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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, spectral studies and biological evaluation of a novel series of 2-substituted-5,6-diarylsubstituted imidazo(2,1-b)-1,3,4-thiadiazole derivatives as possible anti-tubercular agents

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Abstract A novel series of 18 analogs of 2-substituted-5,6-diarylsubstituted imidazo(2,1-b)-1,3,4-thiadiazole **6a–r** have been synthesized by the reaction of 2-amino-5-substituted-1,3,4-thiadiazoles **5a–d** and an appropriately substituted α-bromo-1,2-(*p*-substituted)diaryl-1-ethanones **4a–e**. Structures of these compounds were established by physiochemical, elemental analysis and spectral data. All the title compounds were tested for their in-vitro anti-tubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$ using Alamar Blue susceptibility test and the activity expressed as the minimum inhibitory concentration (MIC) in μg/ml. Among synthesized compounds, compound **6h** (MIC = 1.25 μg/ml) exhibited excellent anti-tubercular activity with respect to other synthesized compounds and reference drugs. Compounds **6c**, **6f**, and **6g** have also displayed an encouraging anti-tubercular activity profile. Further, some title compounds were also assessed for their cytotoxic activity (IC_{50}) against in a mammalian Vero cell line using MTT assay. The results reveal that these compounds exhibit anti-tubercular activity at non-cytotoxic concentrations.

Keywords Anti-tubercular activity · Cytotoxicity · 2-Amino-5-substituted-1,3,4-thiadiazoles · Imidazo-(2,1-b)-1,3,4-thiadiazoles

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Introduction

Tuberculosis (TB) is the oldest documented infectious disease. It is a chronic necrotizing bacterial infection with wide variety of manifestations caused by *Mycobacterium tuberculosis*, which has plagued humans throughout recorded and archeological history (Dutt and Stead, 1999). The introduction of the first line drugs like streptomycin, para-aminosalicylic acid, isoniazid etc., for treatment some 50 years ago has witnessed in a remarkable decline in TB cases all over the world. The active TB is currently treated with a four-drug regimen comprising mainly isoniazid, rifampicin, pyrazinamide and ethambutol for a period of at least 6 months (Snider and Roper, 1992; Bass *et al.*, 1994).

Having these powerful weapons in armamentarium, the battle against TB at least in industrialized countries seemed to be over during 1970s. But it did not take a long time for *M. tuberculosis* to find its way around these compounds, since the mid of 1980s, the disease has been undergoing a resurgence driven by variety of changes in social, medical and economic factors. The long treatment regime can be difficult to fully complete, fueling the development of multi-drug resistant (MDR) TB that allowed *M. tuberculosis* to escape the current drug arsenal and created frightening epidemics in affected countries. In the absence of an effective therapy, patients with MDRTB continue to spread the disease, producing new infections with the

resistant strains. Since the MDRTB microbe is more infectious and virulent than the sensitive *M. tuberculosis*, and until new drugs that are effective against MDR strains are introduced, TB epidemic will grow at an exponential level (Rattan *et al.*, 1998 and Weil, 1994). The other alarming fact is that the outbreak of HIV during the 1980s has exposed the frailties of the current drug armamentarium and has been accelerating the spread of TB all over the world (Davies, 2000).

Today, TB is among the top five causes of global mortality. One third of the world's population is currently infected with *M. tuberculosis*; 10% of infected cases will develop clinical disease. According to the World Health Organization (WHO) facts sheets, TB kills 2 million people each year. It is estimated that between 2000 and 2020, nearly 1 billion people will be newly infected; in other words, someone in the world is newly infected with TB every second. 200 million people will get sick and 35 million will die from TB if control is not further strengthened (Maher, 2002; Dye, 2002). No new class of anti-tuberculosis agents has been developed since the introduction of rifampin into clinic in 1960s. Therefore, there is an urgent need for development of innovative anti-TB agents to effectively combat TB, with improved properties such as enhanced activity against MDR strains, reduced toxicity and shortened duration of therapy. From the mid-1990s, this infectious disease was the focus of renewed scientific interest. In the last 10 years, the research on *M. tuberculosis* has undergone much progress. The thriving accomplishment of the genome of *M. tuberculosis* has offered a promise of a new generation of potent drugs to combat the emerging epidemic of TB. The emphasis of mycobacterial research now has shifted from gene hunting to interpretation of the biology of the whole organism in an effort to better define which activities is likely to be critical to survival and thus amenable to the development of new drugs (Cole *et al.*, 1998; Barry *et al.*, 2000). In this regard, there have been few additions of some promising new anti-tuberculosis agents, such as the long acting rifamycins, fluoroquinolones, oxazolidinones and nitroimidazopyrans to the existing main-line drugs (Ian, 2001).

