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Antitubercular Evaluations of Pyrazolyl Bis Indolyl Methane Synthesized by Using Novel Clay Catalyst

Ismail Shaikh¹, Muktar Shaikh¹, Syed Abed³, Sheshrao Pawar*¹

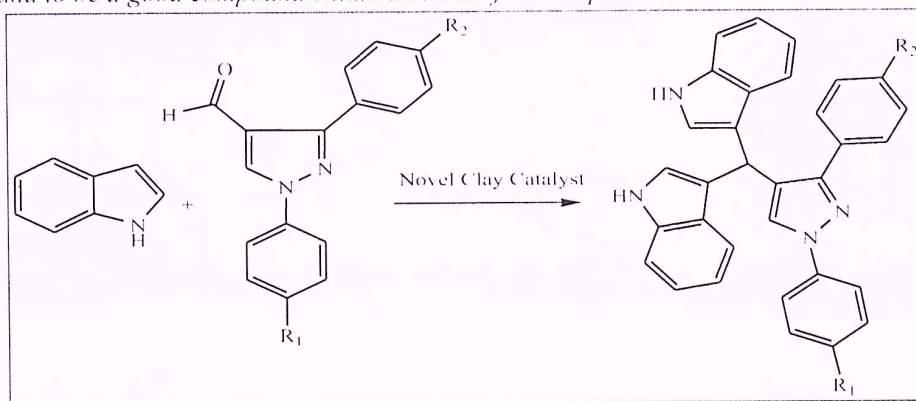
¹P.G. Department of Chemistry, ShriAnand College, Pathardi, Dist. Ahmednagar (M.S.) 414102.

²Dr. Rafiq Zakaria College for Womens, Navkhanada Aurangabad, Dist. Aurangabad, (M.S.).

³Government College of Arts and Science, Aurangabad, Dist. Aurangabad (M.S.).

Abstract

A series of eleven Pyrazolyl Bis indolyl methane derivatives were prepared using novel clay catalyst, characterized by 1H NMR, mass spectra (ES-MS). The synthesized compounds were then evaluated for their growth inhibitory activity against *M. smegmatis* mc2 155 initially. Rifampicin was used as standard reference. In this screening, derivative 5 and 3 presented superior inhibition compared to others within series (MIC = 54 and 59 µg/mL). Compound No 5 was found to be a good compound within a series of 11 compounds.



Introduction

In clinics, the universal era of antitubercular drugs remains insufficient to tackle the rising populations of patients with multi-drug resistant (MDR) and extensively drug-resistant (XDR) *Mycobacterium tuberculosis*. World Health Organization (WHO) reports tuberculosis (TB) as a second leading reason for mortality from an infective disease. Current documents record about 9 million new patients and 1.5 million deaths owing to this infection, including 360,000 deaths among HIV-positive people [1]. It is also mentioned that one-third of the world's people is infected with latent TB and 10% of which is expected to develop active TB at some point in their lives [1]. The strategy of vaccination and current antibiotic therapy to treat the disease is not effective in current scenario due to multi-drug resistance strains of *M. tuberculosis* [2]. In the early 1950s, there was a gradual decline in the number of cases of TB, but there has been resurgence since from 1984 [3]. This growth of TB: all the way through the recent years was mainly due to HIV-1 infection, immigration, increased trade, and globalization [4]. Due to emergence of MDR and XDR TB in 1993 WHO declared TB a global emergency [5]. Market is lack of new anti-TB agent since from last forty years except Bedaquiline, which was approved in year 2012 by USFDA [6]. Hence advancement in anti-TB drugs is a clinical need to reduce toxicity as well as treatment time. Several nitrogen containing heterocycles play important roles

in medicinal chemistry. Indole and its derivatives have wide range of applications in biological and medicinal activities [7]. Bisindole derivatives bears anticancer, anti-bacterial, antitumor properties [8]. Moreover, bis-indoles are members of promising new drug class these are diarylamidine derivatives that target DNA synthesis, providing a broad-spectrum antibacterial activity [9]. 1,4 azaindole derivatives was reported as potent against MDR-TB with MIC value ranging from 0.78-1.56 μM , along with low cytotoxicity against human leukemia monocytic [10]. In a similar structural analogy some indole derivatives were found to be potent against INH resistant strains (MIC 14.3 μM) [11]. N-phenyl pyrazole nucleus is well known moiety in drug discovery research. Derivatives of N-phenyl pyrazole were documented for their various biological potentials [12-14]. In our design, we utilised drug designing approach and accordingly, N-phenyl pyrazole and bisindole moiety were clubbed together within a molecular frame using a linker. Also, additional benzene ring was inserted at C-3 position of pyrazole ring for lipophilicity and possible interactions with receptor. Resulting hybrid molecules (1-11) were exposed for their growth inhibitory potentials against *M. smegmatis mc*²155.

