Design, One-pot Synthesis and Biological Evaluation of Imidazo[2,1-b] [1,3,4] Thiadiazole Derivatives for their Anti-Tubercular and Anti-Fungal Activity

Mukesh Bugalia[1*], Sangamesh B. Puranik[2], Rohit Saraswat[3], Mahesh Jhajharia[4], Prashant Sharma[5]

School of Pharmacy, OPJS University, Churu, Rajasthan, India
*Corresponding Author

Abstract**—** *In the present designed work, we have synthesized imidazo[2,1-b][1,3,4]thiadiazole derivatives* ***(6a1-a6 to 6d1-d6)*** *by reaction of compound* ***3*** *with appropriate α-haloaryl ketones produce substituted imidazo thiadiazole derivatives* ***(4a-d)****. In the next step, these compounds* ***(4a–d)*** *undergoes the Vilsmeier reaction to introduce formyl group on the substituted arylimidazo[2,1-b][1,3,4]thiadiazole derivatives to form carbaldehyde derivatives* ***(5a–d)*** *and finally in the last step of the reaction for the synthesis of designed molecules, a one-pot synthetic procedure was used. For this, the one-pot reaction of* ***5a–d****, thiosemicarbazide and substituted α-haloaryl ketones were reacted together in different reaction condition in ethanol solvent at an optimum temperature around 80°C produces a corresponding derivatives* ***(6a1-a6 to 6d1-d6)*** *with a better yield. The IR,* ^{1}*H-NMR, and mass spectroscopy techniques were used to confirm the structure of final products and all synthesized molecules were tested for anti-TB and anti-fungal activity. The compounds* ***6a1, 6a2, 6a3, 6c1, 6c6*** *and* ***6d1*** *with MIC* ***1.6-6.25*** *µgm/ml displayed very good antitubercular and* ***6a1, 6a4, 6a5,*** *and* ***6d1*** *displayed very good antifungal activity with MIC 5 µgm/ml due to electron withdrawing groups at* 4^{th} *position to both phenyl rings which are attached to the thiazole of the imidazo thiadiazole and imidazo thiadiazole ring.*

Keywords— Anti-fungal, Anti-tubercular, Imidazo[2,1-b][1,3,4], thiadiazole-5-carbaldehyde, One-pot synthesis, Vilsmeier reaction.

I. INTRODUCTION

Worldwide, Tuberculosis is utmost top ten causes of death, 1.72 million of people were died around the world in 2016, among them 0.42 million of people suffering from HIV along with TB especially in low and middle-class income countries like India, China, Indonesia, Nigeria, South Africa, Philippines, and Pakistan. The 96% of death occurred due to TB in the countries mentioned above among those India stands first with most deaths. In general, the main compliance of TB is a synergy with HIV, Multidrug-resistant strain development and patient non- compliance due to MTB has made the circumstance ever more risk and it is extensively recognized that innovative intrusion tactics are required (Zheng and Blanchard, 2001). The basic imidazothiadiazole is an interesting and more demanding moiety with exciting biotic properties such as antimicrobial (Gwande *et al.*, 1987; Desai and Baxi, 1992; Mamolo *et al.*, 1996; Gadad *et al.*, 2000; Alireza *et al.*, 2003), antifungal (Alagawadi and Alegaon, 2011; Alagawadi and Alegaon, 2011), anti-tubercular (Manjoor *et al.*, 2013; Arya *et al.*, 1972; Gadad *et al.*, 2004), anti-inflammatory (Labanauskas *et al.*, 2001), antihyperlipidemic (Patel *et al.*, 2013), antihypertensive (Turner *et al.*, 1988; Turner *et al.*, 1988) and anticancer (Noolvi *et al.*, 2011; Noolvi *et al.*, 2012; Gireesh *et al.*, 2011; Gireesh *et al.*, 2013; Kumar *et al.*, 2014; Chou *et al.*, 2003). In the recent years, many antitubercular drugs reported which includes Rhodanine acetic acid carrying designed

imidazothiadiazole moieties (Alegaon *et al.*, 2012), Scaffolds of imidazothiazole and thiadiazole (Romeo *et al.*, 2015), thiazole, imidazothiadiazole hybrids (Ramprasad *et al.*, 2015). In the present scenario, the compounds which has the imidazothiadiazole as a basic moiety have fascinated the attention of investigators in antitubercular agents. The study of the present work was to discover, advance the novel derivatives with upgraded potential for curing tuberculosis and anti-fungal assessment of various imidazothiadiazole molecules. We have designed, synthesized twenty-four derivatives of substituted imidazo[2,1-b][1,3,4] thiadiazole and they assessed for anti-tubercular and anti-fungal activity.

II. MATERIALS AND METHODS

2.1 Chemicals and instruments

The majority of the chemicals required and solvents are found from marketable sources and they are used without additional purification. The Thin Layer Chromatography (Silica gel coated on aluminium plates) is used to monitor reaction condition of synthesized compounds. Bruker AM-400 and 100 MHz spectrometers were used to record the ^{1}H-NMR and ^{13}C-spectra of synthesized compounds. DMSO was used as a solvent for recorded by Shimadzu, LCMS-2020 to determine the Molecular weight of the compounds by ESI-MS method.

