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Synthesis and in vitro Evaluation of the Antimycobacterial Activity of N-aryl-1,4-dihydropyridine-3,5-dicarboxamides

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Abstract

Dihydropyridines (DHPs) with carboxamides in the 3 and 5 positions show anti-tuberculosis activity. The purpose of the present study was to synthesize new DHPs that would possibly possess anti-tuberculosis activity. So a series of N-aryl-1,4-dihydropyridine-3,5-dicarboxamides (**3-38**) was prepared. They were screened as antitubercular agents against one type of fast growing Mycobacterium (*M. smegmatis*). The compounds that passed this first screening were then tested against slow-growing Mycobacterium (BCG). Minimum bacteriocidal concentrations (MBCs) were determined using the agar proportion method. The cytotoxic effect of two active compounds against HeLa cell lines was determined using an MTT assay method. N,N-Bisphenyl-4-[1-(4-fluorobenzyl)-2-methylthioimidazole-5-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide **27** and N,N-bisphenyl-4-[1-(2-chlorobenzyl)-2-methylthioimidazole-5-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide **33** were the most potent compounds tested, showing MBCs of 16 and 32 μg/ml, respectively. Their activities were comparable to that of isoniazid (32 μg/ml). In the MTT assay, these two compounds showed moderate toxicity.

Keywords: Dihydropyridine; Antituberculosis; Synthesis; BCG

Introduction

The acid-fast bacillus *Mycobacterium tuberculosis* is the causative agent of tuberculosis (TB). The tubercle bacillus is a slow-growing organism that does not produce any substance that is toxic to the normal host. On the other hand, this organism acts as an irritating foreign body, and virulent, avirulent and nonpathogenic types can produce tubercle formation. The tubercle bacillus is an intracellular parasite that lives and grows within the host's tissue, macrophages and epithelial cells [1].

The tubercle bacillus cell wall consists of mycolyl arabinogalactan units that are bonded to a peptidoglycan

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nucleus through covalent bonds. Mycolated 1,3-branched arabinofuranoside-based hexasaccharide motifs form the mycolyl arabinan domain in this structure [2, 3]. The mycolyl moieties are high molecular weight α-alkyl, β-hydroxy fatty acids that are responsible for the hydrophobic character of the cell envelope. The unique hydrophobic properties of the mycobacterium envelope protect the bacterium from its environment and provide a barrier against the diffusion of commonly used hydrophilic antimicrobial agents [4]. Globally, tuberculosis remains the leading infectious killer of adults, killing an estimated three million people per year [5]. An inactive form of the disease develops to the active form in one-tenth of the affected population [6]. The current antitubercular drugs that are available for treatment were discovered from 1945–1965. No new drugs have been synthesized during the last few decades [7]. The recent emergence of outbreaks of multi-drug-resistant tuberculosis (MDR-TB) poses a serious threat to the successful treatment of the disease. TB will undoubtedly become more prevalent in most countries due to the presence of the human immunodeficiency virus (HIV) epidemic [8].

There are millions of patients suffering from tuberculosis, which makes it necessary to discover and synthesize novel compounds that are more potent and less prone to promote resistance and have fewer side effects. The number of publications providing better insights into M. tuberculosis and disease progression has increased since the mid-1990s along with the number of novel molecules reported as potential leads for TB drug discovery [9-14].

The search for new antitubercular drugs may use both biochemical and chemical methods. It has been shown that the substitution of a carboxylate ester with an aryl carboxamide group in the usual cardiovascular 1,4-dihydropyridines reduced calcium channel blocking but provided significant antitubercular properties [15]. Preparation and antimycobacterial evaluation of some new 1,4-dihydropyridine-3,5-dicarboxamide derivatives with lipophilic groups were reported by Desai et al. in 2001 [12]. They concluded that these compounds may act as precursors. After penetration into the cell wall, the 3,5-carboxylate anions may be formed by enzymatic hydrolysis. Previous studies have shown moderate to good activity for 1,4-dihydropyridine-3,5-dicarboxamide compounds with a phenyl or substituted phenyl at the C-4 position when compared to rifampicin [12]. There are a few reports that evaluate 1,4-dihydropyridine-3,5-dicarboxamide derivatives containing heteroaromatic rings at the C-4 position. Recently, Amini et al. (2008) reported the synthesis of new 1,4-dihydropyridine-3,5-dicarboxamide derivatives with a 4-(4,5-dichloroimidazole-2-yl) moiety [13]. The surveyed compounds were weak to moderate inhibitors of *M. tuberculosis* (H37Rv) when compared with rifampicin. To understand the structure–antitubercular activity relationship, we prepared some novel 1,4-dihydropyridine-3,5-dicarboxamide derivatives and determined their inhibitory activity against BCG.

Materials and Methods

Chemicals obtained from Merck and Sigma–Aldrich were used without further purification. Analytical thin layer chromatography (TLC) was performed on Merck silica gel (60F254) plates. ^{1}H-NMR spectra were run on a Bruker AC-80 spectrometer. Infrared spectra were recorded on a FT-IR Perkin-Elmer Paragon 1000 spectrophotometer. Elemental microanalyses were within ±0.4% of the theoretical values for C, H and N. The purity of the compounds was checked by TLC on silica gel plates using chloroform and methanol.

General Procedure for the Preparation of 1,4-Dihydropyridine-3,5-dicarbamoyl Derivatives (3-38)

Imidazole-5-carbaldehydes **1a-d** were prepared as reported previously [16].

A mixture of 1-benzyl-2-methylthio-1H-imidazole-5-carbaldehyde **1** (4 mmol), the appropriate N-arylacetoacetanilide **2** (8.0 mmol) and ammonium acetate (8.0 mmol) was refluxed in 15 mL of methanol overnight. The reaction vessel was protected from light. The solvent was removed under reduced pressure, and the residue was crystallized from methanol to give pure compounds **3-38**.

