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Research Article

Synthesis and Antitubercular Activity of Mannich bases of imidazo[2,1-*b*][1,3,4]thiadiazoles

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ABSTRACT

A series of 5,6-disubstituted imidazo[2,1-*b*][1,3,4]thiadiazoles were synthesized. The structures of newly synthesized compounds were characterized by spectral and analytical data. All the title compounds were tested for their *in-vitro* antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$ using Alamar-Blue susceptibility test, and the activity is expressed as the minimum inhibitory concentration (MIC) in µg/mL. Among the series, compounds **3a, 3c, 4a, 5c** and **6a** displayed an encouraging antitubercular activity profile as compared to the reference drug, rifampicin.

INTRODUCTION

Tuberculosis (TB) is a chronic necrotizing bacterial infection with wide variety of manifestations caused by *Mycobacterium tuberculosis*, which has been a scourge of humanity for thousands of years and remains one of the prevalent health tribulations in the world [1]. Tuberculosis (TB) is contagious and airborne. It is a disease of poverty affecting mostly young adults in their most productive years. 95% of TB deaths are in the developing world. TB is among the three greatest causes of death among women aged 15-44, 320,000 women died from TB in 2010 [2]. The WHO estimated that 17% of the 9.2 million new cases of active TB had some form of drug-resistant TB (DR-TB); of these, 3.1% or 440000 individuals had multidrug-resistant (MDR)-TB (defined as resistance to rifampicin and isoniazid) [3].

In developing countries where rates of both infection and active disease have always been high, the number of cases skyrocketed, so dramatic was the increase that the World Health Organization (WHO) declared TB a global health emergency in 1993, for the first time an infectious disease achieved that dubious distinction [4-6].

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Furthermore, the co-infection with human immunodeficiency virus (HIV) has worsened the situation. The convergence of HIV and TB also posses difficult problems, not only because viral infection increase mortality from TB but also optimization of further difficulties such as the rifampicin induces CYP 450 enzymes along with inhibition of RNA polymerization [7,8]. Further, rifampicin is known to have major pharmacokinetic interactions with certain anti-HIV drugs [9, 10].

Almost one in four deaths among people with HIV is due to TB. In 2010 350,000 people died of HIV-associated TB. It is also the most common presenting illness among people living with HIV, including those who are taking antiretroviral treatment. There were an estimated 1.1 million HIV positive new TB cases globally in 2010. Around 82% of patients live in sub-Saharan Africa. At least one-third of the 34 million people living with HIV worldwide are infected with TB. Persons co-infected with TB and HIV are 21-34 times more likely to develop active TB disease than persons without HIV [11]. These problems demand renewed efforts towards the development of novel chemical entities to control the mortality from TB.

Imidazo[2,1-*b*][1,3,4]thiadiazole derivatives were first discovered in the early 1950s and, since then, the research work on this heterocyclic system has led to significant

developments in their chemistry and biology. Imidazo[2,1-*b*][1,3,4]thiadiazole ring systems have been extensively studied and, so far, a variety of biological activities have been reported for a large number of their derivatives, such as antitubercular, antibacterial, anticancer, anthelmintic, antifungal, anticonvulsant, anti-inflammatory, analgesic, antipyretic, local anaesthetic, cardiotonic, diuretic, leishmanicidal and herbicidal activities [12].

Recently, these derivatives have attracted the interest of researchers as antituberculosis agents. Some members of the imidazo[2,1-*b*][1,3,4]thiadiazoles family displayed good activity against *M. tuberculosis* $H_{37}Rv$ [13].

The present work was aim to explore and develop the novel molecules with improved potential for treating tuberculosis. In this paper, we report the synthesis and antitubercular screening of novel Mannich bases of imidazo[2,1-*b*][1,3,4]thiadiazole derivatives.