2.2 Antimicrobial Activity

H37Rv strain (ATCC No-27294) of *Mycobacterium Tuberculi* was used to perform an Anti-tubercular activity in BACTEC medium (Collins *et al.*, 1997; Franzblau *et al.*, 1998) using a broth microdilution assay (Yajko *et al.*, 1995; Suling *et al.*, 2000). The Microplate Alamar Blue Assay (MABA) used to determine the MIC (Minimum Inhibitory Concentration).

The two different fungal strains, *C. Albicans, and A. Fumigatus* were used to perform the Anti-fungal activity by using the standard drug Fluconazole with different series of dilutions (25, 10 and 5 microgram/mL) to read the MIC of the various compounds.

2.3 Brief synthetic procedure for the synthesis of targeted molecules

The key starting material 5-amino-2-mercapto- 1,3,4-thiadiazole (**1**) was synthesized from the reaction of Thiosemicarbazide and CS_2 (Carbon disulfide), compounds (**2) was** synthesized from the reaction of compound **1** against Hydrazine hydrate and compound **3** obtained by the reaction with Acetylacetone. The compound **3rd** react with different α-haloaryl ketones for refluxing about 8 hours to produce compound **4a-d**. In the next step, **4a-d** follows Vilsmeier reaction for the formylation to produce Carbaldehyde derivatives (**5a-d**). For the formation of final products one-pot synthetic reaction was used in that compound **5a-d**, α-haloaryl ketone and thiosemicarbazide were react together under different reaction conditions in solvent ethanol to produce the final products **(6a1-a6 to 6d1-d6)** and they were isolated in good yields. The physical data of the newly synthesized compounds (6a1-a6 to 6d1-d6) are given in Table 1. The detailed procedure for all synthesized compounds is as follows:

2.3.1 Preparation of 5-amino-2-mercapto-1,3,4-thiadiazole

Potassium hydroxide (0.16 mole) was dissolved in anhydrous ethanol (40 ml) and carbon disulfide (0.24 mole) and then add the thiosemicarbazide (0.15 mole) in anhydrous ethanol (40 ml) was added and the mixture was stirred and refluxed for 6 hrs. Under reduce pressure after removing excess of

solvent, the residue was added to the water, dissolve and acidified with Conc. HCl with care. The final precipitate was filtered off to give 5-amino-2-mercapto-1,3,4-thiadiazole **(**Salih *et al.*, 2008).

TABLE 1
PHYSICAL DATA OF THE SYNTHESIZED COMPOUNDS

Product	R1	R2	Melting Point °C	Rf value (n-Hexane:Ethyl acetate) 3:2
6 a1	p-NO_2	p-OCH_3	210	0.28
6 a2	p-NO_2	p-NO_2	220	0.29
6 a3	p-NO_2	p-OCH_3	190	0.41
6 a4	p-NO_2	p-Cl	200	0.55
6 a5	p-NO_2	p-Br	198	0.28
6 a6	p-NO_2	m-NO_2	218	0.57
6 b1	p-Br	p-NO_2	214	0.22
6 b2	p-Br	p-OCH_3	204	0.37
6 b3	p-Br	p-CH_2	229	0.51
6 b4	p-Br	p-Cl	196	0.47
6 b5	p-Br	p-Br	242	0.36
6 b6	p-Br	m-NO_2	236	0.28
6 c1	p-OCH_3	p-NO_2	177	0.42
6 c2	p-OCH_3	p-OCH_3	186	0.26
6 c3	p-OCH_3	p-CH_2	190	0.37
6 c4	p-OCH_3	p-Cl	199	0.22
6 c5	p-OCH_3	p-Br	165	0.59
6 c6	p-OCH_3	m-NO_2	209	0.47
6 d1	p-Cl	p-OCH_3	155	0.41
6 d2	p-Cl	p-NO_2	160	0.55
6 d3	p-Cl	p-CH_2	172	0.47
6 d4	p-Cl	p-Cl	222	0.34
6 d5	p-Cl	p-Br	189	0.47
6 d6	p-Cl	m-NO_2	193	0.37

2.3.2 Preparation of the 2-amino-5-hydrazino-1,3,4-thiadiazole

Add the 0.02 moles of Hydrazine hydrate to 0.01 moles of 5-amino-2-mercapto-1,3,4-thiadiazole which is previously dissolved in absolute ethanol and reflux for 6 hours or until up to hydrogen sulfide gas was completely concluded **(**Salih *et al.*, 2008).

2.3.3 Preparation of compound 3 [2-amino-5-(3,5-dimethyl-1H- pyrazolyl)-1,3,4-thiadiazole]

0.01 moles of 2-amino-5-hydrazino-1,3,4-thiadiazole was taken in absolute ethanol. Further added to the 0.01 moles of Acetyl acetone and refluxed for around 10 hours, concentrate the solution and allowed it to cool, to get the product and the solvent benzene was used to recrystallize the product (Salih *et al.*, 2008).