4-(1-(benzyl)-2-methylthio-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-diphenyl-1,4-dihydropyridine-3,5-dicarboxamide (**3**). IR(KBr): cm_1: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d6): δ 9.0(s, 2H, 2 × amide -NH), 7.8(s, 1H, imidazole C_4-H), 7.8-6.2 (m, 16H, arom, NH), 5.1(m, 3H,-CH_2N, dihydropyridine C_4-H), 2.2(s, 3H, SCH_3), 1.85ppm(s, 6H, dihydropyridine C_2,C_6 CH_3).

4-(1-(benzyl)-2-methylthio-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-di-(2-methoxy–phenyl)-1,4-dihydro pyridine-3,5-dicarboxamide (4). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d_6): δ 9.2(s, 2H, 2 × amide –NH), 7.3-6.4(m, 15H, arom, NH, imidazole C_4-H), 4.5(s, 3H, -CH_2N, dihydropyridine C_4-H), 3.4(s, 6H, OCH_3), 2.2(s, 3H, SCH_3), 1.8ppm(s, 6H, dihydropyridine C_2,C_6 CH_3).

4-(1-(benzyl)-2-methylthio-1H-imidazol-5-yl)-2,6-dimethyl-N3,N5-di-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxamide (5). IR(KBr): cm$^-$

[1]: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d_6): δ 9.5(s, 2H, 2 × amide -NH), 8.15(m, 3H, imidazole C_4-H, arom C-H), 7.7-6.4(m, 12H, arom, NH), 5.0-4.7(m, 3H, dihydropyridine C_4-H, -CH_2N), 2.0(s, 3H, SCH_3), 1.5ppm(s, 6H, dihydropyridine C_2,C_6 CH_3).

4-(1-(benzyl)-2-methylthio-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-di-(4-nitrophenyl)-1,4-dihydro pyridine-3,5-dicarboxamide (6). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d_6): δ 9.8(s, 2H, 2 × amide -NH), 8.4(s, 1H, imidazole C_4-H), 8.2-7.4 (m, 14H, arom, NH), 5.2-4.8(m, 3H, -CH_2N, dihydropyridine C_4-H), 2.0(s, 3H, SCH_3), 1.8ppm(s, 6H, dihydropyridine C_2,C_6 CH_3).

4-(1-(benzyl)-2-methylthio-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-di-(2-chlorophenyl)-1,4-dihydro pyridine-3,5-dicarboxamide (7). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d_6): δ 8.5(s, 2H, 2 × amide -NH), 8.0(s, 1H, imidazole C_4-H), 7.5-6.0 (m, 14H, arom, NH), 4.8(m, 3H, -CH_2N, dihydropyridine C_4-H), 2.0(s, 3H, SCH_3), 1.5ppm(s, 6H, dihydropyridine C_2,C_6 CH_3).

4-(1-(benzyl)-2-methylthio-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-di-(4-chlorophenyl)-1,4-dihydro pyridine-3,5-dicarboxamide (8). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d_6): δ 9.1(s, 2H, 2 × amide -NH), 8.0(s, 1H, imidazole C_4-H), 7.5-6.3 (m, 14H, arom, NH), 4.6-4.8(m, 3H, -CH_2N, dihydropyridine C_4-H), 1.9(s, 3H, SCH_3), 1.6ppm(s, 6H, dihydropyridine C_2,C_6 CH_3).

4-(1-(benzyl)-2-(Ethylthio)-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-diphenyl-1,4-dihydropyridine-3,5-dicarboxamide (9). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d_6): δ 9.3(s, 2H, 2 × amide -NH), 8.0(s, 1H, imidazole C_4-H), 7.6-6.4 (m, 16H, arom, NH), 5.1(m, 3H, dihydropyridine C_4-H, -CH_2N), 2.5(m, 2H, SCH_2), 1.85(s, 6H, dihydropyridine C_2,C_6 CH_3), 0.85ppm(t, 3H, CH_3).

4-(1-(benzyl)-2-(ethylthio)-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-di-(2-methoxyphenyl)-1,4-dihydropyridine-3,5-dicarboxamide (10). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d_6): δ 9.3(s, 2H, 2 × amide -NH), 8.0(s, 1H, imidazole C_4-H), 7.6-6.4 (m, 14H, arom, NH), 5.1(m, 3H, dihydropyridine C_4-H, -CH_2N), 2.5(m, 2H, SCH_2), 1.85(s, 6H, dihydropyridine C_2,C_6 CH_3), 0.85ppm(t, 3H, CH_3).

4-(1-(benzyl)-2-(ethylthio)-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-di-(3-nitrophenyl)-1,4-dihydropyridine -3,5- dicarboxamide (11). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d_6): δ 9.7(s, 2H, 2 × amide -NH), 8.3(m, 3H, imidazole C_4-H, arom C-H), 7.0-6.5 (m, 12H, arom, NH), 5.0(m, 3H, dihydropyridine C_4-H, -CH_2N), 2.5(m, 2H, SCH_2), 1.8(s, 6H, dihydropyridine C_2,C_6 CH_3), 0.9ppm(t, 3H, CH_3).

4-(1-(benzyl)-2-(ethylthio)-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-di-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxamide (12). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d_6): δ 9.8(s, 2H, 2 × amide –NH), 8.5(s, 1H, imidazole C_4-H), 7.6-6.4 (m, 14H, arom, NH), 5.0(m, 3H, dihydropyridine C_4-H, -CH_2N), 2.6(m, 2H, SCH_2), 1.9(s, 6H, dihydropyridine C_2,C_6 CH_3), 0.9ppm(t, 3H, CH_3).

4-(1-(benzyl)-2-(ethylthio)-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-di-(2-chlorophenyl)-1,4-dihydropyridine-3,5-dicarboxamide (13). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d_6): δ 9.6(s, 2H, 2 × amide -NH), 8.3(s, 1H, imidazole C_4-H), 7.6-6.3 (m, 14H, arom, NH), 5.1(s, 3H, dihydropyridine C_4-H, -CH_2N), 2.7(q, 2H, SCH_2), 1.85(s, 6H, dihydropyridine C_2,C_6 CH_3), 1.0ppm(t, 3H, CH_3).

