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SYNTHESIS AND EVALUATION OF SUBSTITUTED MANNICH BASES OF PIPERAZINE AS ANTI TUBERCULAR AGENTS

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ABSTRACT

A series of Bis-Mannich bases of N-phenyl Piperazine and Bis-mannich bases of Piperazine were synthesized, the structures of the compounds were elucidated and screened for antitubercular activity against *M. tuberculosis* strain H37Rv by using radiometric BACTEC 12B medium as broth micro dilution assay (Alamar blue assay). Among the test compounds Compound N-((4-((pyridinylamino) methyl) piperazine-1-yl) methyl)-N-pyridinylamine (**2e)** exhibited highest inhibitory activity (MIC<0.2µ/ml). Compounds (4-(aminomethyl) piperazine-1-yl) methanamine (**2a**) and N-((4-(phenylamino) methyl) piperazine-1-yl) methyl) benzenamine (**2b**) have shown good inhibitory activity (MIC 0.2µ/ml). Compounds **2f** have shown the intermediatory antitubercular activity (MIC 3.125µ/ml).

Keywords: N-phenylpiperazine; Bis-Mannich bases of Piperazine; Mycobacterium tuberculosis; Alamar Blue Assay; Anti-Tuberculosis agents.

1. INTRODUCTION

Tuberculosis is a chronic, serious and contagious bacterial infection. As per the recent reports, it was a much more predominant disease in the past than today and it was responsible for the deaths of approximately one-third of the world population during the last two centuries [Tarek et al.,; Ratten et al.,]. The incidence of Tuberculosis infection has steadily risen in the last decade and this increase can be attributed to a similar increase in human immunodeficiency virus (HIV) infection [Dharmarajan et al.,]. The reemergence of TB infection is further complicated by an increase in cases, which are resistant to conventional antitubercular drug therapy [Sbarbaro et al.,]. A series of pyrazinamide substituted mannich bases of piperazine and 4-substituted piperazine derivatives of 3-hydroxy-6-methyl-4H-pyran-4-one pharmacophore were reported as antimycobacterial agents [Dharmarajan et al.,; Berk et al.,].

In view of all these facts, we report design, synthesis and antitubercular evaluation of bismannich bases of piperazine derivatives in this papper.

2. MATERIALS AND METHODS.

Chemistry

All chemicals and solvents used in this study were supplied by Merck (Darmstadt, Germany), Aldrich Chemicals Co. (Steinheim, Germany) and SD Fine Chemicals, Mumbai.
Melting points were determined in an open capillary on a Heco melting point apparatus and are uncorrected. The compounds were routinely checked for purity by TLC on silica gel plates and their structures were verified by their IR spectra were measured on a SHIMADZU FT-IR model PC spectrometer in KBr disc (□, cm-1), ^{1}H-NMR spectra were obtained on a BRUKER AC 80 MHz spectrophotometer in CDCl3 and DMSO with TMS as an internal standard (Chemical shift in δ, ppm) and mass spectra were recorded on a QUATTRO Micromass Walter instrument (UK Ltd.) by electron impact Technique.

General method for the Synthesis of compounds 2a- 2f

Compounds **2a -2f** were synthesized by taking 0.01 moles of unsubstituted Piperazine in 60ml of Ethyl alcohol with substituted primary

amines in a beaker under perfect Ice-cold conditions to this solution add 0.02 moles of formaldehyde with constant stirring for about half an hour. The reaction mixture was transferred to flask and refluxed for 4-6 hrs. The refluxed mixture was poured in crushed Ice. The resulting solid was collected by filtration, washed with water and recrystallized from ethanol to obtain target compound (Sheela Joshi et al.,).

(4 - (aminomethyl) piperazine – 1 - yl) methanamine (2a).