2.3.4 General procedure for Preparation of 6-(3 or 4-substituted)-2-(3,5-dimethyl-1H-pyrazole-1yl)- imidazo(2,1-b)(1,3,4)-thiadiazole (4a-d)

The 0.01 mole of compound **3** and α-haloaryl ketone were added to the dry ethanol and refluxed for 8 hours to get the solid hydrobromide by removing excess of solvent. The solid hydrobromide was suspended in water, neutralized with the solution of sodium carbonate to obtained free base which is

filtered, washed and dried to get the crystal by recrystallization with solvent ethanol **(**Koalvi *et al.*, 2006).

2.3.5 General synthetic procedure for the preparation of 6-(3 or 4-substituted)-2-(3,5-dimethyl-1H-pyrazolyl)-imidazo(2,1-b) (1,3,4)-thiadiazoles-5-carbaldehy (5a-d)

For the synthesis of 5a-d, vilsmeier reagent was prepared by adding phosphorous oxychloride (54 mmol) to the solution of DMF (65 mmol) in chloroform (5 mL) by maintaining the temperature 0-5°C. The compound 4a-d (5 mmol) was dissolve in chloroform (20 mL) and the same was added to the Vilsmeier reagent with constant stirring and cooling. The reactant mixture which is obtained set a side at room temperature for 3 hours and reflux the reactant mixture for 15-20 hours by monitoring with TLC. The excess of chloroform solvent was removed under reduced pressure to get oily mixture which was poured on to crushed ice and collect the precipitate of aldehyde derivatives 5a-d was filtered and recrystallized with suitable solvents like petroleum ether with chloroform or ethanol **(**Ozadali *et al.*, 2014**)**.

2.3.6 General procedure for the newly synthesized compounds (6a1-a6 to 6d1-d6)

An one-pot synthetic protocol (Scheme) was employed. By taking compound 5a-d (1 mmol), thiosemicarbazide (1 mmol) and different α-haloaryl ketones (1 mmol) in 5 mL ethanol along with few drops of a catalytic amount of acetic acid and refluxed around 30-50 minutes by observing with TLC to get the final derivatives (6a1-a6 to 6d1-d6), that are filtered, washed with hot ethanol and dried (Ozadali *et al.*, 2014**)** which afforded the analytically pure products **(6a1-a6 to 6d1-d6)** in good yields. The spectral characters of newly synthesized compounds **(6a1-a6 to 6d1-d6)** are as follows.

2.3.7 1-((2-(3,5-dimethyl-1-H-pyrazol-1yl)-6-(4-nitrophenyl) imidazo[2,1-b][1,3,4]thiadiazol-5-yl)methylene)-2-(4-(4- methoxyphenyl)thiazol-2yl)hydrazine (6a1)

Brown solid, Yield: 86%, FT-IR: 3428 (NH), 2898 (C-H, aliphatic), 1639 (C=N), 1093 (C–N); ^{1}H-NMR (400 MHz, DMSO-d6): δ 12.14 (S, NH), δ 5.91 (s, 1H, =CH), δ3.78, 3.84 (s,3H, CH), δ 6.97 (s, 1H, CH, thiazole) δ 7.67-7.75 (m, Ar-H, C2, C6) δ 7.75-8.32 (m, Ar-H, C3, C5) δ 4.25 (S, OCH_3); ^{13}C NMR (100 MHz, DMSO) d 171.2, 164.2, 160.2 (2C), 149.2 (2C), 148.2, 134.7 (3C), 133.1, 131.8, 131.5, 130.7, 130.2, 128.7, 122.0 (2C), 121.5, 119.9, 119.8, 107.2, 100.2, 55.1, 22.0, 13.9; MS (ESI) m/z: 571.12 $[M]^+$.

2.3.8 1-((2-(3,5-dimethyl-1-H-pyrazol-1-yl)-6-(4-nitrophenyl) imidazo[2,1-b][1,3,4]thiadiazol-5-yl)methylene)-2-(4-(4- nitrophenyl)thiazol-2-yl)hydrazine (6a2)

Dark brown, Yield: 87%, FT-IR: 3410 (NH), 2878 (C-H, aliphatic), 1641 (C=N), 1095 (C–N); ^{1}H-NMR (400 MHz,DMSO-d6): δ 12.09 (S, NH), δ 5.91 (S, 1H, =CH), δ3.78, 3.84 (s, 3H, CH_3), δ 6.99 (s, 1H, CH, thiazole), δ 7.95-8.09 (m, Ar-H, C2, C6) δ 8.10-8.32 (m, Ar-H, C3, C5); MS (ESI) m/z: 586.09 $[M]^+$.

2.3.9 1-((2-(3,5-dimethyl-1-H-pyrazol-1yl)-6-(4-nitrophenyl) imidazo[2,1-b][1,3,4]thiadiao-5-yl) methylene)-2-(4-p- tolylthiazol-2-yl)hydrazine (6a3)

Dark brown solid, Yield: 86%, FT-IR: 3425 (NH), 2901 (C-H, aliphatic), 1648 (C=N), 1105 (C–N); ^{1}H-NMR (400 MHz, DMSO-d6): δ 12.15 (S, NH), δ 5.96 (s, 1H, =CH), δ3.58-3.74 (s, 3H, CH_3), δ 6.87 (s, 1H, CH, thiazole), δ 7.65-7.75 (m, Ar-H, C2,C6) δ 7.80-8.22 (m, Ar-H, C3, C5); MS (ESI) m/z: 555.12 $[M]^+$.