Imidazoles certainly belong among the most important, significant and abundant five-membered heterocycles, which are constituents of a variety of natural and synthetic products. The advent of sulfur drugs and the later discovery of mesoionic compounds greatly accelerated the rate of progress in the field of thiadiazoles. The thiadiazole and imidazole compounds are extensively studied due to their wide spectrum of bioactivities. Among them the imidazo(2,1-b)-1,3,4-thiadiazole derivatives are pharmacologically important because of their immunostimulant, anti-inflammatory, analgesic, antifungal, antimicrobial, antileishmanial, antitumor, anti-tuberculosis and other activities (Mazzone *et al.*, 1984; Srivastava and Pathak, 1991; Gadad *et al.*, 1999, Terzioglu and Aysel, 2003; Jaquith *et al.*, 2010). In addition, the reports of Kolavi *et al.*, (2006) and Gadad *et al.*, (2004) on the synthesis, antimicrobial and anti-tubercular activity of a series of 2,5,6-trisubstituted imidazo(2,1-b)-1,3,4-thiadiazoles and 2,6-disubstituted imidazo(2,1-b)-1,3,4-thiadiazole derivatives respectively, which have exhibited moderate to excellent anti-tuberculosis activity, are the driving force for selecting imidazo(2,1-b)-1,3,4-thiadiazole nucleus. Although, recently Gadad *et al.*, (2008) have reported the synthesis and biological evaluation of 2-trifluoromethyl/sulfonamido-5,6-diaryl substituted imidazo(2,1-b)-1,3,4-thiadiazole derivatives as cyclooxygenase-2 inhibitors and also Pyl *et al.*, (1963) have reported earlier the synthesis of 2-methyl-5,6-unsubstituted diaryl imidazo(2,1-b)1,3,4-thiadiazole derivatives, but there are no reports on anti-tubercular activity of substituted 5,6-diarylimidazo(2,1-b)1,3,4-thiadiazoles. Therefore, in view of the above facts and in continuation of our search on biologically active heterocyclic systems (Palkar *et al.*, 2010), in this article, we report the synthesis and spectral studies of novel series of 2-substituted-5,6-diarylsubstituted imidazo(2,1-b)1,3,4-thidiazole derivatives (**6a–r**) and preliminary evaluation of in vitro anti-tubercular as well as cytotoxic activity.

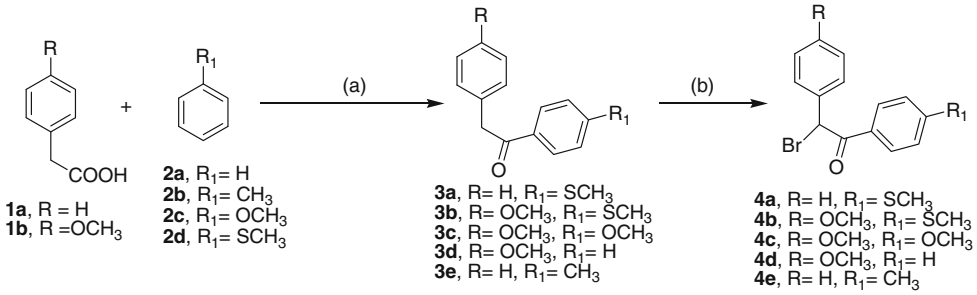
Chemistry

Synthesis of 1,2-(*p*-substituted)diaryl-1-ethanones **3a–e** was carried out by reacting appropriate phenyl acetic acid **1a**, **b** with various substituted aromatic hydrocarbons **2a–d**. Subsequently, **3a–e** were subjected to bromination using liquid bromine in chloroform to obtain α-bromo-1,2-(*p*-substituted)diaryl-1-ethanones **4a–e** as shown in Scheme 1 (Veeramaneni *et al.*, 2003; Shimokawa *et al.*, 1979).