IR (KBr, υ, cm-1): 3372(NH), 2968 (-CH2-), 2914 (C-H), 1472 (-CH2-), 1219 (C-N); Anal. Calcd
(%) for C6H16N4: C, 49.97; H, 11.18; N, 38.85. Found: C, 49.96; H, 11.16; N, 38.84. 1H NMR
(DMSO, δ ppm): 2.45 (s, CH2; 8H), 3.42 (s, CH2; 4H), 3.96 (s, NH; 4H); MASS: m/e M+ 144.2

N-((4-(phenylamino) methyl) piperazine-1-yl) methyl) benzenamine (2b).

IR (KBr, υ, cm-1): 3337 (NH), 3031 (C-H), 2975 (-CH2-), 1624 (C=C), 1487 (-CH2-), 1237 (CN);
Anal. Calcd (%) for C18H24N4: C, 72.94; H, 8.16; N, 18.90. Found: 72.93; H, 8.14; N, 18.89. 1H NMR (CDCl3, δ ppm): 2.42 (s, -CH2; 8H), 4.10 (s, CH2; 4H), 3.99 (s, NH; 2H), 6.32-7.05 (m,
ArH; 10 H); MASS: m/e M^+ 296.4

N-((4-((4-chlorophenylamino) methyl) piperazine-1-yl) methyl) – 4 - chlorobenzenamine (2c).

IR (KBr, υ, cm-1): 3387 (NH), 2953 (C-H), 2981 (-CH2-), 1597 (C=C), 1458 (-CH2-), 1324 (CN),
787 (C-Cl);Anal. Calcd (%) for C18H22Cl2N4: C, 59.18; H, 6.07; N, 15. 34. Found: C, 59.16; H, 6.05; N, 15. 33. 1H NMR (MeOD, δ ppm): 2.43 (s, -CH2-; 8H), 3.84 (s, -CH2-; 4H), 4.42 (s, -NH; 2H), 6.51-7.01 (s, ArH; 8H); MASS: m/e M+ 365.3.

N-((4-((2-hydroxy phenylamino) methyl) piperazine-1-yl) methyl) – 2 - hydroxy benzenamine (2d).

IR (KBr, υ, cm-1): 3356 (NH), 3509 (OH), 2976 (-CH2-), 2974 (C-H), 1604 (C=C), 1467 (-CH2-),
1224 (C-N); Anal. Calcd (%) for C18H24N4O2: C, 65.83; H, 7.37; N, 17.06. Found: C, 65.81; H, 7.35; N, 17.05. 1H NMR (MeOD, δ ppm): 2.49 (s, -CH2-; 8H), 4.1 (s, -CH2-; 4H), 4.27 (s, -NH; 2H), 5.7 (s, OH; 2H), 6.75-7.1 (s, ArH; 8H); MASS: m/e M+ 328.2

N-((4-((pyridinylamino) methyl) piperazine-1-yl) methyl) -N-pyridinylamine (2e).

IR (KBr, υ, cm-1): 3345 (NH), 2989 (C-H), 2918 (-CH2-), 1600 (C=C), 1485 (-CH2-), 1219 (CN); Anal. Calcd (%) for C16H22N6: C, 64.40; H, 7.43; N, 28.17. Found: C, 64. 39; H, 7.41; N, 28.15.1H NMR (DMSO, δ ppm): 2.44 (s, -CH2-; 8H), 4.07 (s, -CH2-; 4H), 4.16 (s, -NH; 2H), 6.75-7.90 (s, ArH; 8H); MASS: m/e M+ 298.3

N-((4-((diphenylamino) methyl) piperazine-1-yl) methyl) – N - phenylbenzenamine (2f).

