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RESEARCH ARTICLE

Bis dihydropyrimidine: synthesis and antimycobacterial activity

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Abstract

A series of bis dihydropyrimidine compounds were synthesised by reacting dapsone with acetylacetoacetate to produce *N*1-4-[4-(2-oxopropylcarboxamido) phenylsulphonyl] phenyl-3-oxobutanamide, then treated with guanidine hydrochloride and an appropriate aldehyde with a catalytic amount of p-toluene sulphonic acid (PTSA) in the presence of methanol to afford the title compounds. The synthesised compounds were evaluated for their antimycobacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$ and isoniazid (INH) resistant *M. tuberculosis*. Among the synthesised compounds, compound *N*5-(4-4-[6-(4-fluorophenyl)-2-imino-4-methyl-1,2,3,4-tetrahydro-5-pyrimidinylcarboxamido]phenylsulphonylphenyl)-6-(4-fluorophenyl)-2-imino-4-methyl-1,2,3,4-tetrahydro-5-pyrimidine carboxamide (**3g**) was found to be the most promising compound with activity against *M. tuberculosis* $H_{37}Rv$ and INH resistant *M. tuberculosis* with a minimum inhibitory concentration (MIC) between 0.08 and 0.10 μM.

Keywords: Bis dihydropyrimidine, dapsone, antitubercular agents

Introduction

Tuberculosis is a bacterial disease caused by *Mycobacterium tuberculosis*, a relatively slow-growing, aerobic and acid-fast bacillus. Classically, tuberculosis is a pulmonary disease but disseminated and extrapulmonary manifestations may also occur, especially in immunocompromised persons. Tuberculosis is transmitted person to person and is usually contracted by inhalation of *M. tuberculosis* droplet nuclei generated by an infectious person.

Tuberculosis (TB) has infected about one-third of the world's 5 billion people. In 1995, from this pool of infected people, about 3.3 million new active smear-positive cases were reported, along with an estimated 4 million other cases, which led to about two to three million deaths [1].

Currently TB is a silent threat to industrialised people. The World Economic Forum (WEF) [2] report found that nearly one third of over 11,000 business leaders from more than 130 developed and developing countries said they expected TB to affect their business in the next five years. The report states that one in ten said they expected the effect to be serious, particularly because three-quarters of those who fall sick or die of TB are people of prime working age, between 15 and 54 years. TB claims millions of lives every year with 1.6 million people dying of the disease in 2006 and 98% of these were in developing countries.

Although the immediate tuberculosis threat is chiefly to people infected with the human immunodeficiency virus, which causes AIDS, participants at a meeting [2] here expressed alarm that the spread of tuberculosis from such patients also threatened the health workers who care for them and ultimately healthy people who are not infected with H.I.V.

The currently available regimens to treat multi-drug resistant TB (MDR-TB) are four to ten times more likely to fail than the standard therapy for patients with drug-susceptible organisms [3–6]. After the introduction of rifampicin, no worthwhile anti-tuberculosis drug with a new mechanism of action has been developed in over forty years. Along with HIV/AIDS, MDR-TB is the most

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important threat to TB control. Countries with a high MDR-TB prevalence generally have a history of poor TB control. The major barrier to MDR-TB treatment is the high cost of the second-line drugs which are at least 300 times more expensive than the first-line drugs based on the Green Light Committee (GLC) prices and between 1000-3000 times more expensive when market prices are used.