2.3.10 1-(4-(4-chlorophenyl)thiazol-2-yl)-2-((2-)3,5-dimethyl-1-H-pyrazol-1-yl)-6-(4-nitrophenyl) imidazo[2,1-b][1,3,4] thiadiazol-5-yl) methylene) hydrazine (6a4)

Brown solid, Yield: 84%, FT-IR: 3405 (NH), 2911 (C-H, aliphatic), 1618 (C=N), 1095 (C–N); ^{1}H-NMR (400 MHz, DMSO-d6): δ 12.09 (S, NH), δ 6.06 (s, 1H, =CH), δ3.78, 3.84 (s,3H, CH) δ 6.71 (s, 1H, CH, thiazole), δ 7.85-7.99 (m, Ar-H, C2 C6) δ 8.01-8.32 (m, Ar-H, C3, C5); MS (ESI) m/z: 575.07 [M].

2.3.11 1-(4-(4-bromophenyl)thiazol-2-yl)-2-((2-(3,5-dimethyl-1H-pyrazol-1-yl)-6-(4-nitrophenyl) imidazo[2,1-b][1,3,4] thiadiazol-5-yl) methylene) hydrazine (6a5)

Brown solid, Yield: 86%, FT-IR: 3416 (NH), 2899 (C-H, aliphatic), 1611 (C=N), 1081 (C–N); ^{1}H-NMR (400 MHz, DMSO-d6): δ 12.11 (s, NH), δ 5.90 (s, 1H, =CH), δ3.68, 3.71 (s, 3H, CH_3), δ 6.89 (s, 1H, CH, thiazole), δ 7.86-7.99 (m, Ar-H, C2, C6) δ 8.02-8.26 (m, Ar-H, C3, C5); MS (ESI) m/z: 619.02 $[M]^+$.

2.3.12 1((2-(3,5-dimethyl-1-H-pyrazol-1-yl)-6-(4-nitrophenyl) imidazo[2,1-b][1,3,4]thiadiazol-5yl)methylene)-2-(4-(3- nitrophenyl)tolythiazole-2-yl)hydrazine (6a6)

Dark brown, Yield: 87%, FT-IR: 3418 (NH), 2828(C-H, aliphatic), 1650 (C=N), 1097 (C–N); ^{1}H-NMR (400 MHz, DMSO-d6): δ12.08 (S, NH), δ 5.93 (s, 1H, =CH), δ3.78, 3.84 (s,3H, CH_3) δ 6.99 (s, 1H, CH, thiazole), δ 7.80-7.89 (m, Ar-H, C2, C6) δ 8.10-8.32 (m, Ar-H, C4, C5); MS (ESI) m/z: 586.09 $[M]^+$.

2.3.13 1-((6-(4-bromophenyl)-2-(3,5-dimethyl-1-H-pyrazol-1-yl) imidazo[2,1-b][1,3,4]thiadiazol-5-yl)methylene)-2-(4-(4- nitrophenyl)thiazol-2-yl)hydrazine (6b1)

Dark green solid, Yield: 85%, FT-IR: 3416 (NH), 2899(C-H, aliphatic), 1611 (C=N), 1081 (C–N); ^{1}H-NMR (400 MHz, DMSO-d6): δ 12.11 (s, NH), δ 5.90 (s, 1H, =CH), δ3.16, 3.27 (s, 3H, CH_3), δ 7.49 (s, 1H, CH, thiazol), δ 7.68-7.90 (m Ar-H, C2, C6) δ 8.00-8.11 (m, Ar-H, C3, C5); ^{13}C-NMR (100 MHz, DMSO) d 171.8, 162.8, 148.4, 148.2, 145.7, 144.3, 139.3 (2C), 138.4,136.2 (2C), 130.1, 129.6 (2C), 128.4 (3C), 127.4 (2C), 121.6(2C),105.3, 100.0, 22.5, 17.2, 12.1; MS (ESI) m/z: 619.02 $[M]^+$.

2.3.14 1-((6-(4-bromophenyl)-2-(3,5-dimethyl-1-H-pyrazol-1-yl) imidazo[2,1-b][1,3,4]thiadiazol-5-yl) methylene)-2-(4-(4- methoxyphenyl)thiazol-2yl)hydrazine (6b2)

Dark brown solid, Yield: 84%, FT-IR: 3442 (NH), 2903 (C-H, aliphatic), 1640 (C=N), 1096 (C–N); ^{1}H-NMR (400 MHz, DMSO-d6): δ 12.10 (S, NH), δ 5.96 (s, 1H, =CH), δ3.79, 3.84 (s, 3H, CH_3), δ 6.96 (s, 1H, CH, thiazole) δ 7.67-7.75 (m, Ar-H, C2,C6) δ 7.75-8.33 (m, Ar-H, C3, C5) δ 4.25 (S, OCH3); MS (ESI)m/z: 604.04 $[M]^+$.

