



Design, synthesis, characterization and biological evaluation of indole derivative as antitubercular agents

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Abstract

Tuberculosis is a chronic granulomatous disease which is a major health problem in developing countries. The Anti-tubercular drugs for the treatment of Tuberculosis are complicated by the existence of Multi Drug Resistance (MDR) tuberculosis, Extensively Drug Resistance (XDR) tuberculosis and Totally Drug Resistant (TDR) tuberculosis. In this research work, indole derivative. Anti-tubercular activity was evaluated against *Mycobacterium tuberculosis* Compounds, synthesized in the present investigation were screened for anti-tubercular activity against *M. tuberculosis* H37Ra strain (*Mycobacterium tuberculosis* H37Ra is an attenuated tubercle bacillus closely related to the virulent type strain MTB H37Rv) in the Middlebrook 7H9 (MB 7H9 broth) by using Isoniazid as standard drug at a concentration of 25 µg/ml. In the present study, the synthesized compounds (a,) show promising activity between 6.25 µg/ml to 1.6 µg/ml against *Mycobacterium tuberculosis* were performed.

Keywords: indoles, organic products, bioactive compounds, heterocyclic synthesis, annulations, nitrogen-containing functional groups, named reactions, aldehyde

Introduction

Tuberculosis (TB) is the second leading cause of human death from infectious diseases worldwide. Current estimates suggest that one third of the world's population are infected resulting in some 2 million deaths per year. The Nineteenth World Health Organization (WHO) Tuberculosis Report indicates that TB is one of the world's deadliest communicable diseases. According to Global Tuberculosis Report 2017, by the World Health Organization, TB the chronic granulomatous disease is the leading cause of death worldwide in developing countries. [1] The Anti-tubercular drugs for the treatment of Tuberculosis are rendered ineffective by the development of Multi Drug Resistance (MDR) tuberculosis, Extensively Drug Resistance (XDR) tuberculosis and Totally Drug Resistant (TDR) tuberculosis. TB treatment is challenging and time-consuming. It requires appropriate treatment regimens for at least six months via directly observed therapy (DOT) and follow-up support. Treatment requires a minimum of six months in two separate phases. The duration of phase one is about two months and involves four drugs (Isoniazid, Rifampicin, Pyrazinamide And Ethambutol), followed by four months of phase two (using Isoniazid and Rifampicin). The present treatment has some limitations such as drug resistances, drug toxicity and intolerance, drug-drug interactions and poor patient adherence due to the long duration of the treatment. Therefore, there is an urgent need of new drug molecules with lesser side effects of treatment which are more effective. [2]

Materials and Methods

Materials

All the chemicals and solvents were purchased from Harish lab in Gandhi nagar Vellore, Industrial & Laboratory, chemicals Etc. The IR spectrum for the synthesized were study using ATR-FTIR spectroscopical (Bruker model) The samples were made in DMSO-d₆/ ¹H NMR and ¹³C NMR

spectra were recorded on CDCl₃/ D₂O solvents using tetramethylsilane as the internal standard and were expressed in δ scale. The standard abbreviations s, d, t, q, m, dd, ABq and brs refer to singlet, doublet, triplet, quarter, and multiplet, doublet of doublet, AB quartet, and broad singlet, respectively. Mass spectra were recorded on Agilent MSD-VL, AB SCIEX-API 2000 Mass spectrometer.

Methods

- To establish the method of synthesis for proposed compounds.
- To synthesis the indole compound by appropriate establish method.
- To carry out the preliminary screening such as physical constant determination, solubility test for the prepared compound.
- To confirm the structures of synthesized compounds by advanced spectroscopic techniques like ATR-FTIR, H NMR, CNMR, Mass spectroscopy.
- To confirm the target binding efficiency of synthesized compounds by docking Studies