Globally, 400 000 new cases of MDR-TB occur each year. Currently, drug-sensitive TB can be treated with first-line drugs for 6 to 9 months, and 95% of patients can be cured with this regimen [7]. The current work describes the synthesis of a novel bis dihydropyrimidine moiety with encouraging antimycobacterial activity against *M. tuberculosis* H_{37}Rv and isoniazid (INH) resistant *M. tuberculosis.*

Materials

All chemicals were supplied by Merck (New Delhi, India) and SD fine chemicals (Delhi, India). Melting points were determined by the open tube capillary method and are uncorrected. The purity of the compounds were checked using thin layer chromatography (TLC) plates (silica gel G) using solvent system toluene-ethyl formate- formic acid (5:4:1) and benzene-methanol (8:2) solvent systems, the spots were located under iodine vapours or UV light. IR spectra were obtained on a Perkin-Elmer 1720 FT-IR spectrometer (New Delhi, India) (KBr Pellets). The ^{1}H–NMR spectra were recorded on a Bruker AC 300 MHz spectrometer (New Delhi, India) using TMS as the internal standard in DMSO/$CDCl_3$.

Methods

Chemistry

General method for the preparation of N1-4-[4-(2-oxopropylcarboxamido) phenylsulphonyl] phenyl-3-oxobutanamide (2)

Ethyl acetoacetate (0.01 mol) and dapsone (0.01 mol) were mixed and refluxed for 15 h. The blackish liquid formed was then heated on a water bath to remove the alcohol formed during the reaction. After allowing the reaction mixture to cool, the crude crystals were obtained and then filtered. The residue was stirred for 20 min with diethyl ether, the solid separate was filtered off, dried and purified from the ethanol.

IR:(KBr) cm^{-1}: 3307 (NH), 3042 (CH), 1642 (C=O) ^{1}H NMR (DMSO-d_6) ppm: 2.19 (6H, s, CH_3), 3.62 (4H, s, CH_2), 7.31–7.92 (8H, m, aromatic), 8.09, 10.33 (2H,s,NH); Mass (*m/z*): 417 (M^{+1}); Cal/Ana:[C (57.53) 57.67, H (4.8) 4.79, N (6.73) 6.72]

General method for the preparation of compounds (3a–m)

The compound *N*1-4-[4-(2-oxopropylcarboxamido) phenylsulphonyl]phenyl-3-oxobutanamide (0.01 mol) was treated with guanidine hydrochloride (0.015 mol) and an appropriate aldehyde (0.01 mol) with a catalytic amount of p-toluene sulphonic acid (PTSA) in the presence of ethanol to afford the title compounds in a 57–94% yield after recrystalisation with ethanol.

N5-(4-4-[6-(4-methoxyphenyl)-2-imino-4-methyl-1,2,3,4-tetrahydro-5-pyrimidinyl carbo-xamido] phenyl sulphonylphenyl)-6-(4-methoxyphenyl)-2-imino-4-methyl-1,2,3,4-tetra hydro-5-pyrimidine carboxamide (3a)

IR: (KBr) cm^{-1}: 3307 (NH), 3218 (OH), 3042 (CH), 1642 (C=O), 1320 (C-N). ^{1}H NMR (DMSO-d_6) ppm: 1.29–1.36 (6H,d, CH_3), 2.27 (2H, s, CO=NH), 3.8 (6H,s, OCH_3), 5.26–5.3 (2H, m, CH-CH_3), 7.42–7.88 (16H, m, aromatic), 9.92 (6H, s, NH).Mass (*m/z*): 734 (M^+); Cal/Ana: [C (62.12) 62.15, H (5.17) 5.2,N (15.25) 15.2]

N5-(4-4-[6-(4-chlorophenyl)-2-imino-4-methyl-1,2,3,4-tetrahydro-5-pyrimidinyl carbo-xamido] phenyl sulphonylphenyl)-6-(4-chlorophenyl)-2-imino-4-methyl-1,2,3,4-tetrahydro-5-pyrimidine carboxamide (3b)

IR: (KBr) cm^{-1}: 3307 (NH), 3216 (OH), 3042 (CH), 1642 (C=O), 1320 (C-N), 766(C-Cl). ^{1}H NMR (DMSO-d_6) ppm: 1.29–1.33 (6H, d, CH_3), 2.37 (2H, s, CO=NH), 5.18–5.22 (2H, m, CH-CH_3), 7.62–7.78 (16H, m, aromatic), 10.16 (6H, s,NH). Mass (*m/z*): 743 (M^+); Cal/Ana: [C (58.14) 58.1, H (4.3) 4.35, N (15.73) 15.76]