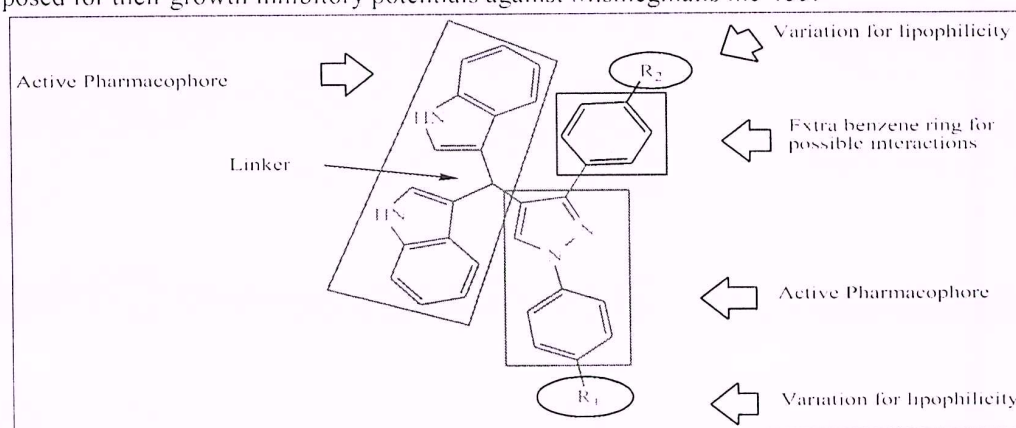


Figure 1: Ligand based designing for compound No1-1

Materials and methods:

Biology

Assay for growth Inhibition against *M. smegmatis mc*²155.

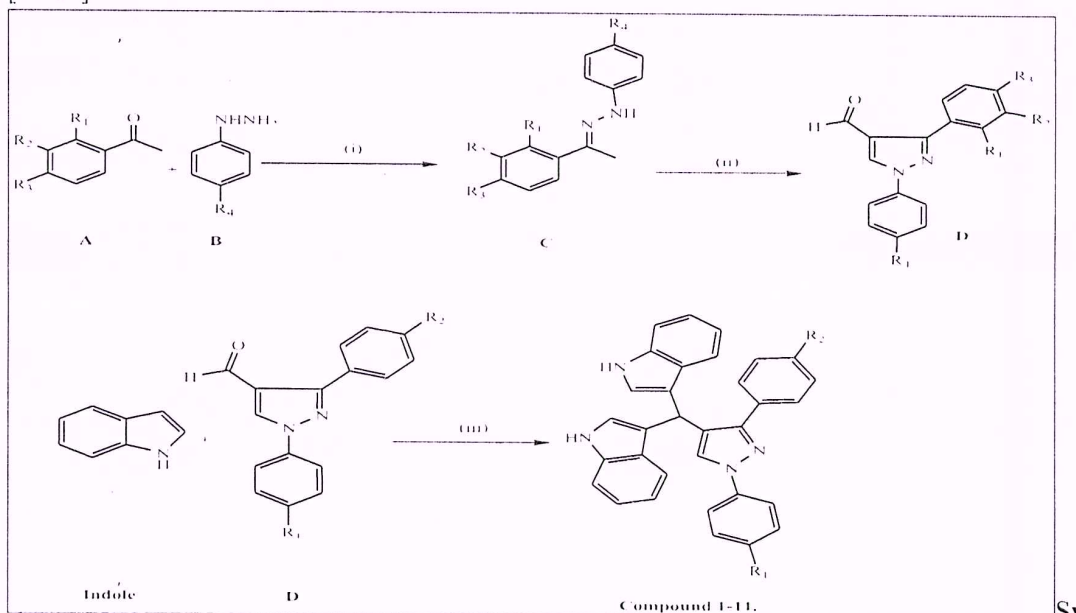
Evaluation of anti-tubercular activity against *M. smegmatis mc*²155 was carried out for synthesized compounds (1-11) by using Rifampicin as standard drug. Compounds were dissolved in the solvent at the concentration of 1.0 mg/ml and were further serially two fold diluted. From each dilution, a 100 μL was incorporated into 2ml of nutrient agar medium. Same procedure was followed for Rifampicin. After adding compounds, the medium was allowed to solidify in the tubes to make slant. Culture of *M. smegmatis mc*²155 were grown nutrient agar was harvested in 0.9% Saline to make single cell suspension of 2×10^5 cfu/mL. To each slant 10 μL of suspension was spread on the surface of the medium and the tubes were kept at 37 $^{\circ}\text{C}$ for 24 hours for the appearance of colonies. Tubes containing no drug served as control. The minimum concentration of the drug Rifampicin and test compounds were recorded, which completely inhibited the growth of *M. smegmatis* and was termed as Minimum Inhibitory Concentration (MIC) of bacteria (Table 2).