Experimental procedure

Step 1

The respective Methyl- piperazine (10 mmol) was added to a stirred mixture of 2- phenylindole (10 mmol) and 35% aqueous formaldehyde solution (10 mmol) in anhydrous ETOH (12 ml) at 25°C. The reaction mixture changed its colour from dark-orange to bright- orange, and significant precipitation occurred, stirring was continued for the time indicated. After that, the precipitate was filtered off, washed with cold EtOH (10 ml), and dried

Step 2

1-((4-methylpiperidin-1yl) methyl)-2-phenyl-1 H-indole (2mmol) ethanol (15ml) was treated with LiOH.H₂O

(2mmol) followed by the appropriate aromatic aldehyde (2mmol). The reaction mixture was refluxed on a water bath for 1h. After the completion of the reaction, a precipitate was formed and this served as an indicator for reaction monitoring

the reaction visually. The formed precipitate was filtered off and recrystallised from ethanol/benzene to give the product

SCHEME

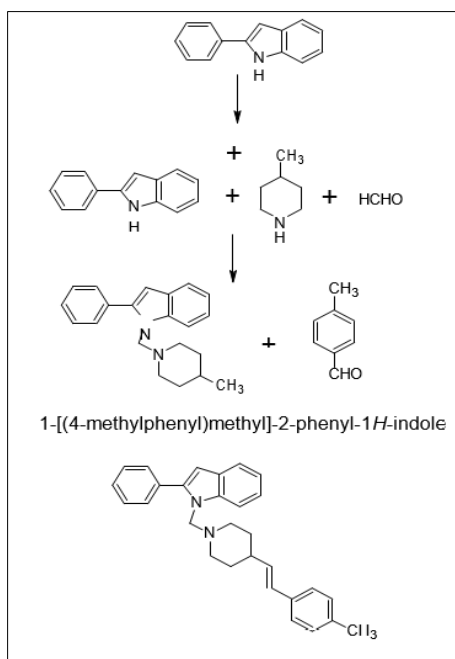


Fig 1

Results and Discussion

Spectral studies

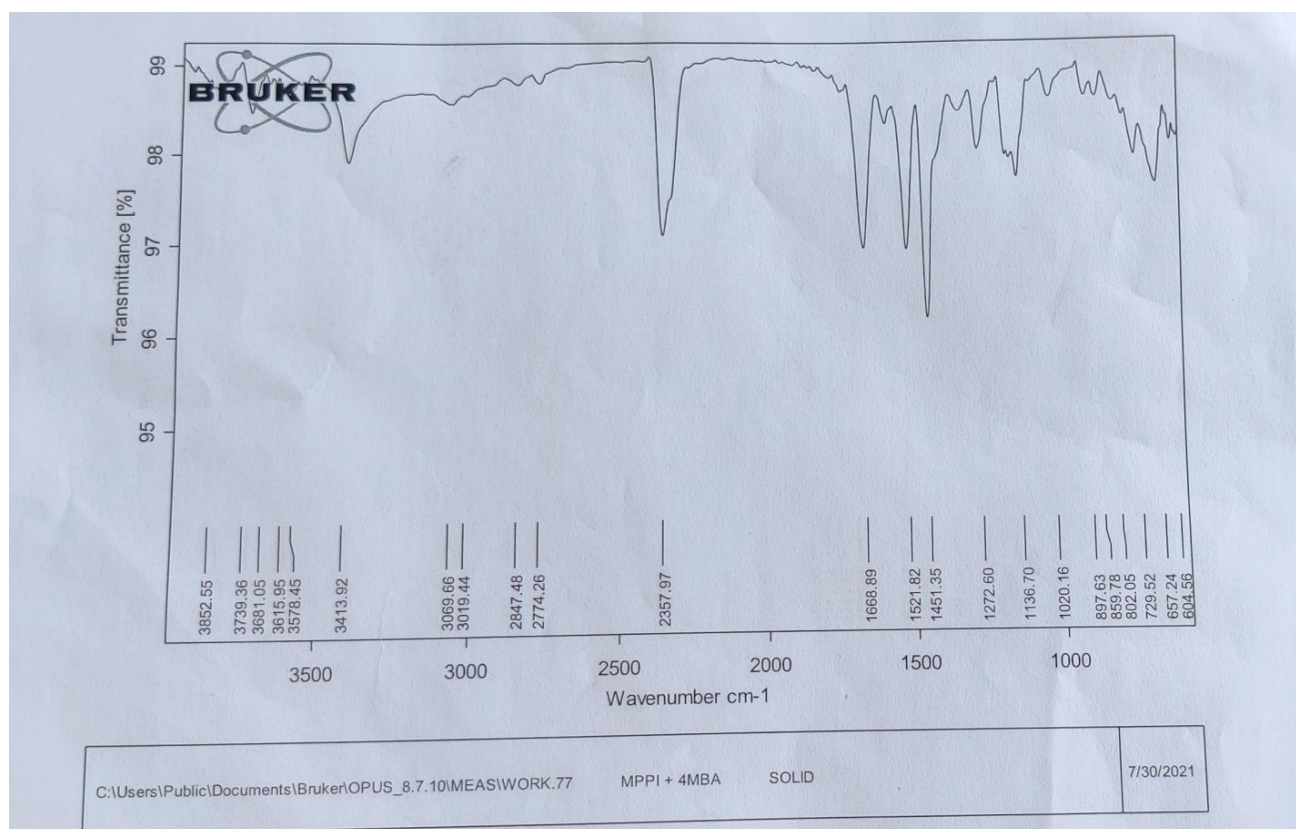


Fig 2: IR spectrum of synthesized Compound (E)-1-((4-(4-methylstyryl) piperidin-1-yl) methyl)-2-phenyl-1H-indole

Compound: IR: 3112.17 cm⁻¹(Ar-C-H str), 2885.30 cm⁻¹ (Al-CH₂-str), 1604.66 cm⁻¹ (C=N str), 1257.50 cm⁻¹ (C-O-C str), 694.32cm⁻¹ (C-S-C str)

1 HNMR Studies for the Synthesized Compounds

1HNMR spectral data of synthesized compounds are shown in Table 3 and the spectra are shown in figure 2

Table 2: m/z values of synthesized compounds by ESI Mass spectra

Compound Name	m/z values
(E)-1-((4-(4-methylstyryl)piperidin-1-yl)methyl)-2-phenyl-1H-indole	407.52 [M+H] ⁺

Table 3: The interaction energies (kcal mol⁻¹) of 1TPY and ligands obtained from the molecular docking with Auto Dock vina with PyRx

S. No	Compound Name	Binding Energy (kcal/mol)
01	(E)-1-((4-(4-methylstyryl)piperidin-1-yl)methyl)-2-phenyl-1H-indole	-6.6
02	Isoniazid (Standard)	-4.7

In Auto dock study of the compound was screened against specific target protein 1TPY was carried out. The compound was docked at the active site of the protein and by analyzing it we can calculate the compounds that have maximum binding energy compare with standard (Isoniazid). From

those results we found that the compound (-6.6) shows highest binding energies as compared to STANDARD Isoniazid (-4.7). The entire compound has good binding with protein.