EXPERIMENTAL

Chemistry

General details

Melting points were determined in open capillaries and are uncorrected. The IR spectra were recorded on Nicolet Impact 410 FT-IR spectrophotometer (Model-410, USA) using KBr pellets. ^{1}H NMR was recorded on Bruker 300 MHz NMR spectrometer (Model RX-300, Switzerland) in $CDCl_3$ with TMS as internal standard. Mass spectra were recorded on GCMS Schimadzu Japan QP-2010S and elemental analyses were carried out using Heraeus CHN rapid analyzer.

Synthesis of 2-((2-chlorophenoxy)methyl)-5,7a-dihydro-6-arylimidazo[2,1-b][1,3,4]thiadiazoles (2a-c)

General method: A mixture of equimolar quantities of 2-amino-5-(2-chlorophenoxy methyl)-1,3,4-thiadiazole (**1**) (0.01mol) and bromoacetyl compound (0.01mol) was refluxed in dry ethanol for 18 hrs. 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 (**2a-c**). It was filtered, washed with water, dried and recrystallized from suitable solvent.

2-((2-chlorophenoxy)methyl)-5,7a-dihydro-6-(4-bromophenyl)imidazo[2,1-b][1,3,4]thiadiazole (2a). Yield 77%; colorless solid (ethanol); mp 186–188 ^{0}C; IR (KBr) νcm^{-1}: 2924, 1621, 1498, 1137; ^{1}HNMR ($CDCl_3$) δ 4.11 (*s,* 2H, CH_2), 7.89 (*s*, 1H, C5-H, imidazole), 7.21-7.89 (*m,* 8H, Ar-H); Mass *m/z*: 420.32(m+). Anal. Calcd. For $C_{17}H_{13}BrClN_3OS$: C, 48.30; H, 3.10; N, 9.94; Found: C, 47.13; H, 3.32; N, 9.68.