IR (KBr, υ, cm-1): 3032 (ArH), 2921 (-CH2-), 1593 (C=C), 1487 (-CH2-), 1274(C-N). Anal.
Calcd (%) for C30H32N46: C, 80.32; H, 7.19; N, 12.49. Found: C, 80.30; H, 7.18; N, 12.47. 1H NMR (MeOD, δ ppm): 2.53 (s, -CH2-; 8H), 4.72 (s, -CH2-; 4H), 6.5-7.5 (m, ArH; 20H); MASS: m/e M+ 448.1

ANTITUBERCULAR ACTIVITY

The Antitubercular activity was done at Maratha Mandals Research Institute, Belgum, Karnataka (India). The synthesized eight derivatives were assessed against Mycobacterium tuberculosis by using microplate Alamar blue assay (MABA) using Isoniazid as a reference drug. The *In vitro* antitubercular screening was conducted at100 to 0.2 µg/ml against *M. tuberculosis* H37Rv in BACTEC 12B medium using a broth micro dilution assay [Gundurao et al.]. All the derivatives exhibiting fluorescence were tested in BATEC 460 radiometric system. Compounds demonstrating at least 90% inhibition were tested at lower concentrations by serial dilution against *M. tuberculosis*H37Rv to establish the minimum inhibitory concentration (MIC) using microplate Alamar blue assay (MABA). Compound **2e** was selected for further screening, where it showed promising inhibitory activity. The MIC is defined as the lowest drug concentration required to complete inhibition of bacterial growth [Maria et al.,]. The MICs of all the compounds were reported in **Table 1**.

3. RESULTS AND DISCUSSION

Bis-Mannich bases of Piperazine derivatives **were synthesized by refluxing pipperazine with** with formaldehyde and

amine in Ethanol provided the compounds 2a-2f in good yeilds(scheme 1). The detailed synthesis, physical data (table 1), spectral and analytical data are listed in the experimental protocol section.

The IR spectra of the compounds displayed a broad band at 3385-3387 cm^{-1} indicate –NH stretching and aromatic CH stretching at 3032-2935 cm^{-1}. A triplet at δ value 2.46 ppm representing 8 protons, and a singlet at 4.1 ppm indicates 4 methylenyl protons. The mass spectra of compounds showed the base peak at their corresponding m/z values. The elemental analysis results were within ± 0.3% of the theoretical values.

The synthesized six compounds were screened for their *In vitro* antituberculosis activity against *M. tuberculosis* strain H37Rv by using radiometric BACTEC 12B medium as broth micro dilution assay. The data of the antitubercular activity screening reveals that the compounds showed significant inhibitory activity except **2c** and **2d**. Compound **2e** bearing 2-pyrimidinyl amino methyl substitution on either sides displayed very good antimycobacterial activity, and more comparable to isoniazid (MIC, 0. 2µg/ml). Compounds bearing the substitutions aminomethyl and phenylaminomethyl substitutions on either sides of piperazine as in **2a** and **2b** showed second promising activity (MIC, 0. 2µg/ml). Moreover, the second phenyl ring substitution in compound **2f** on the nitrogen of either ends decreased the antimycobacterial activity (MIC 3.125µg/ml). Substitution of chloro and hydroxyl substitution on phenyl of the derivative, as in compounds **2c** and **2d** showed sudden decline in antimycobacterial activity (MIC 6.25µg/ml).

4. CONCLUSION

The results obtained revealed that compound 2e showed maximum inhibitory activity, may be due to the 2-pyrimidinyl amino methyl substitution. Other compounds bearing phenyl substitution also supported the activity but any electron withdrawing substitutions on phenyl ring not supported the activity.

Table 1: The *In vitro* antitubercular activity of compounds against *M. tuberculosis* $H_{37}Rv$

Compound	R	R'	MIC (µ/ml)
2a	Aminomethyl	Aminomethyl	**0.2**
2b	N-methyl benzenamine	N-methyl benzenamine	**0.2**
2c	4-chloro-N-methyl benzenamine	4-chloro-N-methyl benzenamine	6.25
2d	2-hydroxy-N-methyl benzenamine	2-hydroxy-N-methyl benzenamine	6.25
2e	N-methylpyridin-2-amine	N-methylpyridin-2-amine	**<0.2**
2f	N-methyl-N-phenyl benzenamine	N-methyl-N-phenyl benzenamine	3.125
INH			0.2

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