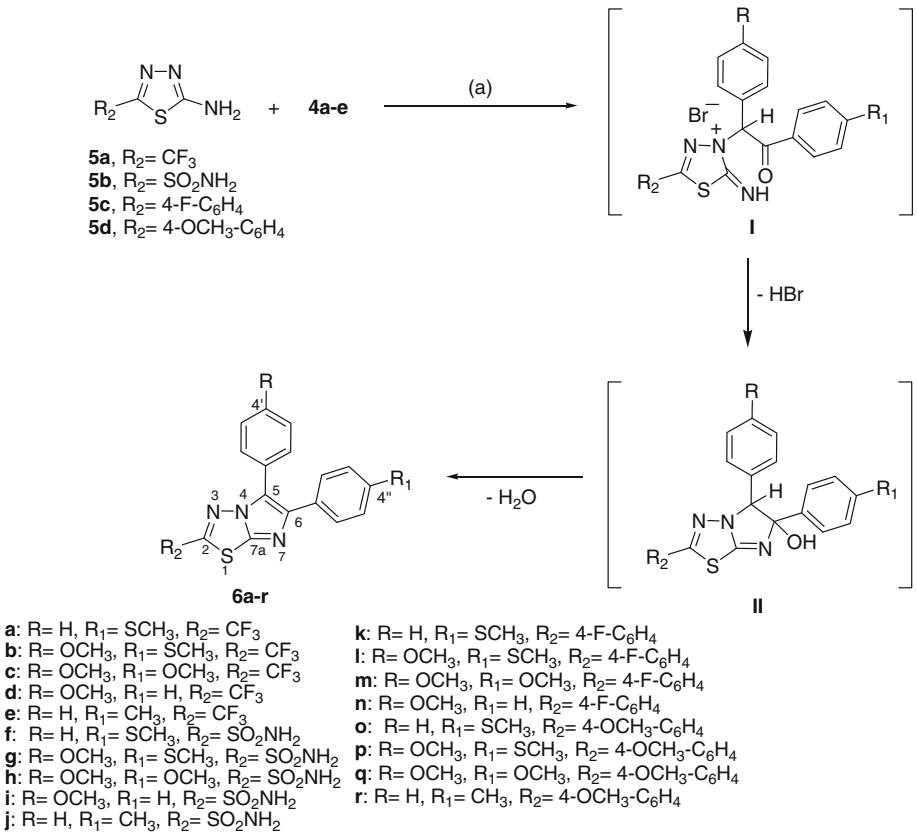
The synthesis of 2-substituted-5,6-diarylsubstituted imidazo(2,1-b)-1,3,4-thiadiazole derivatives **6a–r** was carried out by the condensation of **4a–e** with 2-amino-5-substituted-1,3,4-thiadiazole **5a–d** under reflux in dry ethanol (as depicted in Scheme 2). This reaction proceeds via intermediate iminothiadiazole **I**, which spontaneously undergoes ring closer to **II** under reflux temperature to afford the desired fused heterocycles **6a–r** with good yields.

The substitution at fifth position of 2-amino-5-substituted-1,3,4-thiadiazole is crucial in determining the course of its reaction with substituted α-bromo-1,2-(*p*-substituted)diaryl-1-ethanone, such type of reactions have been studied by deStevens *et al.*, (1993) in case of 2-amino-5-substituted-1,3,4-thiadiazole derivatives. Pyl *et al.*, (1963) have described the synthesis 5,6-unsubstituted diaryl

Scheme 1 Synthetic route of α-bromo-1-(4″-substituted)phenyl-2-(4′-substituted)phenyl-1-ethanones (**4a–e**). Reagents and conditions: (*a*) H_3PO_4, $(CF_3CO)_2O$, 25°C, stir, 1 min; (*b*) Br_2, $CHCl_3$, 50°C, stir, 0.5 h



Scheme 2 Synthetic route of a novel series of 2-substituted-5,6-diarylsubstituted imidazo-(2,1-b)-1,3,4-thiadiazole (**6a–r**). Reagents and conditions: (*a*) absolute alcohol, reflux, 6–12 h, P_2O_5, reflux, 4–9 h, Na_2CO_3



a: R= H, R_1= SCH_3, R_2= CF_3
b: R= OCH_3, R_1= SCH_3, R_2= CF_3
c: R= OCH_3, R_1= OCH_3, R_2= CF_3
d: R= OCH_3, R_1= H, R_2= CF_3
e: R= H, R_1= CH_3, R_2= CF_3
f: R= H, R_1= SCH_3, R_2= SO_2NH_2
g: R= OCH_3, R_1= SCH_3, R_2= SO_2NH_2
h: R= OCH_3, R_1= OCH_3, R_2= SO_2NH_2
i: R= OCH_3, R_1= H, R_2= SO_2NH_2
j: R= H, R_1= CH_3, R_2= SO_2NH_2
k: R= H, R_1= SCH_3, R_2= 4-F-C_6H_4
l: R= OCH_3, R_1= SCH_3, R_2= 4-F-C_6H_4
m: R= OCH_3, R_1= OCH_3, R_2= 4-F-C_6H_4
n: R= OCH_3, R_1= H, R_2= 4-F-C_6H_4
o: R= H, R_1= SCH_3, R_2= 4-OCH_3-C_6H_4
p: R= OCH_3, R_1= SCH_3, R_2= 4-OCH_3-C_6H_4
q: R= OCH_3, R_1= OCH_3, R_2= 4-OCH_3-C_6H_4
r: R= H, R_1= CH_3, R_2= 4-OCH_3-C_6H_4

imidazo(2,1-b)-1,3,4-thiadiazole derivatives starting with 2-amino-5-substituted-1,3,4-thiadiazole.

Biological activity

Anti-tubercular activity

Minimum inhibitory concentration (MIC) values presented in Table 3 were determined for title compounds **6a–r** against *M. tuberculosis* strain $H_{37}Rv$ using the Micro plate Alamar Blue assay (MABA) (Franzblau *et al.*, 1998). This methodology is nontoxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric methods (Vanitha and Paramasivan 2004; Reis *et al.*, 2004). The purpose of the screening program is to provide a resource whereby new experimental compounds can be tested for their capacity to inhibit the growth of virulent *M. tuberculosis*.