N5-(4-4-[6-(4-4-dimethylamino)-2-imino-4-methyl-1,2,3,4-tetrahydro-5-pyrimidinyl carboxamido] phenyl sulphonylphenyl)-6-(4-4-Dimethylamino)-2-imino-4-methyl-1,2,3,4-tetrahydro-5-pyrimidine carboxamide (3c)

IR: (KBr) cm^{-1}: 3307 (NH), 3218 (OH), 3042 (CH), 1642 (C=O), 1320 (C-N). ^{1}H NMR (DMSO-d_6) ppm: 1.31–1.34 (6H, d, CH_3), 2.37 (2H, s, CO=NH), 3 (12H, s, N $(CH_3)_2$), 5.16–5.2 (2H, m, CH-CH_3), 7.52–7.98 (16H, m, aromatic), 10.12 (6H, s, NH). Mass (*m/z*): 760 (M^+) Cal/Ana: [C (63.15) 63.18, H (5.78) 5.8, N (18.42)18.4]

N5-4-[4-(2-imino-4-methyl-6-phenyl-1,2,3,4-tetrahydro-5-pyrimidinylcarboxamido) phenyl sulphonyl]phenyl-2-imino-4-methyl-6-phenyl-1,2,3,4-tetrahydro-5-pyrimidine carboxamide (3d)

IR: (KBr) cm^{-1}: 3307 (NH), 3218 (OH), 3042 (CH), 1642 (C=O), 1320 (C-N). ^{1}H NMR (DMSO-d_6) ppm: 1.29–1.31 (6H, d, CH_3), 2.37 (2H, s, CO=NH), 5.16–5.2 (2H, m, CH-CH_3), 7.52–7.98 (18H, m, aromatic), 10.12 (6H, s, NH). Mass (*m/z*): 674 (M^+); Cal/Ana: [C (64.09) 64.1, H (5.04) 5.05, N (16.61) 16.6]

N5-(4-4-[6-(3,4-dimethoxyphenyl)-2-imino-4-methyl-1,2,3,4-tetrahydro-5-pyrimidinyl carboxamido] phenyl sulphonylphenyl)-6-(3,4-Dimethoxy phenyl)-2-imino-4-methyl-1,2,3,4-tetrahydro-5-pyrimidine carboxamide (3e)

IR: (KBr) cm^{-1}: 3307 (NH), 3218 (OH), 3042 (CH), 1642 (C=O), 1320 (C-N). ^{1}H NMR (DMSO-d_6) ppm: 1.32–1.37(6H, d, CH_3), 2.37 (2H, s, CO=NH), 3.8 (12H, s, OCH_3), 5.16–5.2 (2H, m, CH-CH_3), 7.52–7.98 (14H, m, aromatic), 10.12 (6H, s, NH).Mass (*m/z*): 794 (M^+); Cal/Ana: [C (60.45) 60.44, H (5.28) 5.25,N (14.1) 14.08]

N5-(4-4-[6-(3,4,5-trimethoxy phenyl)-2-imino-4-methyl-1,2,3,4-tetrahydro-5-pyrimidinyl carboxamido] phenyl sulphonylphenyl)-6-(3,4,5-Trimethoxy phenyl)-2-imino-4-methyl-1,2,3,4-tetrahydro-5-pyrimidine carboxamide (3f)
IR: (KBr) cm^{-1}: 3307 (NH), 3218 (OH), 3042 (CH), 1642 (C=O), 1320 (C-N). ^1H NMR (DMSO-d_6) ppm: 1.29–1.37 (6H, d, CH_3), 2.37 (2H, s, CO=NH), 3.8 (18H, s, OCH_3), 5.16–5.20 (2H, m, CH-CH_3), 7.52–7.98 (12H, m, aromatic), 10.12 (6H, s, NH). Mass (*m/z*):854 (M^+); Cal/Ana: [C (59.01) 59.02, H (5.38) 5.37, N (13.11) 13.1]