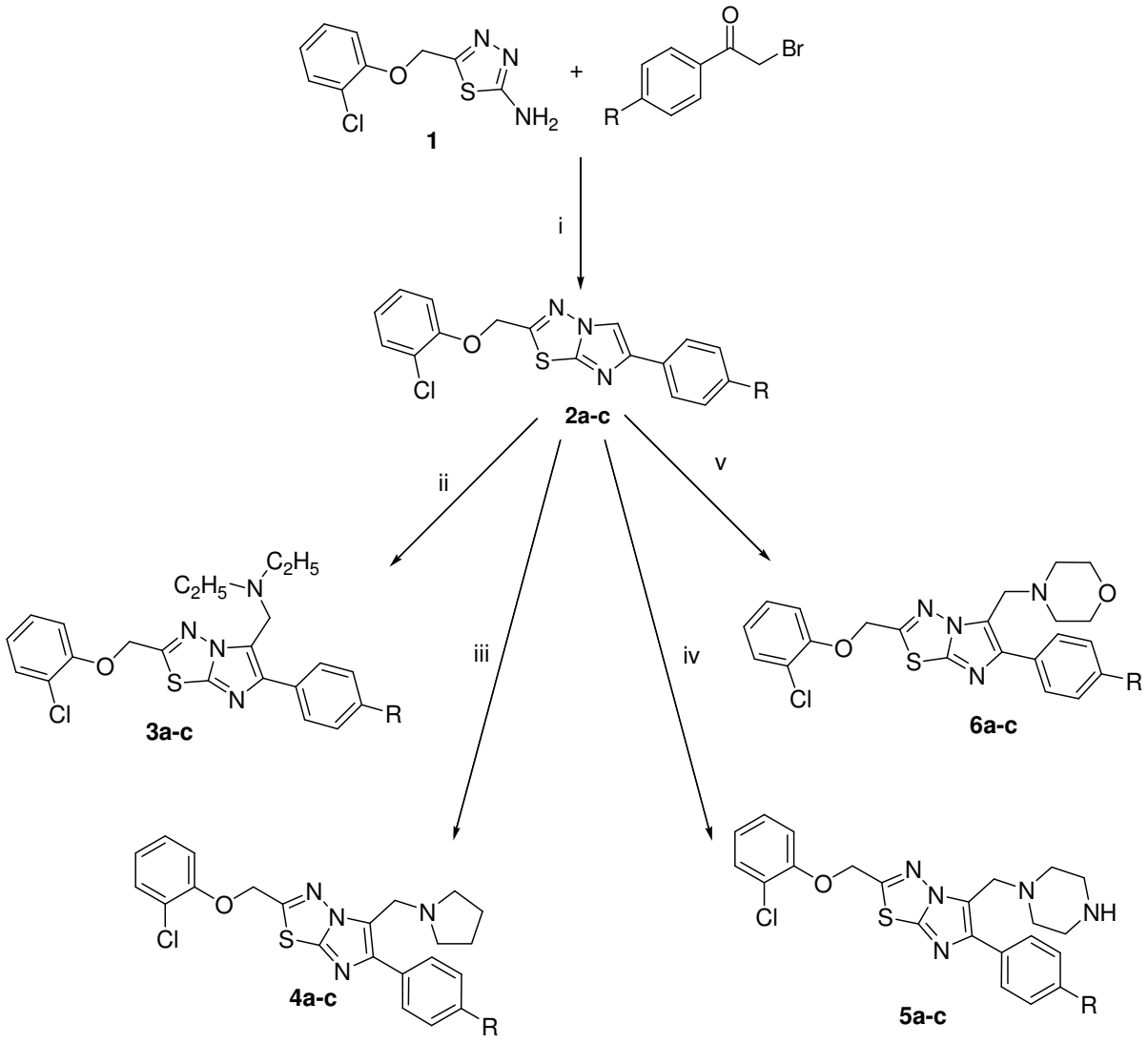
2-((2-chlorophenoxy)methyl)-5,7a-dihydro-6-(4-methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazole (2b). Yield 78%; light yellow solid (ethanol); mp 184–186 ^{0}C; IR (KBr) νcm^{-1}: 2956, 1635, 1485, 1124; ^{1}HNMR ($CDCl_3$) δ 3.89 (*s,* 3H, OCH_3), 4.14 (*s,* 2H, CH_2), 7.82 (*s*, 1H, C5-H, imidazole), 7.08-7.79 (*m,* 8H, Ar-H); Mass *m/z*: 373.13(m+). Anal. Calcd. for $C_{18}H_{16}ClN_3O_2S$; C, 57.83; H, 4.31; N, 11.24.Found: C, 57.65; H, 4.15; N, 11.31.

2-((2-chlorophenoxy)methyl)-5,7a-dihydro-6-(4-nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazole (2c). Yield 82%; pale yellow solid (ethanol); mp 178–180 ^{0}C; IR (KBr) νcm^{-1}: 2965, 1643, 1453, 1125; ^{1}HNMR ($CDCl_3$) δ 4.23 (*s,* 2H, CH_2), 7.82 (*s*, 1H, C5-H, imidazole), 7.10-7.65 (*m,* 8H, Ar-H); Anal. calcd. for $C_{17}H_{13}ClN_4O_3S$: C, 52.51; H, 3.37; N, 14.41. Found: C, 56.83; H, 3.13; N, 14.12

Synthesis of 2-(2-chlorophenoxymethyl)-6-aryl-5,7a-dihydro-5-diethylamine-1-ylmethylimidazo [2,1-b][1,3,4]thiadiazole (3a-c)

General method: A mixture of 2-((2-chlorophenoxy)methyl)-5,7a-dihydro-6-arylimidazo[2,1-*b*][1,3,4]thiadiazoles (**2**) (0.005 mol), diethylamine (0.71g, 0.01 mol), formalin (1 mL) and acetic acid (1 mL) in methanol (20 mL) was refluxed for 10 hrs (monitored by TLC). Reaction mixture was diluted with water and extracted with chloroform (3x30mL). The combined chloroform extract was washed with water (3x30 mL) and dried over anhydrous sodium sulfate. The solvent was removed under vacuum and the residue was recrystallized from chloroform and hexane mixture.