Cytotoxic activity

The cellular conversion of MTT [3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyl-tetrazolium bromide] into a formazan product (Mosmann, 1983) was used to evaluate cytotoxic activity (IC_{50}) of some synthesized compounds (**6a–c**, **f–j**, **l**, **m** and **o–q**) against a mammalian Vero cell line upto concentrations of 62.5 μg/ml using the Promega Cell Titer 96 non-radioactive cell proliferation assay (Gundersen *et al.*, 2002). The IC_{50} values were determined and are presented in Table 3.

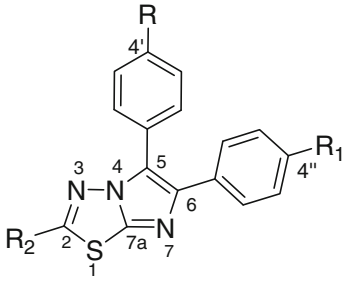
Results and discussion

Synthetic and spectral studies

We have synthesized a novel series of 2-substituted-5,6-diarylsubstituted imidazo(2,1-b)-1,3,4-thiadiazole derivatives (**6a–r**) by reacting 2-amino-5-substituted-1,3,4-thiadiazole (**5a–d**) with an appropriately substituted α-bromo-1,2-(*p*-substituted)diaryl-1-ethanones (**4a–e**) as illustrated in Scheme 2. All the newly synthesized compounds gave satisfactory analysis for the proposed structures, which were confirmed on the basis of physicochemical, elemental analysis and spectral data that are summarized in Tables 1 and 2, respectively.

In general, the IR spectra of these compounds showed moderately strong bands around 3232–3454, 2921–2969 and 1594–1618 cm^{-1}, characteristic of the SO_2NH_2, CH_3 and C=N groups, respectively. In the nuclear magnetic resonance spectra (^1H NMR), the signals of the respective protons of the synthesized compounds were verified on the basis of their chemical shifts, multiplicities and coupling constants. In ^1H-NMR spectra, the synthesized compounds showed prominent signals for the aromatic protons as multiplets between δ 6.77–8.10 ppm. Compounds **6f–j** showed a characteristic singlet between δ 8.60–8.82 ppm indicating the presence of SO_2NH_2 group. The peaks appeared around δ 1.15–1.22, 1.92–2.03 and 3.75–3.88 ppm confirms the presence of CH_3, SCH_3 and OCH_3 groups, respectively.

Table 1 Physico-chemical data of a novel series of 2-substituted-5,6-diarylsubstitued imidazo(2,1-b)-1,3,4-thiadiazole derivatives (**6a–r**)



Compound	Structure			Yield (%)	Mp (°C)	Rf value[c]	Mol. weight
	R	R_1	R_2				
6a	H	SCH_3	CF_3	72.50	162–164	0.42[a]	391.40
6b	OCH_3	SCH_3	CF_3	60.90	142–144	0.54[a]	421.50
6c	OCH_3	OCH_3	CF_3	57.35	110–112	0.50[a]	405.40
6d	OCH_3	H	CF_3	63.50	138–140	0.62[b]	375.40
6e	H	CH_3	CF_3	46.52	178–180	0.52[c]	359.40
6f	H	SCH_3	SO_2NH_2	55.30	290–292	0.49[c]	402.50
6g	OCH_3	SCH_3	SO_2NH_2	43.68	314–318	0.68[b]	432.50
6h	OCH_3	OCH_3	SO_2NH_2	39.90	298–300	0.60[b]	416.50
6i	OCH_3	H	SO_2NH_2	68.91	302–304	0.55[b]	386.45
6j	H	CH_3	SO_2NH_2	61.25	276–280	0.48[a]	370.45
6k	H	SCH_3	4-F-C_6H_4	49.34	210–212	0.53[a]	417.50
6l	OCH_3	SCH_3	4-F-C_6H_4	57.26	196–200	0.71[b]	447.55
6m	OCH_3	OCH_3	4-F-C_6H_4	62.78	222–224	0.59[b]	431.50
6n	OCH_3	H	4-F-C_6H_4	44.56	216–218	0.43[a]	401.45
6o	H	SCH_3	4-OCH_3-C_6H_4	52.89	232–234	0.65[b]	429.60
6p	OCH_3	SCH_3	4-OCH_3-C_6H_4	66.02	204–206	0.57[b]	459.60
6q	OCH_3	OCH_3	4-OCH_3-C_6H_4	59.23	184–188	0.62[b]	443.50
6r	H	CH_3	4-OCH_3-C_6H_4	69.62	190–194	0.74[b]	397.50

All synthesized compounds were purified by flash chromatography using 200–400 mesh silica gel

[a] Mobile phase choloroform:hexane (1:9)

[b] Mobile phase ethyl acetate:hexane (2:8)

[c] Iodine vapors or 10% aqueous potassium permanganate solution was used as visualizing agent

Table 2 Spectral and elemental analysis data of a novel series of 2-substituted-5,6-diarylsubstitued imidazo(2,1-b)-1,3,4-thiadiazole derivatives (**6a–r**)