N5-(4-4-[6-(4-fluorohenyl)-2-imino-4-methyl-1,2,3,4-tetrahydro-5-pyrimidinylcarbox- amido] phenyl sulphonylphenyl)-6-(4-fluoroophenyl)-2-imino-4-methyl-1,2,3,4-tetrahydro-5-pyrimidine carboxamide (3g)
IR: (KBr) cm^{-1}: 3307 (NH), 3218 (OH), 3042 (CH), 1642 (C=O), 1320 (C-N), 786(C-F). ^1H NMR (DMSO-d_6) ppm: 1.29–1.31(6H, d, CH_3), 2.37 (2H, s, CO=NH), 5.16–5.2 (2H, m, CH-CH_3), 7.52–7.98 (16H, m, aromatic), 10.12 (6H, s, NH). Mass (*m/z*): 710 (M^+); Cal/Ana: [C (60.84) 60.81, H (4.5) 4.49, N (15.77) 15.76]

N5-(4-4-[6-(2-chlorophenyl)-2-imino-4-methyl-1,2,3,4-tetrahydro-5-pyrimidinylcarbox- amido] phenyl sulphonylphenyl)-6-(2-Chloro phenyl)-2-imino-4-methyl-1,2,3,4-tetrahydro-5-pyrimidine carboxamide (3h)
IR: (KBr) cm^{-1}: 3307 (NH), 3218 (OH), 3042 (CH), 1642 (C=O), 1320 (C-N), 766 (C-Cl). ^1H NMR (DMSO-d_6) ppm: 1.29–1.31(6H, d, CH_3), 2.37 (2H, s, CO=NH), 5.18–5.22 (2H, m, CH-CH_3),7.62–7.78 (16H, m, aromatic),10.16 (6H, s, NH). Mass (*m/z*): 743 (M^+); Cal/Ana: [C (58.14) 58.13, H (4.3) 4.31, N (15.73) 15.72]

N5-(4-4-[6-(2,6-dichlorophenyl)-2-imino-4-methyl-1,2,3,4-tetrahydro-5-pyrimidinyl carboxamido] phenyl sulphonylphenyl)-6-(2,6-Dichloro phenyl)-2-imino-4-methyl-1,2,3,4-tetrahydro-5-pyrimidine carboxamide (3i)
IR: (KBr) cm^{-1}: 3307 (NH), 3218 (OH), 3042 (CH), 1642 (C=O), 1320 (C-N), 766 (C-Cl). ^1H NMR (DMSO-d_6) ppm:1.29–1.32 (6H, d, CH_3), 2.37 (2H, s, CO=NH), 5.18–5.22 (2H, m, CH-CH_3), 7.62–7.78 (14H, m, aromatic), 10.16 (6H, s, NH). Mass (*m/z*): 813 (M^+); Cal/Ana: [C (53.20) 53.21, H (3.69) 3.68, N(13.79) 13.78]

N5-(4-4-[6-(4-nitrophenyl)-2-imino-4-methyl-1,2,3,4-tetrahydro-5-pyrimidinylcarbox-amido] phenyl sulphonylphenyl)-6-(4-Nitro phenyl)-2-imino-4-methyl-1,2,3,4-tetrahydro-5-pyrimidine carboxamide (3j)
IR: (KBr) cm^{-1}: 3307 (NH), 3218 (OH), 3042 (CH), 1642 (C=O), 1320 (C-N). ^1H NMR (DMSO-d_6) ppm: 1.3–1.34 (6H, d, CH_3), 2.47 (2H, s, CO=NH), 5.26–5.3 (2H, m, CH-CH_3), 7.82–8.18 (16H, m, aromatic), 10.12 (6H, s, NH). Mass (*m/z*): 764 (M^+); Cal/Ana: [C (56.54) 5 56.52, H (4.18) 4.17, N(18.32) 18.3]