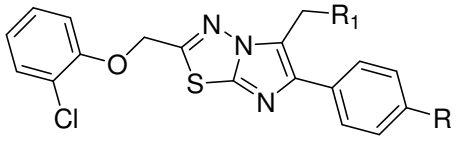
2-((2-chlorophenoxy)methyl)-6-(4-bromophenyl)-5,7a-dihydro-5-((diethylamine-1-yl)methyl)imidazo[2,1-b][1,3,4]thiadiazole (3a). Yield 74%; colorless solid (ethanol); mp 108–110 ^{0}C; IR (KBr) νcm^{-1}: 2985, 2956, 1614, 1201; ^{1}HNMR ($CDCl_3$) δ 1.23 (*t*, 6H, CH_3), 2.42 (*q,* 4H, CH_2), 2.61 (*s*, 2H, CH_2), 4.21 (*s*, 2H, CH_2), 7.03-7.46 (*m,* 8H, Ar-H); Mass *m/z*: 506.12(m+). Anal. Calcd. For $C_{22}H_{24}BrClN_4OS$: C, 52.03; H, 4.76; N, 11.03; Found: C, 52.14; H, 4.52; N, 11.15.



a, R = Br**; b**, R = OMe; **c**, R = NO_2;

Scheme 1:
Reagents and Conditions:
(i) Dry ethanol, reflux, 18hr, Na_2CO_3
(ii) Diethylamine, HCHO, AcOH, MeOH, reflux, 4h.
(iii) Pyrrolidine, HCHO AcOH, MeOH, reflux, 4h
(iv) Piperazine, HCHO, AcOH, MeOH, reflux, 4h.
(v) Morpholine, HCHO, AcOH, MeOH, reflux, 4h.

Table 1: The *in vitro* antitubercular activity of compounds against



Compound	R	R_1	MIC µg/mL
3a	Br	Diethylamine	6.25
3b	OMe	Diethylamine	12.5
3c	NO_2	Diethylamine	6.25
4a	Br	Pyrrolidine	3.125
4b	OMe	Pyrrolidine	6.25
4c	NO_2	Pyrrolidine	6.25
5a	Br	Piperazine	3.125
5b	OMe	Piperazine	6.25
5c	NO_2	Piperazine	3.125
6a	Br	Morpholine	1.6
6b	OMe	Morpholine	3.125
6c	NO_2	Morpholine	3.125
Rifampicin (standard)			0.2

2-((2-chlorophenoxy)methyl)-5,7a-dihydro-6-(4-methoxyphenyl)-5-((diethylamine-1-yl)methyl) imidazo[2,1-b][1,3,4]thiadiazole (3b). Yield 76%; colorless solid (ethanol); mp 156–158 ^{0}C; IR (KBr) νcm^{-1}: 2987, 2945, 1637, 1198; ^{1}HNMR ($CDCl_3$) δ 1.23 (*t*, 6H, CH_3), 2.42 (*q,* 4H, CH_2), 2.61 (*s*, 2H, CH_2), 3.81 (*s*, 3H, OCH_3), 4.21 (*s*, 2H, CH_2), 7.23-7.68 (*m,* 8H, Ar-H); Anal. Calcd. For $C_{23}H_{27}ClN_4O_2S$: C, 60.18; H, 5.93;N, 12.21; Found: C, 60.02; H, 5.72; N, 12.05.

2-((2-chlorophenoxy)methyl)-5,7a-dihydro-6-(4-nitrophenyl)-5-((diethylamine-1-yl)methyl)imid azo[2,1-b][1,3,4]thiadiazole (3c). Yield 68%; pale yellow solid (ethanol); mp 154–156 ^{0}C; IR (KBr) νcm^{-1}: 2983, 2921, 1640, 1210; ^{1}HNMR ($CDCl_3$) δ 1.25 (*t*, 6H, CH_3), 2.51 (*q,* 4H, CH_2), 2.65 (*s*, 2H, CH_2), 4.31 (*s*, 2H, CH_2), 7.21-7.83 (*m,* 8H, Ar-H); Mass *m/z*: 473.02(m+). Anal. Calcd. For $C_{22}H_{24}ClN_5O_3S$: C, 55.75; H, 5.10; N, 14.78; Found: C, 55.62; H, 5.02; N, 14.54.