Chemistry

All the reagents and solvents were purchased from commercial sources and were dried and purified when necessary by standard methods. The melting points of the synthesized compound were determined in open capillary tubes using VMP-D melting point apparatus (Veeco Instrument Corporation, Mumbai, India) and are uncorrected. The ^1H NMR, X-Ray powder diffraction, Energy-dispersive X-Ray Spectroscopy (EDS) and Field Emission Scanning Electron Microscope (FESEM) by using instrument Nova Nano SEM 450 UOP for the compound synthesized were recorded from central instrumentation facility, Savitribai Phule Pune University, Pune. The follow up of reactions was monitored by thin layer chromatography (TLC) on silica gel-precoated aluminum sheets (Type 60, F254, Merck, Germany) and the spots were detected by exposor to UV lamp at λ 254 nm for 20-30 seconds.

Preparation of clay catalyst

Novel Clay was collected from the hilly areas of Bashir farm Jatadevala, Pathardi, Dist. Ahmednagar Maharashtra, India. It was treated and characterized as per work published earlier [15-16].



Synthetic Scheme 1. Reagents and conditions: (i) AcOH/EtOH, reflux, 30 minutes; (ii) DMF/ POCl_3 , 0°C - 30°C , 12-14 h; (iii) Ethanol, clay, RT-reflux, 24-48 hrs.

Table 1: Substitution of Compound No 1-11

Sr.No.	R ₁	R ₂	Sr.No.	R ₁	R ₂
1	H	H	7	OCH ₃	H
2	Cl	H	8	Br	H
3	F	H	9	H	F
4	CH ₃	H	10	H	CF ₃
5	CF ₃	H	11	H	CH ₃
6	OCF ₃	H	-	-	-



General Procedure for synthesis of pyrazole aldehyde D

A mixture of different acetophenone **A** (1.0 mole) and phenyl hydrazine **B** (1.0 mole) were heated in ethanol (10 volumes) in presence of (1 ml) acetic acid at reflux temperature for 30 minutes. Resulting yellow solid product was separated by the filtration. This solid was washed by cold ethanol (minimum) and was suck dried under vacuum. A mixture of N,N-dimethylformamide (2.5 mole) and POCl_3 (2.5 mole) was stirred together at 0°C for 30 minutes. To this reaction mixture the above solid was added at 0°C with constant stirring. The reaction mixture was then allowed to warm up to room temperature and stirred for 12-14 hrs. After completion of reaction, reaction mixture was poured on crushed ice where upon the solid was separated. It was filtered and washed by saturated aq. NaHCO_3 solution followed by water. Solid was crystallized from ethanol to get white crystalline product **D** in an average 70% yield.

General procedure for the synthesis of compounds 1-11.

The mixture of various pyrazole aldehyde (1 Mole), indole (2 Mole) and 0.10 mg of catalyst was taken in mortar and it was pestlegrindwell for specific period in minimum amount of ethanol. Further, the reaction was heated at reflux for 4-5 hrs. The conversion was monitored by TLC. Upon completion, clay catalyst was separated by filtration and reused again for the reaction. Ethanol layer (filtrate) was allowed to cool at RT. Solid precipitated was filtered.

Solid cake was taken in ethanol and it was heated to become a clear solution. It was then cooled to RT and solid precipitated was filtered to get pure desired compound 1-11.