N5-(4-4-[6-(2-furyl)-2-imino-4-methyl-1,2,3,4-tetrahydro-5-pyrimidinylcarbox-amido] phenylsulphonylphenyl)-6-(2-furyl)-2-imino-4-methyl-1,2,3,4-tetrahydro-5-pyrimidine carboxamide (3k)
IR: (KBr) cm^{-1}: 3307 (NH), 3218 (OH), 3042 (CH), 1642 (C=O), 1320 (C-N), 786(C-F). ^1H NMR (DMSO-d_6) ppm:1.37(6H, s, CH_3), 2.37 (2H, s, CO=NH), 5.16–5.2 (2H, m, CH-CH_3), 6.52–7.12 (6H, m, furan), 7.52–7.98 (8H, m, aromatic), 10.12 (6H, s, NH). Mass (*m/z*): 654 (M^+); Cal/Ana: [C (58.71) 58.7,H (4.58) 4.56, N(17.12) 17.10]

N5-(4-4-[2-imino-4-methyl-6-(2-thienyl)-1,2,3,4-tetrahydro-5-pyrimidinylcarbox-amido] phenylsulphonylphenyl)-2-imino-4-methyl-6-(2-thienyl)-1,2,3,4-tetrahydro-5-pyrimidine carboxamide (3l)
IR: (KBr) cm^{-1}: 3307 (NH), 3218 (OH), 3042 (CH), 1642 (C=O), 1320 (C-N), 786(C-F). ^1H NMR (DMSO-d_6) ppm:1.37 (6H, s, CH_3), 2.37 (2H, s, CO=NH), 5.16–5.2 (2H, m, CH-CH_3), 6.08–6.92 (6H, m, thiophene),7.52–7.98 (8H, m, aromatic), 10.12 (6H, s, NH). Mass (*m/z*): 686 (M^+);Cal/Ana: [C (55.97) 55.95, H (4.37) 4.36, N (18.32) 18.3]

N5-(4-4-[6-(3-hydroxy,4-methoxy)-2-imino-4-methyl-1,2,3,4-tetrahydro-5-pyrimi- dinylcarboxamido] phenyl sulphonylphenyl)-6-(3-Hydroxy,4-methoxy)-2-imino-4-methyl-1,2,3,4-tetrahydro-5-pyrimidine carboxamide (3m)
IR: (KBr) cm^{-1}: 3307 (NH), 3218 (OH), 3042 (CH), 1642 (C=O), 1320 (C-N), 786 (C-F). ^1H NMR (DMSO-d_6) ppm:1.37 (6H, s, CH_3), 2.37 (2H, s, CO=NH), 3.8 (6H, s, OCH_3), 8.42 (2H, s, OH), 5.16–5.2 (2H, m, CH-CH_3), 7–7.88 (14H, m, aromatic), 8.42 (6H, s, NH). Mass (*m/z*): 766 (M^+); Cal/Ana: [C (59.53) 59.52, H (4.96) 4.95, N (14.62) 14.63]

Biology

The primary screen was conducted at 6.25μg/ml (or at the molar equivalent of the highest molecular weight compound in a series of congeners) against *Mycobacterium tuberculosis* $H_{37}RV$ (ATCC27294) in BACTEC 12B medium using the BACTEC 460 radiometric system (Alwar, Rajasthan, India)[8].

Cytotoxicity

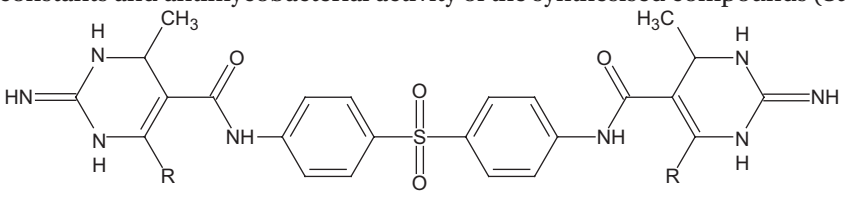
All the compounds were tested for cytotoxicity using the half maximal inhibitory concentration ($1C_{50}$) in VERO cells at concentrations at 62.5μg/mL or 10 times. After a 72 h exposure, viability was assessed on the basis of cellular conversion of 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye into the formazan product using the Promega CellTiter 96® Non-radioactive Cell proliferation method [9].