Synthesis of 2-(2-chlorophenoxymethyl)-6-aryl-5,7a-dihydro-5-pyrrolidin-1-ylmethylimidazo [2,1-b][1,3,4]thiadiazole (4a-c).

General method: A mixture of 2-((2-chlorophenoxy)methyl)-5,7a-dihydro-6-arylimid azo[2,1 *b*][1,3,4]thiadiazoles (**2**) (0.005 mol), pyrrolidine (0.71g, 0.01 mol), formalin (1 mL) and acetic acid (1 mL) in methanol (20 mL) was refluxed for 10 hrs (monitored by TLC). Reaction mixture was diluted with water and extracted with chloroform (3x30mL). The combined chloroform extract was washed with water (3x30 mL) and dried over anhydrous sodium sulfate. The solvent was removed under vacuum and the residue was recrystallized from chloroform and hexane mixture.

2-((2-chlorophenoxy)methyl)-6-(4-bromophenyl) -5,7a-dihydro-5-((pyrrolidin-1-yl)methyl)imid azo[2,1-b][1,3,4]thiadiazole (4a). Yield 61%; pale yellow solid (chloroform + pet ether); mp 136–138 ^{0}C; IR (KBr) νcm^{-1}: 3005, 1618, 1428, 1195; ^{1}HNMR ($CDCl_3$) δ 2.13-2.60 (*m*, 4H, C3, C4-H, pyrrolidine), 3.28-3.42 (*m*, 4H, C2, C5-H, pyrrolidine), 3.89 (*s*, 2H, CH_2), 4.21 (*s*, 2H, CH_2), 7.21-7.56 (8H, Ar-H); Mass *m/z*: 504.21(m+). Anal. Calcd for $C_{22}H_{22}BrClN_4OS$: C, 52.24; H, 4.38; N, 11.08. Found: C, 52.10; H, 4.16; N, 11.21.

2-((2-chlorophenoxy)methyl)-5,7a-dihydro-6-(4-methoxyphenyl)-5-((pyrrolidin-1-yl)methyl)imid azo[2,1-b][1,3,4]thiadiazole (4b). Yield 72%; colorless solid (chloroform + pet ether); mp 144–146 ^{0}C; IR (KBr) νcm^{-1}: 3010, 1612, 1435, 1201; ^{1}HNMR ($CDCl_3$) δ 2.15-2.57 (*m*, 4H, C3, C4-H, pyrrolidine), 3.08-3.31 (*m*, 4H, C2, C5-H,

pyrrolidine), 3.21 (*s,* 3H, OCH_3), 3.75 (*s*, 2H, CH_2), 4.25 (*s*, 2H, CH_2), 7.31-7.68 (8H, Ar-H); Anal. Calcd for $C_{23}H_{25}ClN_4O_2S$: C, 60.45; H, 5.51; N, 12.26. Found: C, 60.13; H, 5.37; N, 12.31.

2-((2-chlorophenoxy)methyl)-5,7a-dihydro-6-(4-nitrophenyl)-5-((pyrrolidin-1-yl)methyl)imidazo [2,1-b][1,3,4]thiadiazole (4c). Yield 68%; colorless solid (chloroform + pet ether); mp 152–156 ^{0}C; IR (KBr) νcm^{-1}: 3045, 1624, 1420, 1198; ^{1}HNMR ($CDCl_3$) δ 2.17-2.63 (*m*, 4H, C3, C4-H, pyrrolidine), 3.21-3.44 (*m*, 4H, C2, C5-H, pyrrolidine), 3.78 (*s*, 2H, CH_2), 4.31 (*s*, 2H, CH_2), 7.29-7.72 (8H, Ar-H); Mass *m/z*: 471.03(m+). Anal. Calcd for $C_{22}H_{22}ClN_5O_3S$: C, 55.99; H, 4.70; N, 14.84. Found: C, 55.72; H, 4.56; N, 14.62.