4-(1-(benzyl)-2-(ethylthio)-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-di-(4-chlorophenyl)-1,4-dihydro-pyridine -3,5-dicarboxamide (14). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d_6): δ 9.4(s, 2H, 2 × amide –NH), 8.2(s, 1H, imidazole C_4-H), 7.7-6.4 (m, 14H, arom, NH), 5.0(s, 3H, dihydropyridine C_4-H, -CH_2N), 2.5(q, 2H, SCH_2), 1.85(s, 6H, dihydropyridine C_2,C_6 CH_3), 0.85ppm(t, 3H, CH_3).

4-(1-methyl-2-methylthio-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-diphenyl-1,4-dihydropyridine-3,5-dicarboxamide (15). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d_6): δ 9.0(s, 2H, 2 × amide -NH), 7.8(s, 1H, imidazole C_4-H), 7.4-6.5 (m, 10H, arom), 6.2(s, 1H, NH), 4.8(s, 1H, dihydropyridine C_4-H), 3.1(s, 3H, NCH_3), 1.9(s, 3H, SCH_3), 1.6ppm(s, 6H, dihydropyridine C_2,C_6 CH_3).

4-(1-methyl-2-methylthio-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-di-(2-methoxyphenyl) -1,4-dihydro -pyridine-3,5-dicarboxamide (16). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); 1H-NMR (DMSO-d_6): δ 9.0(s, 2H, 2 × amide –NH), 7.8(s, 1H, imidazole C4-H), 7.3-6.4 (d, 8H, arom), 6.25(1H, dihydropyridine NH), 4.8(s, 1H, dihydropyridine C4-H), 3.5(s, 6H, OCH_3), 3.15(s, 3H, NCH_3), 1.9(s, 3H, SCH_3), 1.6ppm(s, 6H, dihydropyridine C_2,C_6 CH_3).

4-(1-methyl-2-methylthio-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-di-(3-nitrophenyl)-1,4-dihydro-pyridine-3,5-dicarboxamide (17).IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); 1H-NMR (DMSO-d6): δ 9.5(s, 2H, 2 × amide –NH), 8.2-7.9 (m, 3H, imidazole C_4-H, C2-H arom), 7.7-6.8 (m, 6H, arom), 6.2(s, 1H, NH), 4.8(s, 1H, dihydropyridine C_4-H), 3.1(s, 3H, NCH_3), 1.9(s, 3H, SCH_3), 1.6ppm(s, 6H, dihydropyridine C_2,C_6 CH_3).

4-(1-methyl-2-methylthio-1H-imidazol-5-yl)-2,6-

dimethyl-N^3,N^5-di-(4-nitrophenyl)-1,4-dihydro-pyridine-3,5-dicarboxamide (18). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); 1H-NMR (DMSO-d6): δ 9.6(s, 2H, 2 × amide –NH), 8.0(s, 1H, imidazole C4-H), 7.7(d, 4H, arom), 7.3(d, 4H, arom), 6.2(s, 1H, NH), 4.7(s, 1H, dihydropyridine C4-H), 3.1(s, 3H, NCH_3), 1.9(s, 3H, SCH_3), 1.5ppm(s, 6H, dihydropyridine C_2,C_6 CH_3).

4-(1-methyl-2-methylthio-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-di-(2-chlorophenyl)-1,4-dihydropyridine-3,5-dicarboxamide (19). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d_6): δ 9.4(s, 2H, 2 × amide -NH), 8.3(s, 1H, imidazole C_4-H), 7.6-6.6(m, 9H, arom, NH), 5.0(m, 1H, dihydropyridine C_4-H), 3.2(s, 3H, NCH_3), 2.2(s, 3H, SCH_3), 1.8ppm(s, 6H, dihydropyridine C_2,C_6 CH_3)

4-(1-methyl-2-methylthio-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-di-(4-chlorophenyl)-1,4-dihydropyridine-3,5-dicarboxamide (20). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); 1H-NMR (DMSO-d6): δ 9.1(s, 2H, 2 × amide -NH), 7.8(s, 1H, imidazole C_4-H), 6.7-7.4(m, 8H, arom), 6.2(1H, dihydropyridine NH), 4.6(s, 1H, dihydropyridine C_4-H), 3.1(s, 3H, NCH_3), 1.9(s, 3H, SCH_3), 1.6ppm(s, 6H, dihydropyridine C_2,C_6 CH_3).

4-(2-(Ethylthio)-1-methyl-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-diphenyl-1,4-dihydro-pyridine-3,5-dicarboxamide (21). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); 1H-NMR (DMSO-d6): δ 9.0(s, 2H, 2 × amide -NH), 7.8(s, 1H, imidazole C_4-H), 6.4-7.3(d, 10H, arom), 6.25(s, 1H, NH), 4.8(s, 1H, dihydropyridine C_4-H), 3.15(s, 3H, NCH_3), 2.4(q, 2H, SCH_2), 1.6(s, 6H, dihydropyridine C2,C6 CH_3), 0.6ppm(t, 3H, CH3).

4-(2-(Ethylthio)-1-methyl-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-di-(2-methoxyphenyl)-1,4-dihydropyridine-3,5-dicarboxamide (22). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); 1H-NMR (DMSO-d6): δ 8.0(s, 3H, 2 × amide -NH, imidazole C4-H), 7.4(m, 2H, arom), 6.5(m, 7H, arom, NH), 4.6(s, 1H, dihydropyridine C4-H), 3.4(s, 6H, OCH_3), 3.1(s, 3H, NCH_3), 2.5(q, 2H, SCH_2), 1.7(s, 6H, dihydropyridine C_2,C_6 CH_3), 0.7ppm(t, 3H, CH_3).

4-(2-(Ethylthio)-1-methyl-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-di-(3-nitrophenyl)-1,4-dihydro-pyridine-3,5-dicarboxamide (23). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d_6): δ 9.5(s, 2H, 2 × amide -NH), 8.25(s, 2H, C_2-H arom),8.0(s, 1H, imidazole C_4-H), 7.0 -7.6(m, 6H, arom), 6.4(s, 1H, NH), 4.9(s, 1H, dihydropyridine C_4-H), 3.2(s, 3H, NCH_3), 2.4(q, 2H, SCH_2), 1.6(s, 6H, dihydropyridine C_2,C_6 CH_3), 0.5ppm(t, 3H, CH_3).

