brown solid crystals (67%), m.p. 105-107°C, IR (KBr) ν cm⁻¹ (C=N)1683, (C-N)1105, (C-H)2900, 600(C-Br). ¹H NMR (400MHz, CDCl₃) δ 3.85 (s, 2H, CH₂) δ 7.98 (d, 2H, Ar-H, C2, C6), δ 7.28 (d, 2H, Ar-H, C3, C5), δ 1.25 (m, 6H, C3,C4,C5-H), δ 3.22 (m, 4H, C2, C6-H), δ 2.10, 2.51 (s, 3H, CH₃), δ 5.96 (s, =CH, pyrazole); Anal. Calcd for C₂₁H₂₃N₇O₂S: C, 57.65; H, 5.30; N, 22.41. Found: C, 57.67; H, 5.30; N, 22.43.

2-(3,5-dimethyl-1H-pyrazol-1-yl)-6-phenyl-5-(piperidin-1-ylmethyl)imidazo[2,1-b][1,3,4]thiadiazole.(5d)

Brown solid crystals (63%), m.p. 90-95°C, IR (KBr) ν cm⁻¹ (C=N)1679, (C-N)1156, (C-H)2923, ¹H NMR (400MHz, CDCl₃) δ 3.85 (s, 2H, CH₂) δ 7.98 (d, 2H, Ar-H, C2, C6), δ 7.28 (d, 2H, Ar-H, C3, C5), δ 1.25 (m, 6H, C3,C4,C5-H), δ 3.22 (m, 4H, C2, C6-H), δ 2.10, 2.51 (s, 3H, CH₃), δ 5.96 (s, =CH, pyrazole); Anal. Calcd for C₂₁H₂₄N₆S: C, 64.26; H, 6.16; N, 21.41. Found: C, 64.29; H, 6.17; N, 21.43.

2-(3,5-dimethyl-1H-pyrazol-1-yl)-6-(4-methylphenyl)-5-(piperidin-1-ylmethyl)imidazo[2,1-b][1,3,4]thiadiazole.(5e)

Brown solid crystals (63%), m.p. 110-115°C, IR (KBr) ν cm⁻¹ (C=N)1670, (C-N)1183, (C-H)2932, ¹H NMR (400MHz, CDCl₃) δ 4.00 (s, 2H, CH₂) δ 7.98 (d, 2H, Ar-H, C2, C6), δ 7.88 (d, 2H, Ar-H, C3,

C5), δ 1.26&1.6 (m, 6H, C3,C4,C5-H), δ 2.57 (m, 4H, C2, C6-H), δ 2.10, 2.51 (s, 3H, CH₃), 3.30(s, 3H, Ar-CH₃), δ 5.50(=CH, pyrazole); Anal. Calcd for C₂₂H₂₆N₆S: C, 65.00; H, 6.45; N, 20.67. Found: C, 65.00; H, 6.45; N, 20.70.

Antimicrobial activity

The newly synthesized compounds were screened for their antitubercular and antifungal screening. Antitubercular activity done against Mycobacterium tuberculosis of H37Rv Strain in BACTEC[15,16] medium using a broth micro dilution assay.[17,18] and antifungal screening using agar well diffusion method.

Antitubercular activity

Antitubercular activity done against Mycobacterium tuberculosis of H37Rv Strain in BACTEC[15,16] medium using a broth micro dilution assay. The Microplate Alamar Blue Assay (MABA)[8] Compounds exhibiting fluorescence were tested in the BACTEC 460 radiometric system. Compounds effecting <90% inhibition in the primary screen were not evaluated further. Compounds demonstrating at least 90% inhibition were tested at lower concentrations by serial dilution against M. tuberculosis H37Rv to determine the minimum inhibitory concentration (MIC) using MABA.

Antifungal activity

Antifungal activity was screened against two fungal strain, Candida albicans and Aspergillus fumigatus using Clotrimazole as standard drug. Dimethylformamide was used as solvent control 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 mg. Each sample (100 mL) was loaded into the wells of agar plates directly. The fungal culture was incubated at 25°C for 72 hrs. The standard antibiotic, fluconazole (100 mg/ml) for fungal were used as positive controls and 100 ml of DMSO used as a negative control, zone of inhibitions were recorded in mm. Preliminary screening was conducted for all compounds at 100 mg/mL concentration, against the above-mentioned microorganisms. Different series of dilutions of compounds were made (25, 10 and 5 mg/mL) to determine the MIC.

RESULTS AND DISCUSSION

Antitubercular activity

All synthesized compounds (2a-f and 3a-e to 5a-e) were screened for their antitubercular activity against Mycobacterium tuberculosis. Compounds 2a, 2b, 3a, 4a and 5a have shown promising antitubercular activity. Mycobacterium tuberculosis H37Rv strain was used as standard tubercular organism. Streptomycin and pyrazinamide was used as standard drug. The MIC of streptomycin and pyrazinamide is 7.5mcg/ml. The results are given in table 1.

Table 1: Antitubercular activity of synthesized compounds

Compound code	MIC μ gm/ml
2a	10
2b	10
3a	10
3b	25
4a	10
4b	25
5a	10
5b	25
5c	25
5e	25
Streptomycin	7.5 μ gm/ml

Antifungal activity: All synthesized compound (2a-f and 3a-e to 5a-e) were evaluated for in-vitro antifungal activity against Candida albicans and Aspergillus fumigatus, Fluconazole was used as standard drug. Compounds 2a, 2f, 3a, 3b, and 4e have shown promising antifungal activity. The results are given in table 2.

Table 2: Antifungal activities of the compounds (2a-f, 3a-e, 4a-e and 5a-e) [zone of inhibition in mm and MIC values]

Compound code	<i>C. albicans</i>	<i>A. fumigates</i>
2a	20 (16)	19 (500)
2b	(62)	125
2c	(125)	(500)
2d	(250)	-
2f	19 (16)	18 (500)
3a	25 (4)	24 (32)
3b	20(8)	20(16)
3c	20(125)	18(500)
3e	(250)	-
4a	22(62)	21(125)
4b	(125)	(500)
4c	(250)	-
4d	(500)	-
4e	20(4)	19(8)
5a	20(125)	19(500)
5b	(250)	-
5c	(500)	-
5e	(250)	(500)
Fluconazole	8 μ g/ml	16 μ g/ml

MIC values are given in brackets

CONCLUSION

The preliminary in vitro antituberculosis screening and antifungal screening results of novel imidazo[2,1-b][1,3,4]thiadiazole derivatives, reported in the present article, evidenced that many of the compounds from the series have emerged as potent antitubercular and antifungal agents endowed with moderate to good activity. The possible improvements in the activity can be further achieved by slight modifications in the substituents on the basic imidazo- [2,1-b][1,3,4]thiadiazole nucleus. Our findings will have impact on chemists and pharmacists for further investigations in this field in search of potent antitubercular and antifungal agents.

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