Results and discussion

Chemistry

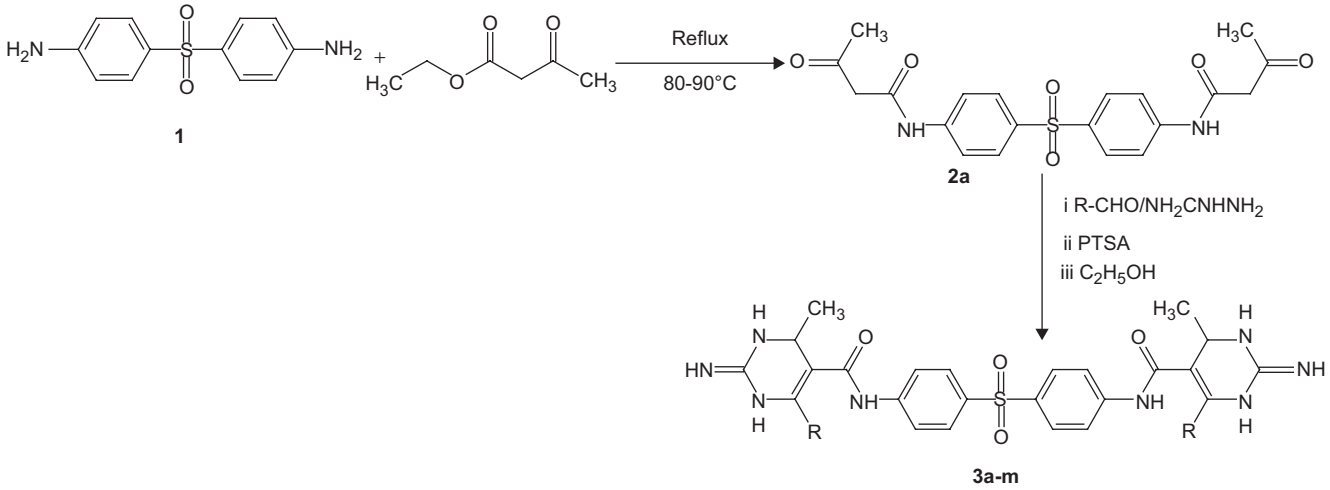
The *N*5-4-[4-(2-imino-4-methyl-6-phenyl-1,2,3,4-tetrahydro-5-pyrimidinylcarboxamido) phenyl sulphonyl] phenyl-2-imino-4-methyl-6-phenyl-1,2,3,4-tetrahydro-5-pyrimidinecarboxamide compounds **3a**–**k** described in this study are shown in Table 1. A reaction sequence for the preparation of the compounds is outlined in Scheme 1. The reaction between dapsone and

Table 1. The physical constants and antimycobacterial activity of the synthesised compounds (**3a**–**m**).



Compound	R	Yield (%)	Mp (°C)	(MIC) μM MTB[a]	MTB[b]
3a	4-Methoxy phenyl	72	235	0.86	2.5
3b	4-Chloro phenyl	78	208	0.32	3.2
3c	4-Dimethylamino phenyl	66	166	0.94	3.78
3d	Phenyl	80	192	0.62	1.56
3e	3,4-Dimethoxy phenyl	82	222	1.48	2.12
3f	3,4,5-Trimethoxy phenyl	86	246	1.56	4.5
3g	4-Fluoro phenyl	94	194	0.08	0.1
3h	2-Chloro phenyl	86	246	0.22	1.72
3i	2,6-Dichloro phenyl	80	226	0.28	1.96
3j	4-Nitro Phenyl	92	212	0.46	3.72
3k	Furyl	78	178	0.59	5.38
3l	Thiophenyl	78	157	0.68	6.23
3m	3-Hydroxy,4-methoxy phenyl	78	192	1.94	7.74
INH	-	-	-	0.73	11.37