4-(2-(Ethylthio)-1-methyl-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-di-(4-nitrophenyl)-1,4-dihydro pyridine-3,5-dicarboxamide (24) IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d_6): δ 9.7(s, 2H, 2 × amide -NH), 8.1(s, 1H, imidazole C_4-H), 7.8(d, 4H, arom), 7.4(d, 4H, arom), 6.3(s, 1H, NH), 4.8(s, 1H, dihydropyridine C_4-H), 3.1(s, 3H, NCH_3), 2.4(q, 2H, SCH_2), 1.6(s, 6H, dihydropyridine C_2,C_6 CH_3), 0.5ppm(t, 3H, CH_3).

4-(2-(Ethylthio)-1-methyl-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-di-(2-chlorophenyl)-1,4-dihydro-pyridine-3,5-dicarboxamide (25) . IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d_6): δ 9.4(s, 2H, 2 × amide -NH), 8.0(s, 1H, imidazole C_4-H), 7.7-6.1(m, 9H, arom, NH), 5.0(s, 1H, dihydropyridine C_4-H), 3.4(s, 3H, NCH_3), 2.6(q, 2H, SCH_2), 1.8(s, 6H, dihydropyridine C_2,C_6 CH_3), 0.8ppm(t, 3H, CH_3).

4-(2-(Ethylthio)-1-methyl-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-di-(4-chlorophenyl)-1,4-dihydro-pyridine-3,5-dicarboxamide (26) IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d_6): δ 9.4(s, 2H, 2 × amide -NH), 8.0(s, 1H, imidazole C_4-H), 7.7-6.8(m, 8H, arom), 6.1(s, 1H, NH), 5.0(s, 1H, dihydropyridine C_4-H), 3.4(s, 3H, NCH_3), 2.6(q, 2H, SCH_2), 1.8(s, 6H, dihydropyridine C_2,C_6 CH_3), 0.8ppm(t, 3H, CH_3).

4-(3-(4-fluorobenzyl)-2-methylthio-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-diphenyl-1,4-dihydro pyridine-3,5-dicarboxamide (27). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d6): δ 9.3(s, 2H, 2 × amide -NH), 8.1(s, 1H, imidazole C_4-H), 7.5-6.4(m, 15H, arom, NH), 5.0(m, 3H, -CH_2-C_6H_5, dihydropyridine C_4-H), 2.2(s, 3H, SCH_3), 1.8ppm(s, 6H, dihydropyridine C_2,C_6 CH_3).

4-(3-(4-fluorobenzyl)-2-methylthio-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-di-(2-methoxyphenyl)-1,4-dihydropyridine-3,5-dicarboxamide (28). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); ^{1}H-NMR (CD_3OD): δ 8.0-6.5(m, 14H, arom, NH, imidazole C_4-H), 6.1(m, 1H, dihydropyridine C_4-H), 5.0(m, 2H, -CH_2N), 3.5(s, 6H, OCH_3), 2.0(s, 3H, SCH_3), 1.5ppm(s, 6H, dihydropyridine C_2,C_6 CH_3).

4-(3-(4-fluorobenzyl)-2-methylthio-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-di-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxamide (29). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d_6): δ 9.6(s, 2H, 2 × amide –NH), 8.3(m, 3H, imidazole C_4-H, arom C-H), 7.9-7.1(m, 6H, arom), 6.5(m, 5H, arom, NH), 5.1(s, 2H, -CH_2N), 4.8(s, 1H, dihydropyridine C_4-H), 2.1(s, 3H, SCH_3), 1.8ppm(s, 6H, dihydropyridine C_2,C_6 CH3).

4-(3-(4-fluorobenzyl)-2-methylthio-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-di-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxamide (30). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d_6): δ 9.8(s, 2H, 2 × amide -NH), 8.5(s, 1H, imidazole C_4-H), 8.0(d, 4H, arom), 7.6(d, 4H, arom), 6.6(m, 5H, arom,

NH), 5.3-4.6(m, 3H, -CH_2N, dihydropyridine C_4-H), 2.2(s, 3H, SCH_3), 1.9ppm(s, 6H, dihydropyridine C_2,C_6 CH_3).

4-(3-(4-fluorobenzyl)-2-methylthio-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-di-(2-chlorophenyl)-1,4-dihydropyridine-3,5-dicarboxamide (31). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d6): δ 8.6(s, 2H, 2 × amide -NH), 8.2(s, 1H, imidazole C_4-H), 7.5-6.5 (m, 13H, arom, NH), 5.0(m, 3H, -CH_2N, dihydropyridine C_4-H), 2.2(s, 3H, SCH_3), 1.8ppm(s, 6H, dihydropyridine C_2,C_6 CH_3).

4-(3-(4-fluorobenzyl)-2-methylthio-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-di-(4-chlorophenyl)-1,4-dihydropyridine-3,5-dicarboxamide (32). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d_6): δ 8.2(s, 2H, 2 × amide -NH), 8.0(s, 1H, imidazole C_4-H), 7.5-6.4(m, 13H, arom, NH), 5.0(m, 3H, -CH_2N, dihydropyridine C_4-H), 1.9(s, 3H, SCH_3), 1.5ppm(s, 6H, dihydropyridine C_2,C_6 CH_3).

4-(1-(2-chlorobenzyl)-2-methylthio-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-diphenyl-1,4-dihydro pyridine-3,5-dicarboxamide (33). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d_6): δ 9.2(s, 2H, 2 × amide -NH), 8.1(s, 1H, imidazole C_4-H), 7.5-6.5(m, 14H, arom), 6.1(m, 1H, NH), 5.0(m, 3H, -CH_2N, dihydropyridine C_4-H), 2.1(s, 3H, SCH_3), 1.5ppm(s, 6H, dihydropyridine C_2,C_6 CH_3).