Synthesis of 2-(2-chlorophenoxymethyl)-6-aryl-5,7a-dihydro-5-piperazine-1-ylmethylimidazo [2,1-b][1,3,4]thiadiazole (5a-c)

General method: A mixture of 2-((2-chlorophenoxy)methyl)-5,7a-dihydro-6-arylimid azo[2,1 *b*][1,3,4]thiadiazoles (**2**) (0.005 mol), piperazine (0.71g, 0.01 mol), formalin (1 mL) and acetic acid (1 mL) in methanol (20 mL) was refluxed for 10 hrs (monitored by TLC). Reaction mixture was diluted with water and extracted with chloroform (3x30mL). The combined chloroform extract was washed with water (3x30 mL) and dried over anhydrous sodium sulfate. The solvent was removed under vacuum and the residue was recrystallized from chloroform and hexane mixture.

2-((2-chlorophenoxy)methyl)-6-(4-bromophenyl) -5,7a-dihydro-5-((piperazin-1-yl)methyl)imidazo [2,1-b][1,3,4]thiadiazole (5a). Yield 68%; pale yellow solid (chloroform + pet ether); mp 144–146 ^{0}C; IR (KBr) νcm^{-1}: 3321, 2985, 1610, 1152; ^{1}HNMR ($CDCl_3$) δ 2.44 (*m*, 8H, piperazine), 4.18 (*s*, 2H, CH_2), 7.24-7.79 (*m*, 8H, Ar-H), 11.51 (Br, 1H, NH); Mass *m/z*: 519.34(m+). Anal. Calcd for $C_{22}H_{23}BrClN_5OS$: C, 50.73; H, 4.45; N, 13.45. Found: C, 50.56; H, 4.27; N, 13.23.

2-((2-chlorophenoxy)methyl)-5,7a-dihydro-6-(4-methoxyphenyl)-5-((piperazin-1-yl)methyl)imid azo[2,1-b][1,3,4]thiadiazole (5b). Yield 71%; colorless solid (chloroform + pet ether); mp 152–154 ^{0}C; IR (KBr) νcm^{-1}: 3315, 2981, 1624, 1201; ^{1}HNMR ($CDCl_3$) δ 2.34 (*m*, 8H, piperazine), 3.78 (S, 3H, OCH_3), 4.24 (S, 2H, CH_2), 7.28-7.81 (*m*, 8H, Ar-H), 11.45 (Br, 1H, NH); Mass *m/z*: 471.05(m+). Anal. Calcd for $C_{23}H_{26}ClN_5O_2S$: C, 58.53; H, 5.55; N, 14.84. Found: C, 58.37; H, 5.35; N, 14.71.

2-((2-chlorophenoxy)methyl)-5,7a-dihydro-6-(4-nitrophenyl)-5-((piperazin-1-yl)methyl)imidazo [2,1-b][1,3,4]thiadiazole (5c). Yield 69%; pale yellow sold (chloroform + pet ether); mp 156–158 ^{0}C; IR (KBr) νcm^{-1}: 3301, 2956, 1613, 1198; ^{1}HNMR ($CDCl_3$) δ 2.37 (*m*, 8H, piperazine), 4.22 (*S*, 2H, CH_2), 7.31- 7.91 (*m*, 8H, Ar-H), 11.57 (Br, 1H, NH); Anal. Calcd for $C_{22}H_{23}ClN_6O_3S$: C, 54.26; H, 4.76; N, 17.26. Found: C, 54.13; H, 4.56; N, 17.09.