2.3.15 1-((6-(4-bromophenyl)-2-(3,5-dimethyl-1-H-pyrazol-1-yl) imidazo[2,1-b][1,3,4]thiadiazol-5yl) methylene)-2-(4-p- tolylthiazol-2-yl)hydrazine (6b3)

Brown solid, Yield: 86%, FT-IR: 3421 (NH), 2903(C-H, aliphatic), 1616 (C=N), 1097 (C–N); ^{1}H-NMR (400 MHz, DMSO-d6): δ 12.03 (s, NH), δ 5.91 (s, 1H, =CH), δ3.18-3.79 (s, 3H, $2CH_3$), δ 6.91 (s, 1H, CH, thiazole), δ 7.72-7.77 (m, Ar-H, C2, C6) δ 7.85-8.12 (m, Ar-H, C3, C5); MS (ESI) m/z: 588.05 $[M]^+$.

2.3.16 1-((6-(4-bromophenyl)-2-(3,5-dimethyl-1-H-pyrazol-1yl) imidazo[2,1-b][1,3,4]thiadiazol-5-yl)methylene)-2-(4-(4- chlorophenyl)thiazol-2-yl)hydrazine (6b4)

Brown solid, Yield: 87%, FT-IR: 3416 (NH), 2899 (C-H, aliphatic), 1611 (C=N), 1081 (C–N); ^{1}H-NMR (400 MHz, DMSO-d6): δ 12.11 (s, NH), δ 5.90 (s, 1H, =CH), δ3.68, 3.71 (s, 3H, CH_3), δ 6.89 (s, 1H, CH, thiazole), δ 7.79-7.88 (m, Ar-H, C2, C6) δ 7.90-8.21 (m, Ar-H, C3, C5); MS (ESI) m/z: 607.99 $[M]^+$.

2.3.17 1-((6-(4-bromophenyl)-2-(3,5-dimethyl-1-H-pyrazol-1-yl) imidazo[2,1-b][1,3,4]thiadiazol-5-yl)methylene)-2-(4-(4- bromophenyl)thiazol-2-yl)hydrazine (6b5)

Dark green solid, Yield: 85%, FT-IR: 3416 (NH), 2899 (C-H, aliphatic), 1611 (C=N), 1081 (C–N); ^{1}H-NMR (400 MHz, DMSO-d6): δ 12.15 δ 5.90 (s, 1H, =CH), δ3.68, 3.71 (s, 3H, CH_3), δ 6.89 (s, 1H, CH, thiazole), δ 7.79-7.88 (m, Ar-H, C2, C6) δ 7.90-8.21m, Ar-H, C3, C5); MS (ESI) m/z: 651.94 $[M]^+$.

2.3.18 1-((6-(4-bromophenyl)-2-(3,5-dimethyl-1-H-pyrazol-1-yl) imidazo[2,1-b][1,3,4]thiadiazol-5-yl)methylene)-2-(4-(3- nitrophenyl)thiazol-2-yl)hydrazine (6b6)

Brown solid, Yield: 86%, FT-IR: 3418 (NH), 2828 (C-H, aliphatic), 1650 (C=N), 1097 (C–N); ^{1}H-NMR (400 MHz, DMSO-d6): δ 12.08 (S, NH), δ 5.93 (s, 1H, =CH), δ3.78, 3.84 (s, 3H, CH_3), δ 6.99 (s, 1H, CH, thiazole), δ 7.77-7.79 (m, Ar-H, C2, C6) δ 8.10-8.32 (m, Ar-H, C4, C5); MS (ESI) m/z: 619.02 $[M]^+$.

2.3.19 1-((2-(3,5-dimethyl-1-H-pyrazol-1yl)6-(4-methoxyphenyl) imidazo[2,1-b][1,3,4]thiadiazol-5-yl) methylene)-2-(4-(4- nitrophenyl)thiazol-2-yl)hydrazine (6c1)

Dark green solid, Yield: 85%, FT-IR: 3428 (NH), 2898 (C-H, aliphatic), 1639 (C=N), 1093 (C–N); ^{1}H-NMR (400 MHz, DMSO-d6) δ 12.14 (S, NH), δ 5.91 (s, 1H, =CH), δ3.78, 3.84 (s, 3H, CH_3), δ 6.97 (s, 1H, CH, thiazole) δ 7.67-7.75 (m, Ar-H, C2, C6) δ 7.75-8.32 (m, Ar-H, C3, C5) δ 4.25 (S, OCH3); ^{13}C NMR (100 MHz, DMSO) d 172.8, 164.8, 160.2, 148.2, 147.2, 145.7, 139.2 (2C), 138.2, 137.2 (2C), 131.1, 129.5 (2C), 128.4 (3C), 127.2 (2C), 121.5 (2C), 104.3, 100.1, 56.1, 22.0, 13.8; MS (ESI) m/z: 571.12 $[M]^+$.