4-(1-(2-chlorobenzyl)-2-methylthio-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-di-(2-methoxyphenyl)-1,4-dihydropyridine-3,5-dicarboxamide (34). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d_6): δ 8.1(s, 2H, 2 × amide –NH), 7.5(m, 1H, imidazole C_4-H), 6.8-6.3(m, 12H, arom), 5.8(m, 1H, NH), 5.0(m, 2H, -CH_2N, dihydropyridine C_4-H), 3.4(s, 6H, OCH_3), 2.1(s, 3H, SCH_3), 1.5ppm(s, 6H, dihydropyridine C_2,C_6 CH_3).

4-(1-(2-chlorobenzyl)-2-methylthio-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-di-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxamide (35). IR(KBr): cm_1: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d_6): δ 9.7(s, 2H, 2 × amide -NH), 8.3(m, 3H, imidazole C_4-H, arom C-H), 8.0-6.5(m, 10H, arom), 5.9(m, 1H, NH), 5.0(m, 3H, -CH_2N, dihydropyridine C_4-H), 2.1(s, 3H, SCH_3), 1.9ppm(s, 6H, dihydropyridine C_2,C_6 CH_3).

4-(1-(2-chlorobenzyl)-2-methylthio-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-di-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxamide (36). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d_6): δ 9.5(s, 2H, 2 × amide -NH), 8.3(s, 1H, imidazole C_4-H), 7.8(d, 4H, arom), 7.4(d, 4H, arom), 7.0-6.4(m, 5H, arom, NH), 5.6(m, 1H, dihydropyridine C_4-H), 4.8(m, 2H, -CH_2N), 1.9(s, 3H, SCH_3), 1.5ppm(s, 6H, dihydropyridine C_2,C_6 CH_3).

4-(1-(2-chlorobenzyl)-2-methylthio-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-di-(2-chlorophenyl)-1,4-dihydropyridine-3,5-dicarboxamide (37). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d6): δ 8.5(s, 2H, 2 × amide -NH), 8.1(s, 1H, imidazole C_4-H), 7.5-6.4(m, 12H, arom), 5.9(m, 1H, NH), 4.8(m, 3H, -CH_2N, dihydropyridine C4-H), 2.0(s, 3H, SCH_3), 1.5ppm(s, 6H, dihydropyridine C_2,C_6 CH_3).

4-(1-(2-chlorobenzyl)-2-methylthio-1H-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-di-(4-chlorophenyl)-1,4-dihydropyridine-3,5-dicarboxamide (38). IR(KBr): cm^{-1}: 3260 (NH), 1660 (C=O); ^{1}H-NMR (DMSO-d_6): δ 9.3(s, 2H, 2 × amide –NH), 8.2(s, 1H, imidazole C_4-H), 7.5-6.5(m, 12H, arom), 6.0(m, 1H, NH), 5.0(m, 3H, -CH_2N, dihydropyridine C_4-H), 2.1(s, 3H, SCH_3), 1.7ppm(s, 6H, dihydropyridine C_2,C_6 CH_3).

Antitubercular Activity

The 31 compounds were tested in vitro against *M. smegmatis*, which is susceptible to both rifampicin and isoniazid. The minimum bactericidal concentration (MBC) was determined using the agar proportion method in Muller Hinton Agar medium. Isoniazid (Sigma Chemical Company) was used as the reference drug at 1.0 μg/mL and 0.2 μg/mL. Solutions of compounds in dimethylsulfoxide were then added. The following concentrations were used: 0.1, 0.2, 0.5, 1, 2, 4, 8, 16 and 32 μg/mL. A culture of fast-growing Mycobacterium cultivated in Muller Hinton (Quelab Co., UK) at 35^0 C for a period of 2 days was adjusted to the optical density of McFarland standard no. 1 using the same medium. One dilution of this suspension, 10^{-2}, was used as an inoculum, 0.1 mL per tube. MBC values were determined after incubation at 35^0 C in the presence of 5–7% CO_2 for a period of 1 day. The colonies on each tube were counted, and the numbers of colonies on drug-containing tubes were compared with that of the drug-free control. Five of them were chosen and tested against BCG. The MBC was determined using the agar proportion method in Lowenstein-Jensen medium. Isoniazid (Sigma Chemical Company) was used as the reference drug.

Solutions of compounds in dimethylsulfoxide were added to Middlebrook 7H9 (Quelab Co., UK) medium with glycerol and enriched with OADC (oleic acid, albumin, dextrose catalase). The following concentrations were used: 0.1, 0.2, 0.5, 1, 2, 4, 8, 16 and 32 μg/mL. A culture of BCG in 7H9 broth (Quelab Co., UK) cultivated at 37 ^{0}C for a period of 7 days was adjusted to the optical density of McFarland standard no. 1 using Lowenstein-Jensen medium. One dilution of this suspension, 10^{-2}, was used as an inoculum, 0.1 mL per tube. MIC values were determined after incubation

at 37 °C in the presence of 5–7% CO_2 for a period of 7 days. The colonies on each tube were counted, and the numbers of colonies on drug-containing tubes were compared with that of the drug-free control.

MTT Assay

Cell viability was determined using a modified MTT assay [17, 18]. Briefly, the cells were seeded (10000 cells per well) onto flat-bottomed 96-well culture plates and allowed to grow for 48 followed by treatment with various concentrations of the test compounds (6.25-200 μM). After removing the medium, the cells were labeled with MTT solution (5 mg/mL in PBS) for 4 h, and the resulting formazan was solubilized with DMSO (100 mL). The absorption was measured at 570 nm (620 nm as a reference) in an ELISA reader.

Results

Chemistry

A mixture of 1-phenylamine (benzyl)-2-methylthio-1H-imidazole-5-carbaldehyde **1**, the appropriate N-arylacetoacetanilide **2** and ammonium acetate was refluxed in methanol overnight. The reaction vessel was protected from light. The solvent was removed under reduced pressure. The residue was crystallized from methanol to give pure compounds **3**-**38** (Fig. 1).