Compound	IR (KBr, cm^{-1})	^1H NMR (DMSO-d_6, δ, ppm)	Elemental analysis
6f	3440, 3020, 2930, 2855, 1610, 1572, 1463, 1239, 1171, 848	8.82 (s, 2H, SO_2NH_2), 7.95 (d, $J = 8.5$ Hz, 2H, aryl-H), 7.79 (d, $J = 8.5$ Hz, 2H, aryl-H), 7.63-7.74 (m, 3H, aryl-H), 7.42 (d, $J = 8.8$ Hz, 2H, aryl-H), 1.97 (s, 3H, 4″-SCH_3)	Anal. calcd. for $C_{17}H_{14}N_4O_2S_3$ C, 50.73; H, 3.51; N, 13.92. Found: C, 50.73; H, 3.48; N, 13.97
6g	3454, 3060, 2961, 2873, 1612, 1578, 1469, 1402, 1235, 1162, 881	8.76 (s, 2H, SO_2NH_2), 8.24 (d, $J = 8.3$ Hz, 2H, aryl-H), 8.08 (d, $J = 8.3$ Hz, 2H, aryl-H), 7.57 (d, $J = 8.5$ Hz, 2H, aryl-H), 7.26 (d, $J = 8.5$ Hz, 2H, aryl-H), 3.84 (s, 3H, 4′-OCH_3), 2.02 (s, 3H, 4″-SCH_3)	Anal. calcd. for $C_{18}H_{16}N_4O_3S_3$ C, 49.98; H, 3.73; N, 12.95. Found: C, 50.03; H, 3.72; N, 12.90
6j	3409, 3115, 2921, 2844, 1618, 1489, 1360, 1243, 1179, 861	8.60 (s, 2H, SO_2NH_2), 7.52 (d, $J = 8.8$ Hz, 2H, aryl-H), 7.46 (d, $J = 8.8$ Hz, 2H, aryl-H), 6.94-7.11 (d, 3H, aryl-H), 6.83 (d, $J = 8.0$ Hz, 2H, aryl-H), 1.15 (s, 3H, CH_3)	Anal. Calcd. for $C_{17}H_{14}N_4O_2S_2$ C, 55.12; H, 3.81; N, 15.12. Found: C, 55.12; H, 3.77; N, 15.09
6l	3029, 2946, 2844, 1600, 1529, 1435, 1228, 1134, 755	7.91 (d, $J = 8.3$ Hz, 2H, aryl-H), 7.82 (d, $J = 8.3$ Hz, 2H, aryl-H), 7.69 (d, $J = 8.9$ Hz, 2H, aryl-H), 7.53 (d, $J = 8.9$ Hz, 2H, aryl-H), 6.93 (d, $J = 8.0$ Hz, 2H, aryl-H), 6.77 (d, $J = 8.0$ Hz, 2H, aryl-H), 3.81 (s, 3H, 4′-OCH_3), 1.92 (s, 3H, 4″-SCH_3)	Anal. calcd. for $C_{24}H_{18}FN_3OS_2$ C, 64.41; H, 4.05; N, 9.39. Found: C, 64.40; H, 4.07; N, 9.39
6m	3010, 2949, 2835, 1596, 1461, 1366, 1269, 1114, 771	7.85 (d, $J = 8.1$ Hz, 2H, aryl-H), 7.76 (d, $J = 8.1$ Hz, 2H, aryl-H), 7.49 (d, $J = 8.0$ Hz, 2H, aryl-H), 7.35 (d, $J = 8.0$ Hz, 2H, aryl-H), 7.05 (d, $J = 8.6$ Hz, 2H, aryl-H), 6.90 (d, $J = 8.6$ Hz, 2H, aryl-H), 3.91 (s, 3H, 4′-OCH_3), 3.83 (s, 3H, 4″-OCH_3)	Anal. calcd. for $C_{24}H_{18}FN_3O_2S$ C, 66.81; H, 4.20; N, 9.74. Found: C, 66.85; H, 4.26; N, 9.79
6n	3050, 2938, 2832, 1606, 1502, 1457, 1392, 1255, 1162, 786	8.02 (d, $J = 8.9$ Hz, 2H, aryl-H), 7.84 (d, $J = 8.9$ Hz, 2H, aryl-H), 7.59 (d, $J = 8.3$ Hz, 2H, aryl-H), 7.33–7.20 (m, 5H, aryl-H), 6.97 (d, $J = 8.3$ Hz, 2H, aryl-H), 3.75 (s, 3H, 4′-OCH_3)	Anal. calcd. for $C_{23}H_{16}FN_3OS$ C, 68.81; H, 4.02; N, 10.47. Found: C, 68.80; H, 4.06; N, 10.47
6o	3035, 2939, 2867, 1603, 1516, 1444, 1287, 1149, 769	8.10 (d, $J = 8.7$ Hz, 2H, aryl-H), 7.92 (d, $J = 8.7$ Hz, 2H, aryl-H), 7.65 (d, $J = 8.2$ Hz, 2H, aryl-H), 7.53–7.38 (m, 5H, aryl-H), 7.05 (d, $J = 8.2$ Hz, 2H, aryl-H), 3.82 (s, 3H, OCH_3), 1.93 (s, 3H, 4″-SCH_3)	Anal. calcd. for $C_{24}H_{19}N_3OS_2$ C, 67.11; H, 4.46; N, 9.78. Found: C, 67.10; H, 4.46; N, 9.73
6p	3029, 2960, 2840, 1613, 1509, 1488, 1359, 1181, 777	7.94 (d, $J = 8.3$ Hz, 2H, aryl-H), 7.79 (d, $J = 8.3$ Hz, 2H, aryl-H), 7.67 (d, $J = 8.5$ Hz, 2H, aryl-H), 7.26 (d, $J = 8.5$ Hz, 2H, aryl-H), 7.13 (d, $J = 8.1$ Hz, 2H, aryl-H), 6.86 (d, $J = 8.1$ Hz, 2H, aryl-H), 3.87 (s, 3H, 4′-OCH_3), 3.80 (s, 3H, OCH_3), 1.99 (s, 3H, 4″-SCH_3)	Anal. calcd. for $C_{25}H_{21}N_3O_2S_2$ C, 65.33; H, 4.61; N, 9.14. Found: C, 65.35; H, 4.66; N, 9.17