2.3.20 1-((2-(3,5-dimethyl-1-H-pyrazol-1yl)-6-(4-methoxyphenyl) imidazo[2,1-b][1,3,4]thiadiazol-5-yl)methylene)-2-(4-(4- methoxyphenyl)thiazol-2-yl)hydrazine (6c2)

Dark orange solid, Yield: 88%, FT-IR: 3428 (Secondary amine N-H), 2898 (C-H, aliphatic), 1639 (C=N), 1093 (C–N); ^{1}H-NMR (400 MHz, DMSO-d6): δ 12.14 (S, NH), δ 5.91 (s, 1H,=CH), δ3.78, 3.84 (s, 3H, CH_3), δ 6.97 (s, 1H, CH, thiazole) δ 7.67-7.75 (m, Ar-H, C2, C6) δ 7.75-8.32 (m, Ar-H, C3, C5) δ 4.36, 4.36 (S, OCH3); MS (ESI) m/z: 556.14 $[M]^+$

2.3.21 1-((2-(3,5-dimethyl-1-H-pyrazol-1yl)-6-(4-methoxyphenyl) imidazo[2,1-b][1,3,4]thiadiazol-5-yl)methylene)-2-(4-p- tolylthiazol-2-yl)hydrazine (6c3)

Brown solid, Yield: 86%, FT-IR: 3411 (NH), 2901 (C-H, aliphatic),1619 (C=N) 1101 (C–N); ^{1}H-NMR (400 MHz, DMSO- d6): δ 12.14 (S, NH), δ 5.91 (s, 1H, =CH), δ2.51, 2.55 (s, 3H, CH_3), δ 6.98 (s, 1H, CH, thiazole) δ 7.22-7.30 (m, Ar-H, C2, C6) δ 7.42-7.68 (m, Ar-H, C3, C5) δ 3.88 (S, OCH3); MS (ESI) m/z: 540.15 $[M]^+$.

2.3.22 1-(4-(4-chlorophenyl)thiazol-2-yl)-2-((2-(3,5-dimethyl-1-H- pyrazol-1-yl)-6-(4-methoxy phenyl)imidazo[2,1-b][1,3,4] thiadiazol-5-yl) methylene) hydrazine (6c4)

Dark green solid, Yield: 86%, FT-IR: 3428 (NH), 2898 (C-H, aliphatic), 1639 (C=N), 1093 (C–N); ^{1}H-NMR (400 MHz, DMSO-d6): δ 12.14 (S, NH), δ 5.86 (s, 1H, =CH), δ3.78, 3.84 (s, 3H, CH3), δ 6.97 (s, 1H, CH, thiazole)δ 7.82-7.96 (m, Ar-H, C2, C6) δ 8.00-8.24 (m, Ar-H, C3, C5) δ 4.27 (S, OCH3); MS (ESI) m/z: 560.09 $[M]^+$.

2.3.23 1-(4-(4-chlorophenyl)thiazol-2-yl)-2-((2-(3,5-dimethyl-1-H-pyrazol-1-yl)-6-(4-methoxy phenyl) imidazo[2,1-b][1,3,4] thiadiazol-5-yl) methylene) hydrazine (6c5)

Dark green solid, Yield: 86%, FT-IR: 3442 (NH), 2903 (C-H, aliphatic), 1640 (C=N), 1096 (C–N); ^{1}H-NMR (400 MHz, DMSO-d6): δ 12.10 (S, NH), δ 5.97 (s, 1H, =CH), δ3.83, 3.84 (s, 3H, CH_3), δ 7.12 (s, 1H, CH, thiazol) δ 7.67-7.75 (m, Ar-H, C2, C6) δ 8.00-8.24 (m, Ar-H, C3, C5) δ 4.29 (S, OCH3); MS (ESI) m/z: 604.04 $[M]^+$.

2.3.24 1-((2-(3,5-dimethyl-1-H-pyrazol-1-yl)-6-(4-methoxyphenyl) imidazo[2,1-b][1,3,4]thiadiazol-1-yl)methylene-2-(4-(3- nitrophenyl)thiazol-2-yl)hydrazine (6c6)

Brown solid, Yield: 83%, FT-IR: 3418 (NH), 2828 (C-H, aliphatic), 1650 (C=N), 1097 (C–N); ^{1}H-NMR (400 MHz, DMSO-d6): δ 12.08 (S, NH), δ 5.93 (s, 1H, =CH), δ3.78, 3.84 (s, 3H, CH_3), δ 6.99 (s, 1H, CH, thiazole), δ 7.77-7.79 (m, Ar-H, C2, C6) δ 8.10-8.32 (m, Ar-H, C4, C5), δ 4.36 (S, OCH3); MS (ESI) m/z: 571.12 $[M]^+$.

2.3.25 1-((6-(4-chlorophenyl)-2-(3,5-dimethyl-1-H-pyrazol-1yl) imidazo[2,1-b][1,3,4]thiadiazol-5-yl)methylene)-2-(4-(4- methoxyphenyl) thiazol-2-yl)hydrazine (6d1)

Light green solid, Yield: 85%, FT-IR: 3428 (NH), 2898 (C-H, aliphatic), 1639 (C=N), 1093 (C–N); ^{1}H NMR (400 MHz, DMSO-d6): δ 12.14 (S, NH), δ 5.58 (s, 1H, =CH), δ3.78, 3.84 (s, 3H, CH_3), δ 6.96 (s, 1H, CH, thiazole), δ 7.11-7.63 (m, Ar-H, C2, C6) δ 7.80-7.88 (m, Ar-H, C3, C5) δ 4.25 (S, OCH3); ^{13}C NMR (100 MHz, DMSO) d 171.7, 163.8, 160.2, 148.2, 145.2, 145.7, 144.2, 139.3 (2C), 136.2, 129.4 (2C), 128.9 (3C), 128.5 (2C), 127.3, 121.5, 114.8 (2C), 104.3, 100.1, 55.9, 17.6, 11.9; MS (ESI) m/z: 560.09 $[M]^+$.