The structures of the title compounds were confirmed by IR and ^{1}H NMR. Some of the characterization data of the prepared compounds are summarized in Table 1-2.

All compounds showed a shouldered absorption band at 1645–1680 cm^{-1} in the IR spectra that is typical of the stretch vibrations of two carboxamide C=O groups. The absorption bond shows a shoulder because of the different geometrical isomerism of the two carbonyl groups (of the carboxamides) at the C-3 and C-5 positions of the 1,4-dihydropyridine ring.

The ^{1}H NMR spectra of the final compounds contained a singlet in the δ 1.95–2.22 ppm region from to the CH_3 protons at the C-2 and C-6 positions and another singlet at 5.05–5.20 ppm from to the C-4 proton of the 1,4-dihydropyridine ring. These two singlets are indicative of a 1,4-dihydropyridine ring. Other signals in the ^{1}H NMR spectra were in accordance with the proposed structures.

Antitubercular Activity

The 31 compounds were tested in vitro against *M. smegmatis*. Five of them were chosen and tested against BCG. Isoniazid (Sigma Chemical Co.) was used as the reference drug. MBC was determined using the agar proportion method in Muller Hinton Agar medium in the first item and then Lowenstein-Jensen medium in the second item. The antimycobacterial activities of the tested compounds are provided in Table 2.

MTT-based Cytotoxicity Assay

The cytotoxic effect of two compounds (**27** and **33**) against HeLa cell lines was determined using the MTT assay method. The IC50s (μg/ml) of the two compounds were found to be 5.149 to 33.07 and 5.209 to 33.37, respectively.

Discussion

Comparison of the antituberculosis activities of the compounds showed that N,N-bisphenyl-4-[1-(4-fluorobenzyl)-2-methylthioimidazole-5-yl]-2,6-dimethyl-1,4-dihydro-pyridine-3,5-dicarboxamide **27**, with an MBC=16 μg/ml, and N,N-bisphenyl4-[1-(2-chlorobenzyl)-2-methylthioimidazole-5-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide **33**, with an MBC=32 μg/ml, were the most potent tested compounds. They were more potent than INH, MBC=32 μg/ml, and other compounds used against *M. tuberculosis* BCG. Compounds with other substituents did not show good antitubercular activity. These results demonstrate that a substituted imidazole group is a suitable equivalent for a nitrophenyl group, which has been previously used on antitubercular 1,4-dihydropyridinedicarboxamides [12].

The front-line antituberculosis drug isoniazid (INH) has been shown to inhibit InhA, the NADH-dependent enoyl ACP-reductase (used in fatty acid biosynthesis), from *Mycobacterium tuberculosis* by covalently bonding with NAD and forming INH-NAD adducts [19].

It seems that these compounds were structurally and functionally the same as NADH and inhibited InhA in the same manner as NADH.

These results indicate that the presence of bulkier substituents at the 4-position of the dihydropyridine ring positively contribute to the antituberculosis activity. This activity may be caused by steric interactions in the polar space that contribute to the effective binding of these molecules with the target [12].

Acknowledgements

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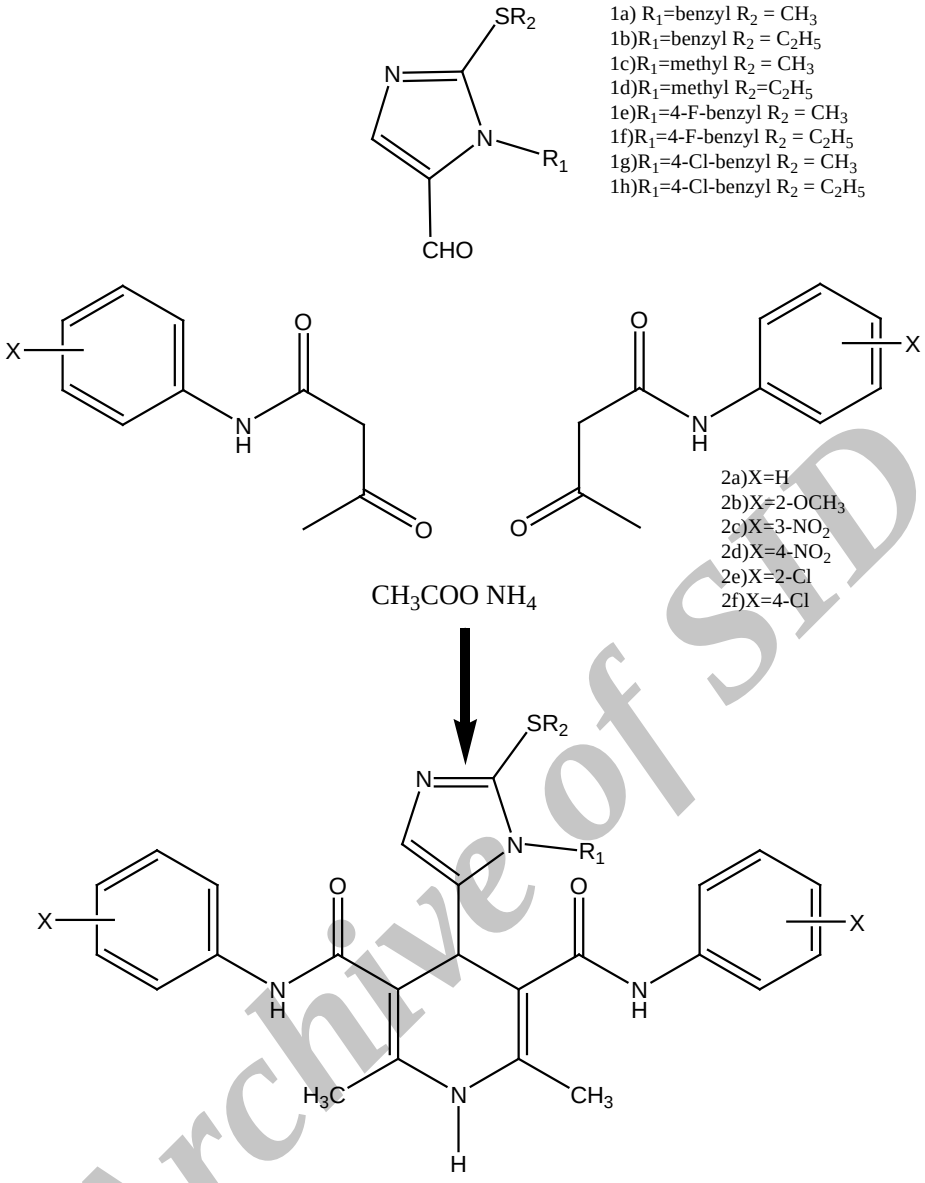