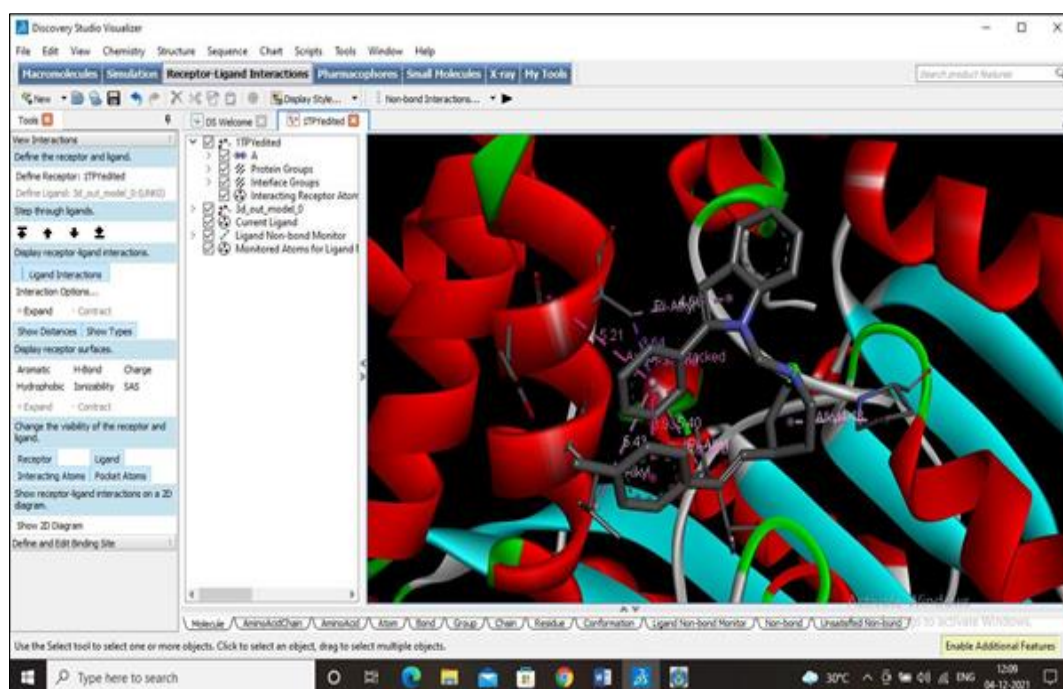
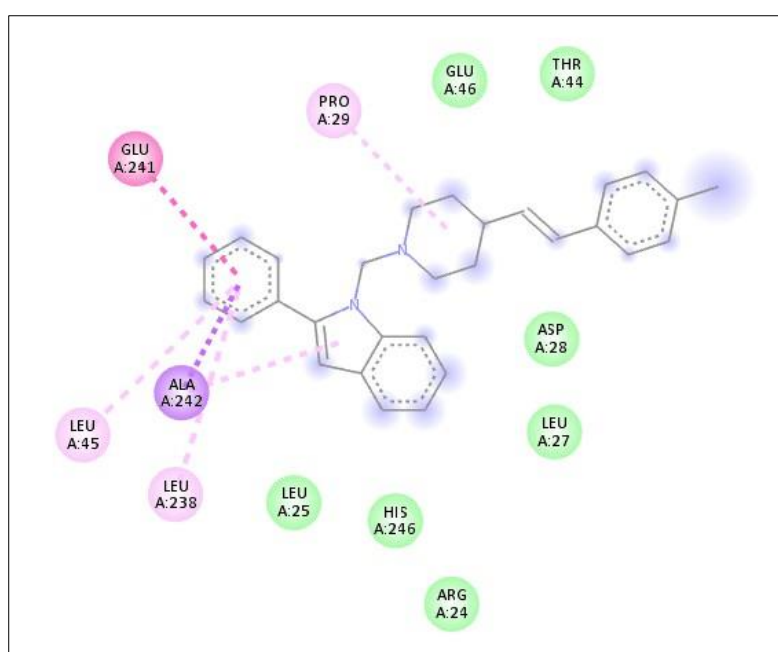
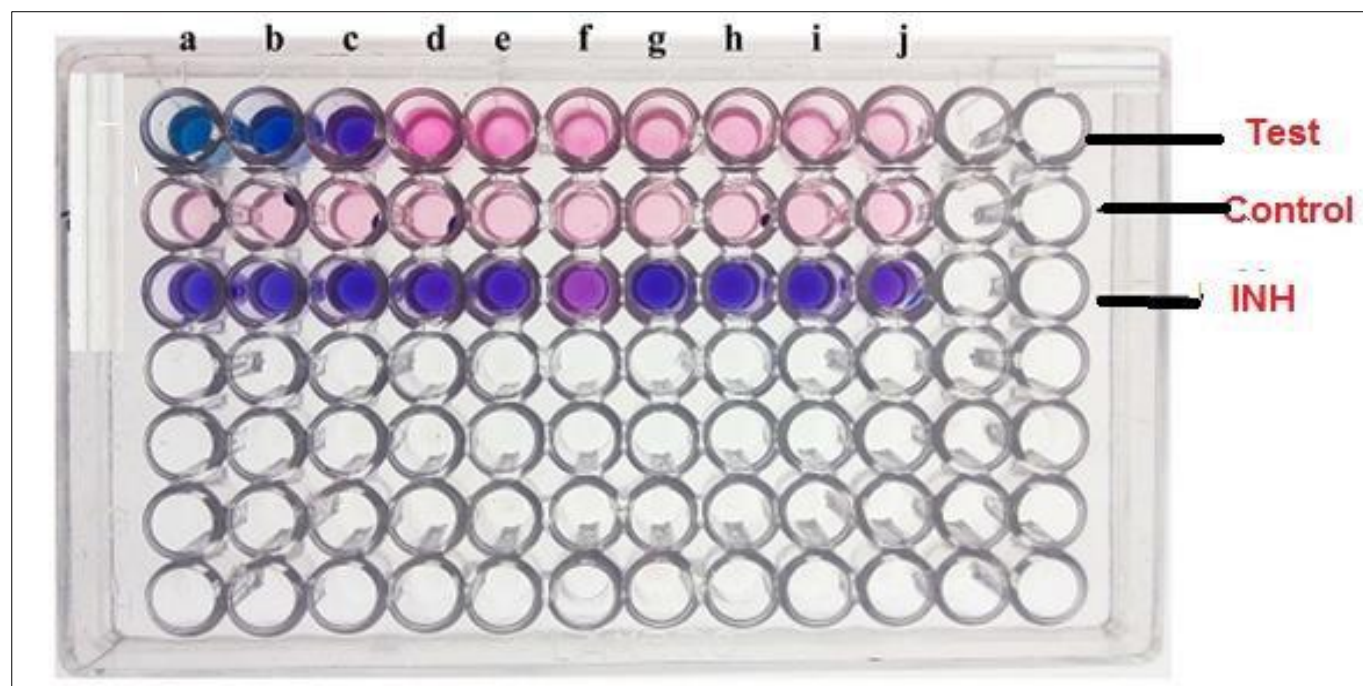
**Fig 5:** 3D Docking Poses of AUTODOCK VINA (Docking pose between lead compound and 1TPY)**Fig 6:** 2D Docking Poses of AUTODOCK VINA (Docking pose between lead compound and 1TPY)