[a] *Mycobacterium tuberculosis* $H_{37}R_v$; [b] INH resistant *Mycobacterium tuberculosis.*



R= 4-methoxy phenyl-,4-chloro phenyl-,4-dimethylamino phenyl-,3,4-dimethoxy phenyl-,3,4,5-trimethoxy phenyl-,4-fluoro phenyl-,2-chloro phenyl-,2,6-dichloro phenyl-,4-nitro phenyl-,furyl-,thiophenyl-,3-hydroxy,4-methoxy phenyl-

Scheme 1. Protocol for the synthesis of the title compounds.

acetylacetoacetate at 80°C to 90°C was performed to produce the *N*1-4-[4-(2-oxopropylcarboxamido) phenylsulphonyl] phenyl-3-oxobutanamide. The product was then treated with guanidine hydrochloride and the appropriate aldehyde with a catalytic amount of p-toluene sulphonic acid (PTSA) in the presence of ethanol to afford the title compounds after recrystalisation with ethanol. The yield of the final products was in the range of 66–94%. The purity of the compounds was checked by TLC and elemental analysis. Both analytical and spectral data (^{1}H-NMR, IR) of all the synthesised compounds were in full agreement with the proposed structures. In general, infra red spectra (IR) revealed NH, CH, C=O, C-N and C-F peak at 3307, 3042, 1642, 1590 and 786 cm^{-1} respectively. In the nuclear magnetic resonance spectra (^{1}H NMR) the signals of the respective protons of the prepared titled compounds were verified on the basis of their chemical shifts, multiplicities and coupling constants. The spectra showed a singlet at δ 1.38 ppm corresponding to a methyl group; a singlet at δ 2.32 ppm corresponding to a CO-NH group; a multiplet at

δ 5.15–5.2 corresponding to a CH-CH_2 group; a multiplet at δ 7.45–7.9 ppm corresponding to an aromatic proton; a singlet at δ 10.12 ppm corresponding to NH protons. The elemental analysis results were within ± 0.4% of the theoretical values.

Antimycobacterial activity

The synthesised compounds **3a**–**m** were tested for their antimycobacterial activity *in vitro* against MTB and INHR-MTB by the agar dilution method using a double dilution technique similar to that recommended by the National Committee for Clinical Laboratory Standards [8]. The INHR-MTB clinical isolate was obtained from the Tuberculosis Research Centre, Alwar, India. The MIC was defined as the minimum concentration of compound required to inhibit 90% of bacterial growth and the MIC results are reported in Table 1 with INH as the standard drug for comparison.