3)R_1=benzyl, R_2 =CH_3 X = H
4)R_1=benzyl , R_2 = CH_3 , X =2-OCH_3
5)R_1=benzyl , R_2 = CH_3 , X =3-NO_2
6)R_1=benzyl , R_2 = CH_3 , X =4-NO_2
7)R_1=benzyl , R_2 = CH_3 , X =2-Cl
8)R_1=benzyl , R_2 = CH_3 , X =4-Cl
9)R_1=benzyl, R_2 = C_2H_3, X = H
10)R_1=benzyl , R_2 = C_2H_5 , X =2-OCH_3
11)R_1=benzyl , R_2 = C_2H_5 , X =3-NO_2
12)R_1=benzyl , R_2 = C_2H_5 , X =4-NO_2
13)R_1=benzyl , R_2 = C_2H_5 , X =2-Cl
14)R_1=benzyl , R_2 = C_2H_5 , X =4-Cl

15)R_1=CH_3, R_2 = CH_3, X = H
16)R_1=CH_3, R_2 = CH_3 , X =2-OCH3
17)R_1=CH_3, R_2 = CH_3 , X =3-NO_2
18)R_1=CH_3, R_2 = CH_3 , X =4-NO_2
16)R_1=CH_3, R_2 = CH_3 , X =2-Cl
20)R_1=CH_3, R_2 = CH_3 , X =4-Cl
21)R_1=CH_3, R_2 = C_2H_3, X = H
22)R_1=CH_3, R_2 = C_2H_5 , X =2-OCH_3
23)R_1=CH_3, R_2 = C_2H_5 , X =3-NO_2
24)R_1=CH_3, R_2 = C_2H_5 , X =4-NO_2
25)R_1=CH_3, R_2 = C_2H_5 , X =2-Cl
26)R_1=CH_3, R_2 = C_2H_5 , X =4-Cl

27)R_1=4-F-benzyl, R_2 = CH_3 X = H
28)R_1=4-F-benzyl , R_2 = CH_3 , X =2-OCH_3
29)R_1=4-F-benzyl , R_2 = CH_3 , X =3-NO_2
30)R_1=4-F-benzyl , R_2 = CH_3 , X =4-NO_2
31)R_1=4-F-benzyl , R_2 = CH_3 , X =2-Cl
32)R_1=4-F-benzyl , R_2 = CH_3 , X =4-Cl
33)R_1=2-Cl-benzyl, R_2 = X = H
34)R_1=2-Cl-benzyl , R_2 = CH_3 , X =2-OCH_3
35)R_1=2-Cl-benzyl , R_2 = CH_3 , X =3-NO_2
36)R_1=2-Cl-benzyl , R_2 = CH_3 , X =4-NO_2
37)R_1=2-Cl-benzyl , R_2 = CH_3 , X =2-Cl
38)$R_1$2-Cl-=benzyl , R_2 = CH_3 , X =4-Cl

Figure 1. Synthesis of 1,4-dihydropyridine3,5-dicarboxamide derivatives.

described in this paper are part of a PharmD thesis. The authors should also appreciate from Ms Atieh Sadat Davari for performing MTT assay.

Table 1- Structural properties of compounds **3-38**

No.	R1	R2	X	Mol. formula	Mol. weight
3	Benzyl	Me	H	$C_{32}H_{31}N_5O_2S$	549.69
4	Benzyl	Me	2-OCH_3	$C_{33}H_{33}N_5O_3S$	579.71
5	Benzyl	Me	3-NO_2	$C_{32}H_{29}N_7O_6S$	639.68
6	Benzyl	Me	4-NO_2	$C_{32}H_{29}N_7O_6S$	639.68
7	Benzyl	Me	2-Cl	$C_{32}H_{29}Cl_2N_5O_2S$	618.58
8	Benzyl	Me	4-Cl	$C_32H_{29}Cl_2N_5O_2S$	618.58
9	Benzyl	Et	H	$C_{33}H_{33}N_5O_2S$	563.71
10	Benzyl	Et	2-OCH_3	$C_{35}H_{37}N_5O4S$	623.76
11	Benzyl	Et	3-NO_2	$C_{33}H_{31}N_7O_6S$	653.71
12	Benzyl	Et	4-NO_2	$C_{33}H_{31}N_7O_6S$	653.71
13	Benzyl	Et	2-Cl	$C_{33}H_{31}Cl_2N_5O_2S$	632.6
14	Benzyl	Et	4-Cl	$C_{33}H_{31}Cl_2N_5O_2S$	632.6
15	Me	Me	H	$C_{26}H_{27}N5O2S$	473.59
16	Me	Me	2-OCH_3	$C_{28}H_{31}N5O4S$	533.64
17	Me	Me	3-NO_2	$C_26H_{25}N_7O_6S$	563.59
18	Me	Me	4-NO_2	$C_{26}H_{25}N_7O_6S$	563.59
19	Me	Me	2-Cl	$C_{26}H_{25}Cl_2N_5O_2S$	542.48
20	Me	Me	4-Cl	$C_{26}H_{25}Cl_2N_5O_2S$	542.48
21	Me	Et	H	$C_{27}H_{29}N_5O_2S$	487.62
22	Me	Et	2-OCH_3	$C_{29}H_{33}N_5O_4S$	547.67
23	Me	Et	3-NO_2	$C_27H_{27}N_7O_6S$	577.61
24	Me	Et	4-NO_2	$C_{27}H_{27}N_7O_6S$	577.61
25	Me	Et	2-Cl	$C_{27}H_{27}Cl_2N_5O_2S$	556.51
26	Me	Et	4-Cl	$C_{27}H_{27}Cl2N_5O_2S$	556.51
27	4-F-benzyl	Me	H	$C_{32}H_{30}FN_5O_2S$	567.68
28	4-F-benzyl	Me	2-OCH_3	$C_{34}H_{34}FN_5O_4S$	627.73
29	4-F-benzyl	Me	3-NO_2	$C_{32}H_{28}FN_7O_6S$	657.67
30	4-F-benzyl	Me	4-NO_2	$C_{32}H_{28}FN_7O_6S$	657.67
31	4-F-benzyl	Me	2-Cl	$C_{32}H_{28}Cl_2FN_5O_2S$	635.57
32	4-F-benzyl	Me	4-Cl	$C_{32}H_{28}Cl_2FN_5O_2S$	635.57
33	2-Cl-benzyl	Me	H	$C_{32}H_{30}ClN_5O_2S$	584.13
34	2-Cl-benzyl	Me	2-OCH_3	$C_{34}H_{34}ClN_5O_4S$	644.18
35	2-Cl-benzyl	Me	3-NO_2	$C_{32}H_{28}ClN_7O_6S$	674.13
36	2-Cl-benzyl	Me	4-NO_2	$C_{32}H_{28}ClN_7O_6S$	674.13
37	2-Cl-benzyl	Me	2-Cl	$C_{32}H_{28}Cl_3N_5O_2S$	653.02
38	2-Cl-benzyl	Me	4-Cl	$C_{32}H_{28}Cl_3N_5O_2S$	653.02