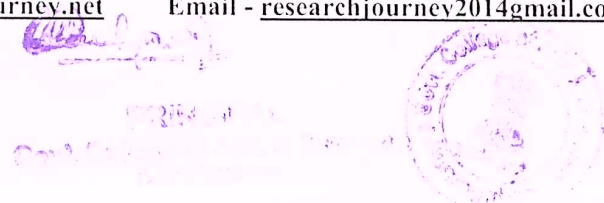
3-(1H-indol-3-yl)(1,3-diphenyl-1H-pyrazol-4-yl)methyl-1H-indole (1): Yield-68%, M.P. $237-239^\circ\text{C}$, $^1\text{H NMR}$ (DMSO-d_6), δ ppm::6.98 (s, 2H, pyrrolH); 7.12 (m, 3H, ArH); 7.38-7.51 (m, 7H, ArH); 7.55-7.68 (m, 6H, ArH); 7.98-8.00 (m, 6H, ArH); 8.78 (s, 1H, PyrazoleH); ES-MS, (m/z) 465.4 (M+1); Anal.Cald.for $\text{C}_{32}\text{H}_{24}\text{N}_4$ (464.4): C, 82.73; H, 5.21; N, 12.06; Found C, 81.52; H, 5.35; N, 12.78.

3-(1-(4-chlorophenyl)-3-phenyl-1H-pyrazol-4-yl)(1H-indol-3-yl)methyl-1H-indole(2): Yield-61%, M.P. $231-233^\circ\text{C}$, $^1\text{H NMR}$ (DMSO-d_6), δ ppm::6.91 (s, 2H, pyrrolH); 7.16-7.18 (m, 3H, ArH); 7.45-7.57 (m, 7H, ArH); 7.59-7.64 (m, 4H, ArH); 7.98-8.00 (m, 6H, ArH); 8.98 (s, 1H, PyrazoleH); ES-MS, (m/z) 450.2 (M+1); Anal.Cald.for $\text{C}_{32}\text{H}_{23}\text{ClN}_4$ (499.2): C, 77.01; H, 4.61; N, 11.23; Found C, 78.02; H, 5.05; N, 12.12.

3-(1-(4-fluorophenyl)-3-phenyl-1H-pyrazol-4-yl)(1H-indol-3-yl)methyl-1H-indole(3): Yield-61%, M.P. $223-225^\circ\text{C}$, $^1\text{H NMR}$ (DMSO-d_6), δ ppm::6.97 (s, 2H, pyrrolH); 7.17-7.19 (m, 3H, ArH); 7.43-7.59 (m, 6H, ArH); 7.62-7.68 (m, 5H, ArH); 7.98-8.02 (m, 6H, ArH); 8.99 (s, 1H, PyrazoleH); ES-MS, (m/z) 483.6 (M+1); Anal.Cald.for $\text{C}_{32}\text{H}_{23}\text{FN}_4$ (482.5): C, 79.65; H, 4.80; N, 11.66; Found C, 78.92; H, 5.01; N, 12.03.

3-(1H-indol-3-yl)(3-phenyl-1-p-tolyl-1H-pyrazol-4-yl)methyl-1H-indole (4): Yield-56%, M.P. $221-222^\circ\text{C}$, $^1\text{H NMR}$ (DMSO-d_6), δ ppm::2.71 (s, 3H, ArCH_3); 6.99 (s, 2H, pyrrolH); 7.11-7.13 (m, 3H, ArH); 7.44-7.54 (m, 8H, ArH); 7.62-7.65 (m, 4H, ArH); 7.98-8.03 (m, 5H, ArH); 8.91 (s, 1H, PyrazoleH); ES-MS, (m/z) 479.4 (M+1); Anal.Cald.for $\text{C}_{33}\text{H}_{26}\text{N}_4$ (478.60): C, 82.82; H, 5.48; N, 11.76; Found C, 83.03; H, 5.41; N, 12.00.