Anti-tubercular activity

All the synthesized compounds (**6a–r**) were evaluated for their preliminary in-vitro anti-tubercular activity against *M. tuberculosis* strain $H_{37}Rv$ by using MABA method. The result of anti-tubercular activity is presented in Table 3. All the synthesized compounds exhibited an interesting activity profile against the tested mycobacterial strain. The brief structural activity relationships reveal that the activity is considerably affected by various substituents at second position of imidazo(2,1-b)-1,3,4-thiadiazole nucleus. It has been observed that among the series, the compounds **6f–j** having sulfonamido group at second position of imidazo(2,1-b)-1,3,4-thiadiazole ring exhibited the significant anti-tubercular activity while other compounds showed poor to moderate activity. It is interesting to note that when both fifth and sixth aromatic rings are substituted with *p*-methoxy (compounds **6c**, **6h**, **6m** and **6q**), it resulted in compounds having an enhanced anti-tubercular activity with MIC values ranging from 1.25–5.0 μg/ml. Amongst them, compounds **6c** and **6h** (MIC = 1.25 μg/ml) exhibited a significant activity. However, when *p*-methoxy on sixth aromatic ring is replaced by *p*-thiomethyl groups (compounds **6b**, **6g**, **6l** and **6p**), it resulted in compounds with decreased anti-tubercular activity. Amongst them, compound **6g** (MIC = 2.5 μg/ml) showed a respectable activity when compared with first line drugs such as isoniazid (MIC = 0.25 μg/ml) and gatifloxacin (MIC = 1.0 μg/ml). However, when a methyl group was introduced on sixth aromatic ring (compounds **6e**, **6j** and **6r**) resulted in either complete loss of anti-tubercular activity or least active derivatives. It was also observed that when there is no substitution on fifth aromatic ring of imidazo(2,1-b)-1,3,4-thiadiazole nucleus (compounds **6e** and **6k**), the anti-tubercular activity was either decreased or lost. Apart from sulfonamide group at second position of imidazo

(2,1-b)-1,3,4-thiadiazole nucleus, we have also studied the influence of trifluoromethyl, *p*-flurophenyl and *p*-methoxyphenyl moities on the biological activity, we observed that such replacement in the core nucleus did not vary the anti-tubercular activity to a greater extent.

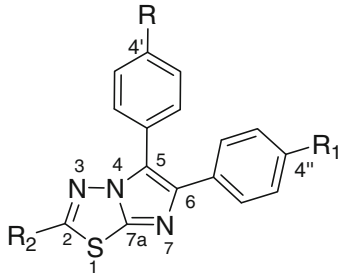
Cytotoxic activity

Some compounds **6a–c**, **f–j**, **l**, **m**, and **o–q** were further examined for toxicity (IC_{50}) in a mammalian Vero cell line upto 62.5 μg/ml concentrations. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 non-radioactive cell proliferation assay and results are summarized in Table 3. Among the thirteen derivatives tested, showed IC_{50} values ranging from 115.9 to 246.6 μM. All compounds did not show significant activity against mammalian Vero cell line at concentrations < 100 μM. Among the test compounds, 2-sulfonamido derivatives **6g–i** showed inferior toxicity with IC_{50} values of >200 μM. A comparison of the substitution pattern at second position of imidazo(2,1-b)thiadiazole nucleus demonstrated that trifluromethyl, *p*-flurophenyl and *p*-methoxyphenyl substituted analogs were more cytotoxic than the sulfonamido substituted analogs. These results are important as these compounds with their increased cytoliability are much attractive in the development of new chemical entities for the treatment of TB. This is primarily due to the fact that the eradication of TB requires a lengthy course of treatment, and the need for an agent with a high margin of safety becomes a primary concern. The IC_{50} for the compounds **6h** was found to be 246.6 μM against the cell line tested.