2.3.26 1((6-(4-chlorophenyl)-2-(3,5-dimethyl-1-H-pyrazol-1yl) imidazo[2,1-b][1,3,4]thiadiazol-5-yl)methylene)-2-(4-(4- nitrophenyl)thiazol-2-yl)hydrazine (6d2)

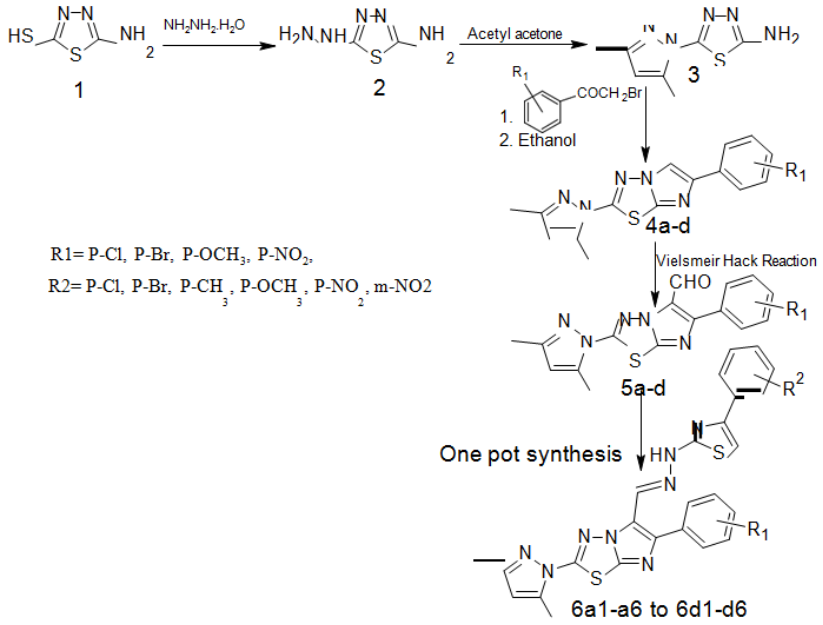
Dark green solid, Yield: 86%, FT-IR: 3405 (NH), 2911 (C-H, aliphatic), 1618 (C=N), 1095 (C–N); ^{1}H-NMR (400 MHz, DMSO-d6): δ 12.09 (S, NH), δ 6.06 (s, 1H, =CH), δ3.78, 3.84 (s, 3H, CH_3), δ 6.71 (s, 1H, CH, thiazole), δ 7.72-7.85 (m, Ar-H, C2, C6) δ 8.02-8.11 (m, Ar-H, C3, C5); MS (ESI) m/z: 575.07 $[M]^+$.

2.3.27 1-((6-(4-chlorophenyl)-2-(3,5-dimethyl-1-H-pyrazol-1yl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)methylene)-2-(4-p- tolylthiazol-2yl)hydrazine (6d3)

Light green solid, Yield: 86%, FT-IR: 3405 (NH), 2911 (C-H, aliphatic), 1618 (C=N), 1095 (C–N); ^{1}H-NMR (400 MHz, DMSO-d6): δ 12.07 (s, NH), δ 5.99 (s, 1H, =CH), δ3.58-3.74 (s, 3H, CH_3), δ 6.71 (s, 1H, CH, thiazole), δ 7.75-7.85 (m, Ar-H, C2, C6) δ 7.85-8.32 (m, Ar-H, C3, C5); MS (ESI) m/z: 544.10 $[M]^+$.

2.3.28 1-((6-(4-chlorophenyl)-2-(3,5-dimethyl-1-H-pyrazol-1yl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)methylene-2-(4-(4- chlorophenyl)thiazol-2-yl)hydrazine (6d4)

Brown solid, Yield: 85%, FT-IR: 3405 (NH), 2911 (C-H, aliphatic), 1618 (C=N), 1095 (C–N); H-NMR (400 MHz, DMSO- d6): δ 12.09 (S, NH), δ 6.06 (s, 1H, =CH), δ3.78, 3.84 (s, 3H, CH_3), δ 6.71 (s, 1H, CH, thiazole), δ 7.75-7.85 (m, Ar-H, C2, C6) δ 7.85-8.32 (m, Ar-H, C3, C5); MS (ESI) m/z: 564.04 $[M]^+$.



SCHEME 1: Synthetic route for the preparation of imidazo-thiadiazole derivatives

2.3.29 1-(4-(4-bromophenyl)thiazol-2-yl)-2-((6-(4-chlorophenyl)-2-(3,5-dimethyl-1-H-pyrazol-1-yl)imidazo[2,1-b][1,3,4] thiadiazol-5-yl)methylene)hydrazine (6d5)

Dark brown solid, Yield: 84%, FT-IR: 3416 (NH), 2899 (C-H, aliphatic), 1611 (C=N), 1081 (C–N); ^{1}H-NMR (400 MHz, DMSO-d6): δ 12.11 (s, NH), δ 5.90 (s, 1H, =CH), δ3.68, 3.71 (s, 3H, CH_3), δ 6.89 (s, 1H, CH, thiazole), δ 7.62-7.99 (m, Ar-H, C2, C6) δ 8.05-8.21 (m, Ar-H, C3, C5); MS (ESI) m/z: 607.99 $[M]^+$.