3-(1-(4-(trifluoromethyl)phenyl)-3-phenyl-1H-pyrazol-4-yl)(1H-indol-3-yl)methyl-1H-indole (5): Yield-59%, M.P. $232-233^\circ\text{C}$, $^1\text{H NMR}$ (DMSO-d_6), δ ppm::6.90 (s, 2H, pyrrolH); 7.01-7.04 (m, 3H, ArH); 7.43-7.51 (m, 7H, ArH); 7.66-7.69 (m, 6H, ArH); 7.98-8.01 (m, 4H,





ArH); 8.99 (s, 1H, PyrazoleH); ES-MS, (m/z) 533.9 (M+1); Anal.Caled.for $C_{33}H_{23}F_3N_4$ (532.5): C, 74.40; H, 4.34; N, 10.52; Found C, 73.98; H, 5.01; N, 10.00.

3-(1H-indol-3-yl)(3-phenyl-1-(4-(trifluoromethoxy)phenyl)-1H-pyrazol-4-yl)methyl)-1H-indole (6): Yield-52%, M.P. 241-243⁰C, ¹H NMR (DMSO-d₆), δ ppm: 6.93(s, 2H, pyrrolH); 7.01-7.04 (m, 3H, ArH); 7.41-7.55(m, 8H, ArH); 7.66-7.69 (m, 5H, ArH); 7.98-8.04 (m, 5H, ArH); 8.99 (s, 1H, PyrazoleH); ES-MS, (m/z) 549.6(M+1); Anal.Caled.for $C_{33}H_{23}F_3N_4O$ (532.5): C, 72.24; H, 4.23; N, 10.22; Found C, 73.08; H, 5.04; N, 10.9

3-(1H-indol-3-yl)(1-(4-methoxyphenyl)-3-phenyl-1H-pyrazol-4-yl)methyl)-1H-indole (7): Yield-54%, M.P. 214-216⁰C, ¹H NMR (DMSO-d₆), δ ppm: 3.79 (s, 3H, ArOCH₃) 6.91(s, 2H, pyrrolH); 7.03-7.07 (m, 5H, ArH); 7.43-7.59(m, 6H, ArH); 7.66-7.74 (m, 6H, ArH); 7.98-8.04 (m, 6H, ArH); 8.91 (s, 1H, PyrazoleH); ES-MS, (m/z) 495.3 (M+1); Anal.Caled.for $C_{33}H_{26}F_3N_4O$ (494.60): C, 80.24; H, 5.23; N, 11.32; Found C, 81.88; H, 5.72; N, 10.88.

3-(1-(4-bromophenyl)-3-phenyl-1H-pyrazol-4-yl)(1H-indol-3-yl)methyl)-1H-indole (8): Yield-54%, M.P. 214-216⁰C, ¹H NMR (DMSO-d₆), δ ppm: 3.79 (s, 3H, ArOCH₃) 6.91(s, 2H, pyrrolH); 7.02-7.04 (m, 3H, ArH); 7.41-7.51 (m, 7H, ArH); 7.61-7.67 (m, 5H, ArH); 7.98-8.02 (m, 5H, ArH); 8.91 (s, 1H, PyrazoleH); ES-MS, (m/z) 496.3 (M+2); Anal.Caled.for $C_{33}H_{26}F_3N_4O$ (494.60): C, 80.14; H, 5.31; N, 11.32; Found C, 81.08; H, 5.67; N, 11.78.

3-(3-(4-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)(1H-indol-3-yl)methyl)-1H-indole (9): Yield-64%, M.P. 221-223⁰C, ¹H NMR (DMSO-d₆), δ ppm: 6.91 (s, 2H, pyrrolH); 7.17-7.19 (m, 3H, ArH); 7.43-7.51 (m, 5H, ArH); 7.62-7.65 (m, 6H, ArH); 7.98-8.00 (m, 7H, ArH); 8.95 (s, 1H, PyrazoleH); ES-MS, (m/z) 483.6 (M+1); Anal.Caled.for $C_{32}H_{23}FN_4$ (482.5): C, 79.65; H, 4.80; N, 11.66; Found C, 78.93; H, 5.12; N, 12.09.

3-(3-(4-(trifluoromethyl)phenyl)-1-phenyl-1H-pyrazol-4-yl)(1H-indol-3-yl)methyl)-1H-indole (10): Yield-51%, M.P. 230-232⁰C, ¹H NMR (DMSO-d₆), δ ppm: 6.92 (s, 2H, pyrrolH); 7.10-7.11 (m, 3H, ArH); 7.40-7.51 (m, 5H, ArH); 7.63-7.68 (m, 6H, ArH); 7.98-8.01 (m, 6H, ArH); 8.91 (s, 1H, PyrazoleH); ES-MS, (m/z) 533.9 (M+1); Anal.Caled.for $C_{33}H_{23}F_3N_4$ (532.5): C, 74.40; H, 4.34; N, 10.52; Found C, 73.91; H, 5.11; N, 10.19.