Synthesis of 2-(2-chlorophenoxymethyl)-6-aryl-5,7a-dihydro-5-morpholine-1-ylmethylimidazo [2,1-b][1,3,4]thiadiazole (6a-c)

General method: A mixture of 2-((2-chlorophenoxy)methyl)-5,7a-dihydro-6-arylimid azo[2,1 *b*][1,3,4]thiadiazoles (**2**) (0.005 mol), morpholine (0.71g, 0.01 mol), formalin (1 mL) and acetic acid (1 mL) in methanol (20 mL) was refluxed for 10 hrs (monitored by TLC). Reaction mixture was diluted with water and extracted with chloroform (3x30mL). The combined chloroform extract was washed with water (3x30 mL) and dried over anhydrous sodium sulfate. The solvent was removed under vacuum and the residue was recrystallized from chloroform and hexane mixture.

2-(2-chlorophenoxymethyl)-6-(4-bromophenyl)-5,7a-dihydro-5-morpholine-1-ylmethylimidazo [2,1-b][1,3,4]thiadiazole (6a). Yield 64%; pale yellow solid (chloroform + pet ether); mp 176–178 ^{0}C; IR (KBr) νcm^{-1}: 3018, 1614, 1425, 1205; ^{1}HNMR ($CDCl_3$) δ 2.51 (*t*, 4H, morpholine), 3.71 (*t*, 4H, morpholine), 4.21 (*s*, 2H, CH_2), 7.14-7.56 (*m*, 8H, Ar-H); Anal. Calcd for $C_{22}H_{22}BrClN_4O_2S$: C, 50.63; H, 4.25; N, 10.74. Found: C, 50.42; H, 4.06;N, 10.65.

2-((2-chlorophenoxy)methyl)-5,7a-dihydro-6-(4-methoxyphenyl)-5-(morpholinomethyl) imidazo [2,1-b][1,3,4]thiadiazole (6b). Yield 67%; pale yellow solid (chloroform + pet ether); mp 188–190 ^{0}C; IR (KBr) νcm^{-1}: 3052, 1608, 1416, 1190; ^{1}HNMR ($CDCl_3$) δ 2.49 (*t*, 4H, morpholine), 3.67 (*t*, 4H, morpholine), 3.81 (*s*, 3H, OCH_3), 4.24 (*s*, 2H, CH_2), 7.24-7.64 (*m*, 8H, Ar-H); Mass *m/z*: 472.41(m+). Anal. Calcd for $C_{23}H_{25}ClN_4O_3S$: C, 58.40; H, 5.33; N, 11.85. Found: C, 58.23; H, 5.13; N, 11.63.

2-((2-chlorophenoxy)methyl)-5,7a-dihydro-5-(morpholinomethyl)-6-(4-nitrophenyl)imidazo [2,1-b][1,3,4]thiadiazole (6c). Yield 71%; pale yellow solid (chloroform + pet ether); mp 182–184 ^{0}C; IR (KBr) νcm^{-1}: 3045, 1638, 1428, 1154;

^{1}HNMR ($CDCl_3$) δ 2.47 (*t*, 4H, morpholine), 3.65 (*t*, 4H, morpholine), 4.27 (*s*, 2H, CH_2), 7.01-7.79 (*m*, 8H, Ar-H); Mass *m/z*: 487.02(m+). Anal. Calcd for $C_{22}H_{22}ClN_5O_4S$: C, 54.15; H, 4.54; N, 14.35. Found: C, 54.31; H, 4.31; N, 14.21.

Antitubercular activity

The antimycobacterial activity of the newly synthesized compounds was assessed against *M. tuberculosis* using the Micro-plate Alamar Blue Assay (MABA) [14]. Succinctly, 200 µL of sterile de-ionized water was added to all outer-perimeter wells of sterile 96-well plates to minimize evaporation of the medium in the test wells during incubation. The 96-well plates received 100 µL of the Middlebrook 7H9 broth and a serial dilution of the tested compounds was made directly on the plate. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37^{0}C for five days. After this time, 25 µL of a freshly prepared 1:1 mixture of Alamar Blue 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.