2.3.30 1-((6-(4-chlorophenyl)-2-(3,5-dimethyl-1-H-pyrazol-1yl) imidazo[2,1-b][1,3,4]thiadiazol-5yl)methylene)-2-(4-(3- nitrophenyl)thiazol-2-yl) hydrazine (6d6)

Brown solid, Yield: 86%, FT-IR: 3418 (NH), 2828 (C-H, aliphatic), 1650 (C=N), 1097 (C–N); ^{1}H-NMR (400 MHz, DMSO-d6): δ 12.08 (S, NH), δ 5.93 (s, 1H, =CH), δ3.78, 3.84 (s, 3H, CH_3), δ 6.99 (s, 1H, CH, thiazole), δ 7.77-7.79 (m, Ar-H, C2, C6) δ 8.10-8.32 (m, Ar-H, C4, C5); MS (ESI) m/z: 575.07 $[M]^+$.

III. RESULTS AND DISCUSSION

In this work, we have successfully synthesized twenty-four new moieties presented in the scheme and their structures were analyzed with the help of various spectroscopic techniques. The ^{1}H-NMR for 6a1-a6 to 6d1-d6 showed a singlet of -NH proton with a broad peak around δ12 ppm and one more proton of the (C=N-) imine linkage shows a singlet of -CH at around δ 8 ppm.

The ^{13}C-NMR of compound **6a1-a6 to 6d1-d6** showed a carbon frequency around 172 ppm due to the presence of secondary nitrogen either side of the carbon of thiazole at a second position which is attached to imine (CH=N−) linkage and the other carbon frequencies around 10 and 20 ppm for methyl groups on the pyrazole ring.

All synthesized compounds **(6a1-a6 to 6d1-d6)** were tested for anti-TB activity, compounds **6a1, 6a2, 6a3, 6c1, 6c6** and **6d1** have shown promising antitubercular activity since the results showed that

amongst all the tested compounds, the compounds **6a1, 6a2, 6a3, 6c1,** and **6d1** with methoxy/nitro substitution at 4th position of phenyl ring at 4th position of thiazole ring of the condensed imidazo[2,1-b][1,3,4]-thiadiazoles moiety and nitro or chloro or methoxy substitution on the 4th position of the phenyl ring at 6th position of the condensed imidazothiadiazole moiety was shown good anti-tubercular activity (1.6 to 6.25 mcg/ml) against *Mycobacterium tuberculosis H37Rv* strain and Streptomycin (6.25 mcg/mL), Ciprofloxacin (3.125 mcg/mL) along with pyrazinamide (3.25 mcg/mL) as standard.

TABLE 2
ANTI-TUBERCULAR AND ANTI-FUNGAL ACTIVITY

	Anti-TubercularActivity	**Anti-fungal activity**	
Product	***M. tuberculosis***	***Candida Albicans***	***A. Flavus***
6 a1	1.6	5	25
6 a2	6.25	10	25
6 a3	6.25	10	50
6 a4	12.5	5	10
6 a5	12.5	5	50
6 a6	12.5	50	75
6 b1	25	25	25
6 b2	12.5	10	25
6 b3	12.5	25	50
6 b4	25	25	25
6 b5	12.5	25	50
6 b6	12.5	50	75
6 c1	6.25	75	75
6 c2	12.5	75	75
6 c3	6.25	25	75
6 c4	12.5	25	50
6 c5	12.5	25	50
6 c6	6.25	50	75
6 d1	6.25	5	25
6 d2	25	25	25
6 d3	25	25	50
6 d4	25	10	25
6 d5	25	25	50
6 d6	25	75	75
Standard values			
Streptomycin	**6.25**	**Fluconazole**	**30**
Ciprofloxacin	**3.125**		

All synthesized compound **(6a1-a6 to 6d1-d6)** were tested for antifungal activity and Fluconazole as a standard drug (MIC **30** µgm/ml). The compounds **6a1, 6a4, 6a5,** and **6d1** have shown promising antifungal activity (MIC **5** µgm/ml) due to the presence of methoxy/chloro/bromo substitution at 4th position of phenyl ring at 4th position of thiazole ring of the basic imidazothiadiazole ring and presence of nitro/chloro groups on to the 4th position of phenyl ring attached to 6th position of basic ring moiety.

But in the presence of electron donating groups in the same position are less reactive along with the substitution at the third position to the phenyl ring. The results are given in Table 2.

IV. CONCLUSION

The present study on the substituted derivatives of imidazo[2,1-b][1,3,4]thiadiazole showed a moderate to finest activity against Mycobacterium tuberculosis and fungal species.

There is a scope with a slight modification on the basic moiety can produce excellent derivatives with better activity and enhanced pharmacokinetic property.

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