3-(1H-indol-3-yl)(1-phenyl-3-p-tolyl-1H-pyrazol-4-yl)methyl)-1H-indole (11): Yield-49%, M.P. 230-232⁰C, ¹H NMR (DMSO-d₆), δ ppm: 2.38 (s, 3H, Ar-CH₃); 6.90 (s, 2H, pyrrolH); 7.09-7.13 (m, 5H, ArH); 7.40-7.45 (m, 5H, ArH); 7.65-7.68 (m, 6H, ArH); 7.98-8.03 (m, 4H, ArH); 8.99 (s, 1H, PyrazoleH); ES-MS, (m/z) 479.9 (M+1); Anal.Caled.for $C_{33}H_{26}N_4$ (478.60): C, 82.80; H, 5.48; N, 11.78; Found C, 83.09; H, 5.41; N, 11.49.

Results and Discussion

Biology

M.smegmatis mc² 155 growth inhibitions

The synthesized molecules (1-11) were examined systematically to define the structure activity relationship influencing potency, using standard, non pathogenic M.smegmatis mc² 155 strain. Strategic modifications were followed wherein R₁ and R₂ were substituted by different groups (Table 2). Rifampicin was used as standard reference in this study.

As per strategy, initially, in compound 1 was synthesized wherein, R₁ and R₂ were kept as H. In the assay, compound 1 demonstrated moderate potency (144 µg/mL) with Rifampicin (29 µg/mL), this initial result gave motivation to us for the further synthesis. In the next level, chloro group was introduced at R₁. In growth inhibitory assay, compound No 2 displayed one



more fold drop in potency compare to compound 1. Hence, in the next compound, electronegative, small F group was inserted at R₁ to increase lipophilicity. Resulting compound No 3 demonstrated very good improvement (59 µg/mL). After this improvement, CH₃ group was inserted at R₁, in the assay, compound No 4 displayed drop in activity when compared with previous compound (84µg/mL). So, considering lipophilic concept, we inserted CF₃ group at R₁ and compound No 5 was synthesized. In test assay, compound NO 5 showed good result when compared with previously synthesized compounds (54 µg/mL). In the next level, compound No 6, 7 and 8 were synthesized wherein, OCF₃, OCH₃ and Br groups were inserted respectively at R₁. These three compounds potency were ranging in 189-432 µg/mL. Further, to look in for the better potency, F, CF₃ and CH₃ groups were shifted at R₂ position (compound 9, 10 and 11), unfortunately, no improvements were noted in compound No 9, 10 and 11. In all, compound No 3 and compound No 5 were better compounds which displayed around two fold less activities when compared with standard Rifampicin (29 µg/mL).

It was in our strategy to explore the lead compounds for the next level (screening of compounds for growth inhibition study against M. tuberculosis H37Rv. Unfortunately, this screening assay was not performed since no one of synthesized compounds displayed better potential than standard Rifampicin.

Table 2. Anti-tubercular activity of 1-11 against M. smegmatis mc² 155

ID	M. smegmatis mc ² 155	
	MIC (µg/mL)	REF ± SD
1	144	1.3±0.09*
2	169	0.13±0.01
3	59	0.68±0.03
4	84	0.78±0.01
5	54	0.42±0.01
6	301	0.86±0.09
7	432	0.79±0.081
8	189	0.85±0.05
9	144	2.19±0.02
10	129	8.3±0.08*
11	169	1.9±0.03*
Rifampicin	29	1.67±0.09*

Conclusion

Present study reported ligand based design and synthesis of novel bisindole derivatives using novel clay catalystand their growth inhibition against M.smegmatis mc²155. Structures of the synthesized compounds were confirmed by their ¹H NMR, mass and elemental analysis. All the synthesized derivatives were successively assayed for their M.smegmatis mc² 155 growth inhibitory potential. Compound 5 and 3 showed moderate inhibitory potentials (54 and 59 µg/mL). None of the synthesized derivative was taken ahead for the next level study since all these derivatives were inferior to standard Rifampicin.

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PRINCIPAL
Govt. College of Arts & Sciences
Aurangabad