Conclusion

In this research article, we report the synthesis, spectral studies, and preliminary biological evaluation of a novel series of 2-substitued-5,6-diarylsubstituted imidazo(2,1-b)-1,3,4-thiadiazole derivatives (**6a–r**) as possible anti-tubercular agents. These fused hetrocyclic compounds were prepared by the cyclodehydration process between 2-amino-5-substituted-1,3,4-thiadiazole derivatives (**5a–d**) and α-bromo-1,2-(*p*-substituted)diaryl-1-ethanones (**4a–e**). The preliminary in-vitro anti-tuberculosis screening of these title molecules has evidenced that 4′-OCH_3 and 4″-OCH_3 substituted analogs **6c**, **6h**, **6m**, and **6q** have emerged as potential compounds endowed with moderate to good anti-tuberculosis activity. However, the replacement of sulfonamido by trifluoromethyl, *p*-flurophenyl and *p*-methoxyphenyl group at second position showed decrease in the anti-tuberculosis activity. Further, some title compounds were also assessed for their cytotoxic activity (IC_{50}) against in a mammalian Vero cell line using MTT assay. The results indicated that these compounds exhibit anti-tubercular activity at non-cytotoxic concentrations. The possible improvements in the anti-tubercular activity can be further achieved by slight modifications in the ring substituents and/or extensive additional functionation warrants further investigations. Our findings will have impact on medicinal chemists and pharmacists for

Table 3 The in vitro anti-tubercular and cytotoxic activity data of a novel series of 2-substituted-5,6-diarylsubstitued imidazo(2,1-b)-1,3,4-thiadiazole derivatives (**6a–r**)



(**6a-r**)

Compound	Structure			MIC[a]	IC_{50} (μM)[b]
	R	R_1	R_2		
6a	H	SCH_3	CF_3	10.0	139.6
6b	OCH_3	SCH_3	CF_3	5.0	122.7
6c	OCH_3	OCH_3	CF_3	1.25	147.3
6d	OCH_3	H	CF_3	Resistant	NT
6e	H	CH_3	CF_3	Resistant	NT
6f	H	SCH_3	SO_2NH_2	2.5	192.2
6g	OCH_3	SCH_3	SO_2NH_2	2.5	213.1
6h	OCH_3	OCH_3	SO_2NH_2	1.25	246.6
6i	OCH_3	H	SO_2NH_2	5.0	204.5
6j	H	CH_3	SO_2NH_2	10.0	168.1
6k	H	SCH_3	4-F-C_6H_4	Resistant	NT
6l	OCH_3	SCH_3	4-F-C_6H_4	10.0	115.9
6m	OCH_3	OCH_3	4-F-C_6H_4	5.0	163.4
6n	OCH_3	H	4-F-C_6H_4	Resistant	NT
6o	H	SCH_3	4-OCH_3-C_6H_4	10.0	134.8
6p	OCH_3	SCH_3	4-OCH_3-C_6H_4	10.0	120.3
6q	OCH_3	OCH_3	4-OCH_3-C_6H_4	5.0	161.9
6r	H	CH_3	4-OCH_3-C_6H_4	Resistant	NT
Isoniazid				0.25	>450
Gatifloxacin				1.0	>550

NT not tested

[a] Minimal inhibition concentration is expressed in μg/ml

[b] Cytotoxicity is expressed as IC_{50}, is the concentration of compound, which is reduced by 50% of the optical density of treated cells with respect to untreated cells using the MTT assay

further investigations in this field in search of potent anti-tubercular agents.

Experimental

Chemistry protocols

All research chemicals/ solvents were purchased from Sigma-Aldrich or Lancaster Co. and used as such for the reactions. Solvents except LR grade were dried and purified according to the literature when necessary. Reactions were monitored by thin layer chromatography (TLC) on pre-coated silica gel plates from E-Merck Co and compounds visualized either by exposure to iodine vapors or dipping in 10% aqueous potassium permanganate solution.

Melting point of synthesized compounds was determined in Thermonik melting point apparatus (Thermonik, Mumbai, India) and is uncorrected. IR spectrum was recorded on Thermo Nicolet IR200 FT-IR Spectrometer (Madison, WI, USA) by using KBr pellets. The ^{1}H-NMR spectra were recorded on Bruker Avance II (400 MHz, (Bruker, Rheinstetten/ Karlsruhe, Germany)) using $CDCl_3$ or DMSO-d_6 as solvent. Chemical shift are given in δ ppm units with respect to TMS. The elemental analysis (CHN) of the compounds was performed on CHN Elemental Analyser (Perkin Elmer 2400, Waltham, Massachusetts, USA). Results of elemental analysis were within $\pm 0.3\%$ of the theoretical values. The purity of the compounds was examined by TLC on silica gel G plate using choloroform:hexane (1:9) or ethyl acetate:hexane (2:8) as mobile phase and iodine vapors or dipping in 10% aqueous potassium permanganate solution as visualizing agent. The compounds were purified by using flash chromatography SP-01 (Biotage, Sweden) using 200–400 mesh silica gel.