Table 4: Study of physico-chemical properties of the synthesized compounds

S. No	Compound Name	Molecular formula	Molecular weight gm	Melting Point (°C)	Rf value	Percentage Yield (%)
1	(E)-1-((4-(4-methylstyryl) piperidin-1-yl)methyl)-2 phenyl-1H-indole	C ₂₈ H ₂₈ N ₂	406.24	270	0.5	68

Table 5: *In-Vitro* Anti Tubercular Activity by Alamar Blue Assay

Sl. No	Cpd. code	Concentration (µg/ml)									
		100	80	60	40	20	10	5	2.5	1.25	0.625
1	Test	90.2	78.0	72.5	64.0	55.2	41.9	35	25.5	14.8	07.8
2	Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	INH	97.9	88.0	83.9	80.3	73.0	62.1	54.4	46.1	36.7	30.5



a – 100µg/ml; b - 80µg/ml; c - 60µg/ml; d - 40µg/ml; e- 20µg/ml; f - 10µg/ml; g - 5µg/ml; h - 2.5µg/ml; i - 1.25µg/ml and j - 0.625µg/ml.

Fig 7: *In-vitro* anti-tubercular activity by Alamar Blue assay

Conclusion

The indole derivative was synthesized, characterized and screened for their anti-tubercular activity. The results of anti-tubercular activity revealed that the compound exhibited significant activity. This study revealed that the necessity of synthesizing many more compounds having these moieties. Such compounds may emerge as much more potent for tuberculosis. The derivative of indole ring structure act as potent anti-tubercular compound.

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