Table 2-Characterization data and antitubercular screening results of the compounds **3**-**38**

Number	MP(°C)	Yield (%)	Analysis			MBC(µg⁄ml)	
			Calculated (Found)				
			C	H	N	*M. smegmatis**	BCG**
3	234-236	70.0	69.92(69.81)	5.68 (5.64)	12.74(12.71)	>32	-
4	198-200	57.5	68.37(68.46)	5.74 (5.71)	12.08(12.06)	>32	-
5	231-233	72.5	60.08(60.13)	4.57(4.54)	15.33(15.36)	ND	ND
6	248-251	97.0	60.08(60.03)	4.57(4.58)	15.33(15.35)	>32	-
7	219-220	80	62.13(62.19)	4.73(4.72)	11.32(11.29)	>32	-
8	230-234	72.8	62.13(62.25)	4.73(4.71)	11.32(11.35)	>32	-
9	224-226	65.25	70.31(70.50)	5.90(5.88)	12.42(12.40)	>32	-
10	228-231	76.5	67.39(67.27)	5.98(583)	11.23(11.21)	>32	-
11	227-223	77.6	60.63(60.75)	4.78(4.89)	15.00(14.98)	>32	-
12	258-260	88.0	60.03(60.21)	4.78(4.69	15.00(15.01)	>32	-
13	169-170	53.0	62.65(62.74)	4.94(4.91)	11.07(11.05)	>32	-
14	232-233	80.0	62.65(62.59)	4.94(4.96)	11.07(11.04)	>32	-
15	245-246	58.25	65.94(66.10)	5.75(5.78)	14.79(14.71)	16	>32
16	235-238	79.5	63.02(62.95)	5.86(5.847)	13.12(13.15)	32	>32
17	229-230	85.4	55.41(55.53)	4.47(4.49)	17.40(17.43)	>32	-
18	181-184	92.0	55.41(55.39)	4.47(4.51)	17.40(17.38)	ND	ND
19	159-162	55.0	57.56(57.48)	4.65(4.61)	12.91(12.93)	>32	>32
20	159-162	55.0	57.56(57.63)	4.65(4.63)	12.91(12.96)	>32	-
21	178-180	42.0	66.50(66.48)	5.99(4.97)	14.36(14.33)	>32	-
22	227-230	96.0	63.60(63.53)	6.07(6.05)	12.79(12.76)	ND	ND
23	231-233	82.0	56.14(56.16)	4.71(4.69)	16.97(17.01)	>32	-
24	241-243	88.8	56.14(56.21)	4.71(4.72)	16.97(16.93)	>32	-
25	158-149	62.5	58.27(58.31)	4.89(4.85)	12.58(12.54)	ND	ND
26	162-165	65.7	58.27(58.32)	4.89(4.87)	12.58(12.56)	>32	-
27	231-233	61.75	67.70(67.66)	5.33(5.36)	12.34(12.35)	32	16
28	93-95	90.0	65.05(64.98)	5.46(5.48)	11.16(11.19)	ND	ND
29	231-234	83.6	58.44(58.55)	4.29(4.31)	14.91(15.01)	>32	-
30	241-243	86.7	58.44(58.49)	4.29(4.27)	14.91(14.98)	>32	-
31	110-112	97.0	60.38(60.47)	4.43(4.41)	11.00(11.03)	>32	-
32	216-220	47.5	60.38(60.45)	4.43(4.40)	11.00(11.05)	>32	-
33	246-248	77.8	65.80(65.73)	5.18(5.21)	11.99(12.02)	4	32
34	245-246	78.5	63.39(63.28)	5.32(5.34)	10.87(10.83)	>32	-
35	237-239	77.8	57.01(57.12)	4.19(4.22)	14.54(14.51)	>32	-
36	215-217	78.5	57.01(57.14)	4.19(4.17)	14.54(14.58)	>32	-
37	234-238	79.0	58.86(58.92)	4.32(4.35)	10.72(10.75)	>32	-
38	242-244	80.0	58.86(58.79)	4.32(4.30)	10.72(10.68)	>32	-
INH	-	-	-	-	-	32	32

MBC (µg⁄ml)*-Determination of MBC with slow-growing Mycobacterium (*M. smegmatis*) in Muller Hinton Agar medium
MBC (µg⁄ml)**- Determination of MBC with fast-growing Mycobacterium (BCG) in Lowenstein-Jensen medium

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