Among the thirteen compounds synthesised, ten compounds were found to be active with minimum inhibitory concentrations of less than 1μM and were more active than INH against MTB. Compounds with an electron withdrawing group substituted phenyl group showed the most activity. Among the thirteen newly synthesised compounds, compound N5-(4-4-[6-(4-fluorophenyl)-2-imino-4-methyl-1,2,3,4-tetrahydro-5-pyrimidinylcarboxamido] phenyl sulphonylphenyl)-6-(4-fluorophenyl)-2-imino-4-methyl-1,2,3,4-tetrahydro-5-pyrimidine carboxamide (**3g)** was found to be most active agent against *Mycobacterium tuberculosis* $H37_{Rv}$ (MTB) and INH resistant *Mycobacterium tuberculosis* (INHR-MTB) with a minimum inhibitory concentration of <0.10 μM. When compared to INH, compound (**3g)** was found to be 9.12 fold and 113.7 fold more active against MTB and INHR-MTB. Compounds 2-chlorophenyl (**3h**) and 2,6-dichlorophenyl (**3i**) substituent were found to be more active than INH against MTB[a] and MTB[b] with MIC results of 0.22 μM, 1.72 μM and 0.28μM, 1.96 μM respectively. The 4-fluoro group substituted (**3g)** derivative displayed relatively higher inhibitory activity in general. However the electron withdrawing groups such as 4-cholrophenyl, 2-chlorophenyl, 2,6-dichlorophenyl, thiophenyl, furyl and 4-nitrophenyl substituted analogues showed good to excellent inhibitory activity against *Mycobacterium tuberculosis* ($H_{37}R_v$) and INH resistant *Mycobacterium tuberculosis* (INHR-MTB). On the other hand the electron donating group containing analogues such as the OCH_3 group substituted 4-methoxy phenyl (**3a**), 3, 4 dimethoxy phenyl (**3e**), 3, 4, 5 trimethoxy phenyl (**3f)** and 3-hydroxyl, 4-methoxy (**3m**) showed moderate antitubercular activity. These reports clearly show that the presence of a phenyl ring with the substitution of a fluoro group at the para position produced a remarkable improvement in antimycobacterial activity.

All the compounds were tested for cytotoxicity ($1C_{50}$) in VERO cells at concentrations of 62.5μg/mL or 10 times. After 72 hours exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega CellTiter 96® non-radioactive cell proliferation method [9]. Most of the active compounds were found to be non-toxic at 62.5μg/mL. The screening of bis dihydropyrimidine derivatives identified novel compounds endowed with antimycobacterial activity and exhibiting MIC values less than 1μM. It is conceivable that derivatives showing more potency, selectivity and low toxicity would be excellent leads for synthesising novel derivatives for antimycobacterial activity against MTB and INHR-MTB. These derivatives could be further modified to exhibit better potency than the standard drugs. Further studies will be performed to acquire more information about quantitative structure-activity relationships (QSAR) and MDR which are in progress in our laboratory. The bis dihydropyrimidine derivatives discovered in this study may provide valuable therapeutic intervention for the treatment of tubercular diseases.

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Declaration of interest

The authors report no conflict of interest.

References

1. Centers for Disease Control and Prevention (CDC). Trends in tuberculosis incidence—United States, 2006. MMWR Morb Mortal Wkly Rep 2007:245–250.
2. World economic forum annual meeting 2009 Davos-Klosters, Switzerland 28 January –1 February.
3. Jain NK, Chopra KK., Prasad G. Multidrug -resistant initial and acquired isoniazid and rifampicin resistance to M. tuberculosis and its implications for treatment. Indian J Tuberc 1992;39:121–124.
4. Goble M, Iseman MD, Medsen LA. Treatment of 171 patients with pulmonary tuberculosis resistant to isoniazid and rifampin. N Engl J Med 1993;328:527–532.
5. Park M, Davis AL, Schluger N, Cohen H, Rom WN. Outcome of MDR-TB patients, 1983–1993. Prolonged survival with appropriate therapy. Am J Respir Crit Care Med 1996;153:317–324.
6. Telzak EE, Sepkowitzk AP, Mannheimer S, Medard F. Successful treatment of multidrug-resistant tuberculosis (MDRTB) among HIV-negative patients. N Engl J Med 1995;333:907–911.
7. Zignol M, Hosseini MS, Wright A. Global incidence of multidrug-resistant tuberculosis. J Infect Dis 2006;194:479–485.
8. Heifets LB, Flory MA, Lindholm-Levy P. Does pyrazinoic acid as an active moiety of pyrazinamide have specific activity against Mycobacterium tuberculosis? J Antimicrob Agents Chemother 1989;33:1252–1254.
9. Gundersen LL, Nissen-Meyer J, Spilsberg B. Synthesis and antimycobacterial activity of 6-arylpurines; the requirements for the N-9 substituent in active antimycobacterial purines. J Med Chem 2002;45:1383–1386.