General procedure for the synthesis of 1-(4″-substituted)phenyl-2-(4′-substituted) phenyl-1-ethanones (**3a**–**e**, Scheme 1)

To a mixture of phenyl acetic acid/*p*-substituted phenyl acetic acid (**1a/b**, 0.073 mol), substituted aromatic hydrocarbon (one of **2a**–**d**, 0.088 mol), 88–93% orthophosphoric acid (0.088 mol) was added, followed by immediate careful addition of trifluoroacetic anhydride (0.0295 mol) with vigorous stirring at 25°C. The mixture turned into a dark colored solution with vigorous exothermic reaction. The reaction mixture was stirred for 1 min at the same temperature and poured into ice-cold water (50 ml) with stirring. Washed with cold hexane (2 × 10 ml) to obtain **3a**–**e** as solid. The percentage yield of compounds **3a**–**e** were found to be 76.3, 68.7, 73.7, 66.9, and 74.1%, respectively.

General procedure for the synthesis of α-bromo-1-(4″-substituted)phenyl-2-(4′-substituted)phenyl-1-ethanones (**4a**–**e**)

To a constantly stirred solution of **3a**–**e** (0.02 mol) in chloroform (30 ml) kept at 50°C was added dropwise bromine (0.022 mol) with stirring. After being stirred at 50°C for 0.5 h, the mixture was washed successively with aqueous 10% sodium thiosulphate (hypo) solution and water. The solvent was removed in-vacuo to obtain the compounds (**4a**–**e**) either as oil/ solid mass/crystaline compounds, which were used in the next step.

General procedure for the synthesis of 2-substituted-5,6-diarylsubstituted imidazo(2,1-b)-1,3,4-thiadiazole (**6a**–**r**, Scheme 2)

A mixture of 2-amino-5-substituted-1,3,4-thiadiazole (one of **5a**–**d**, 0.002 mol) and an appropriate α-bromo-1-(4″-substituted)phenyl-2-(4′-substituted)phenyl-1-ethanones (one of **4a**–**e**, 0.002 mol) in dry ethanol (150 ml) was heated to reflux on a water bath for 6–12 h, phosphorous pentoxide (0.0003 mol) was added and refluxing was continued for another 4–9 h. The reaction mixture was cooled overnight at room temperature. Excess of solvent was removed under reduced pressure and the solid hydrobromide separated was filtered, washed with cold ethanol and dried. Neutralization of hydrobromide salts with cold aqueous solution of Na_2CO_3 yielded the corresponding free bases **(6a**–**r)**, which were recrystallized from dry ethanol. Further, the compounds were purified by flash chromatography using 200–400 mesh silica gel and eluted either with ethyl acetate:hexane (2:8) or choloroform:hexane (1:9) as mobile phase. The physicochemical, spectral, and elemental analysis data of the synthesized compounds are depicted in Tables 1 and 2, respectively.

Anti-tubercular activity

The anti-tubercular activity of title compounds (**6a**–**r**) was assessed against *M. tuberculosis* $H_{37}Rv$ (ATTC 27294) using the Micro plate Alamar Blue assay (MABA, Franzblau *et al.*, 1998). Succinctly, *M. tuberculosis* $H_{37}Rv$ is grown in Middlebrook 7H9 broth (7H9 medium) supplemented with 0.2% (v/v) glycerol, 10% (v/v) ADC (albumin, dextrose, catalase), and 0.05% (v/v) Tween 80. The bacteria are inoculated in 50 ml of 7H9 medium in 1-l roller bottles that are placed on a roller bottle apparatus in an ambient 37°C incubator. When the cells reach an OD_{600} of 0.150 (equivalent to $\sim 1.5 \times 10^7$ CFU/ml), they are diluted 200-fold in 7H9 medium. Further, 200 µl of sterile deionized water was added to all outer-perimeter wells of sterile 96 well plates (falcon, 3072: Becton Dickinson, Lincoln Park, NJ) to minimize evaporation of the medium

in the test wells during incubation. The 96 plates received 93.75 µl of the cell suspension ($\sim 10^4$ bacteria) in Middlebrook 7H9 medium (Difco laboratories, Detroit, MI, USA) and a serial dilution of the compounds **6a–r** was made directly on the plate. The final drug concentrations tested were 0.01–20.0 µg/ml. Plates were covered and sealed with parafilm and incubated at 37°C for 5 days. After this time, 25 µl of a freshly prepared 1:1 mixture of Alamar Blue (Accumed International, Westlake Ohio) reagent and 10% tween 80 was added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth, and a pink color was scored as growth. The MIC was defined as the lowest drug concentration, which prevented a color change from blue to pink.

Cytotoxic activity

The cellular conversion of MTT [3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyl-tetrazolium bromide] into a formazan product was used to evaluate cytotoxic activity (IC_{50}) of some synthesized compounds (**6a–c**, **f–j**, **l**, **m** and **o–q**) against a mammalian Vero cell line upto concentrations of 62.5 µg/ml. After 72 h of exposure, cell viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 non-radioactive cell proliferation assay (Gundersen *et al.*, 2002).

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