RESULTS AND DISCUSSION

Chemistry

During the present investigation required imidazo[2,1-*b*][1,3,4]thiadiazoles were prepared (**Scheme I**) by the reaction of 2-amino-5-(2-chlorophenoxymethyl)-1,3,4-thiadiazole (**1**)[15] with appropriately substituted α-haloketones (phenacylbromides) in dry ethanol as hydrobromides, which on neutralization with aqueous sodium carbonate solution gave corresponding free bases **2a-c** in good yields. The absence of νN-H band in IR spectra of the resulted compounds confirms the formation of product, which exhibits imidazole (C5-H) proton around δ 7.89 in ^{1}H NMR spectra.

Further imidazo[2,1-b][1,3,4]thiadiazoles **2a-c** were subjected to Mannich reaction with four different secondary amines viz. diethylamine, pyrrolidine, piperazine and morpholine to afford corresponding Mannich bases (**3a-c, 4a-c, 5a-c** and **6a-c**). In general the ^{1}H NMR spectra of the products showed the absence of imidazole proton and a singlet is observed around δ 4.0 depending upon substitution, which is assigned to methylene protons bridged to amines and the aliphatic protons of the amine substituent resonated in the expected region along with rest of the protons.

In ^{1}H NMR spectra of morpholine derivatives **6a-c**, 8 protons were observed at δ 2.58 (C3, C5-H; N-CH2) and δ 3.72 (C2, C6-H; O-CH2). The **3a-c**, amine derivatives shows 6 proton at δ 1.23 (triplet) and 2.42 (quadrate). For pyrrolidine derivatives **4a-c**, 8 protons were observed at δ 1.7 (C3, C4-H i.e. -CH2-CH2-) and δ 2.6 (C2, C5-H; N-CH2). For piperazine derivatives **5a-c**, 8 protons show multiplet and NH at 11.51 δ ppm. Mannich products were analyzed for their C, H and N compositions and the values are within the limits.

Antitubercular Activity

The Minimum Inhibitory Concentration (MIC) was determined for compounds **3a-c, 4a-c, 5a-c, 6a-c** against the *M. tuberculosis* strain $H_{37}Rv$ using the Micro-plate Alamar Blue Assay (MABA) (Table 1). This methodology is nontoxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric methods [16-17]. 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*. All the synthesized compounds exhibited an interesting activity profile against the tested mycobacterial strain.

The data of the antitubercular activity screening reveal that the compounds **2a-c** having no substitution at position-5 did not show any considerable activity, in spite of the changes at the at position-6. However, when a secondary amine was introduced at position-5 (compounds **3a-c, 4a-c, 5a-c, 6a-c**), it resulted in compounds having an enhanced antimycobacterial activity. It was found that Mannich bases are superior over imidazo[2,1-*b*][1,3,4] thiadiazoles. Among these the morpholine derivatives were found to have more activity (with MIC values ranging from 1.6-3.125 µg/mL). 2-((2-chlorophenoxy)methyl)-6-(4-bromophenyl) -5,7a-dihydro-5-(morpholino methyl) imidazo[2,1-*b*][1,3,4] thiadiazole **6a** showed excellent inhibition at a concentration of 1.6 µg/mL. The pyrrolidine derivative **4a** and piperazine derivative **5c** showed significant activity (MIC=3.125µg/mL). Where as diethylamine derivatives (**3a**, **3c)** showed good activity (MIC=6.25 µg/mL).

CONCLUSION

In conclusion, this work demonstrates the synthesis, and *in vitro* activity evaluation of novel Mannich bases of imidazo[2,1-*b*][1,3,4] thiadiazoles against *M. tuberculosis*. Amongst, the compound **6a** has shown excellent inhibition against *M. tuberculosis* having 1.6 MIC μg/mL.

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