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Synthesis and Evaluation of Anti-tuberculosis Activity of Substituted 4-(1H-Indol-3-yl)-1,6-Diphenyl-5,6-Dihydropyrimidin-2 (1H) One Derivatives

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ABSTRACT

In the present study novel pyrimidine derivative of 4-(1H-indol-3-yl)-1, 6-diphenyl-5,6-dihydropyrimidin-2 (1H) one were synthesized and evaluated for their in vitro anti tuberculosis activity. A series of chalcones from indole-3-carbaldehyde and substituted aromatic ketone was synthesized and cyclo condensed with phenyl urea to give indoyl-pyrimidone derivatives. All synthesized compounds were characterized by IR spectroscopy and purification determined by melting point and TLC. Compounds were evaluated for their anti-tuberculosis activity against mycobacterium tuberculosis (H37RV Strain). Among the synthesized compounds IP-2 and IP-3 exhibited 6.25 µg/ml and found to be significant in activity when compared with standard drug (3.12 µg/ml).

Keywords: Tuberculosis, World Health Organization (WHO), Anti-tuberculosis

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INTRODUCTION

Tuberculosis, MTB, or TB is a deadly infectious disease caused by various strains of mycobacteria; usually Mycobacterium tuberculosis. 1 According to World Health Organization (WHO) TB is a global pandemic, which has become an important world-wide public health menace with one-third of the world's population infected by the TB bacillus.2 Most infections do not have symptoms, known as latent tuberculosis and about one in ten latent infections eventually progresses to active disease which, if left untreated, kills more than 50% of those so infected. People with weak immune systems (those with HIV/AIDS, those receiving immunosuppressive drugs and chemotherapy) are at a greater risk for developing TB disease. There is currently a growing concern about the progress and spread of multidrug and extensively drugresistant tuberculosis (MDR/XDR-TB), which has the potential to paralyze TB care schemes. The focal theme of this thesis is the exploration of new strategies in the field of modern drug discovery for the development of new drugs, capable of overcoming MDR/XDR-TB [1-5].

History of Tuberculosis

Tuberculosis, an air-borne disease that typically affects the lungs leading to severe coughing, fever and chest pains, but can also affect other parts of the body. It may have killed more persons than any other microbial pathogen. It has been assumed that the genus *Mycobacterium* originated more than 150 million years ago. his peculiar disease, whose Latin-originated name describes the rod shape of the *bacillus* (Figure 1) [6,7], became implicit when the German microbiologist Robert Koch announced that *Mycobacterium tuberculosis* caused TB in the year 1882 [8,9]. This finding, along with the later discoveries of tuberculin in the year 1890 and the BacillusCalmette Guerin (BCG) vaccine in 1908 and anti-tuberculosis drugs starting in 1943, offered hope for the eradication of a disease deadlier than the plaque [10-13].

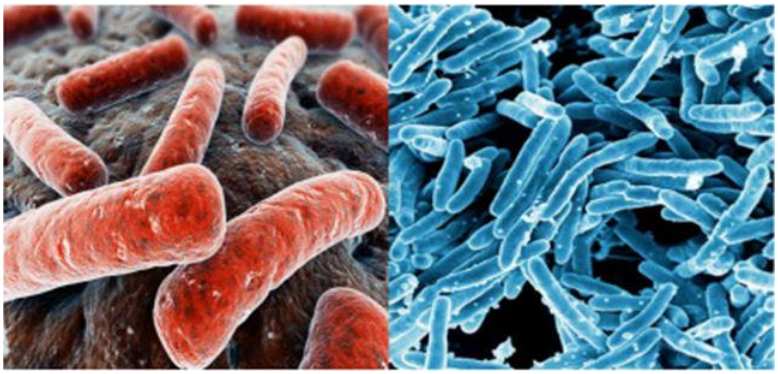


Figure 1: *Mycobacterium tuberculosis.*

Mortality rates significantly turned down from the early to mid-20th century; in spite of this, funding for research was attenuated and between 1970 to 1990, drug and vaccine developments were decelerated. With the advancement of the AIDS pandemic and the emergence of TB resistant strains, interest in TB research and prevention increased. Strategies to control and prevent the disease were developed. The Directly Observed Treatment Short-Course (DOTS) program was introduced in 1993, [14-16] with the addition of a DOTS-plus program to address multidrug resistant (MDR) TB in 1998.3,9 Though current research in recent years has given valuable insight into TB transmission, diagnosis, and treatment, much needs to be done to efficiently decrease the

incidence of and eventually eliminate TB.3,10 The disease still puts a strain on public health, being only second to HIV/AIDS with high mortality rates [17-19].

Treatment and vaccines: Anti-tuberculosis drugs, BCG vaccine, and drug resistance

The choice of TB treatment depends on whether the individual is in the latent or active stage and about the probability of risk. Treatment of TB typically necessitates a drug cocktail, or a combination of multiple drugs, with an intensive initial 2-month phase followed by a slower 4 to 6 months continuation phase [20].

The classical antitubercular agents are divided into two categories: first-line and second-line drugs. The first-line drugs include isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), ethambutol (EMB), and Streptomycin (SM). The second-line drugs including kanamycin, cycloserine, β-aminosalicylic acid, ethionamide, prothionamide, thiacetazone and fluoroquinolones are regarded as a kind of supplement to the first-line drugs. They are usually used in cases of retreatment, resistance, or intolerance to the first-line drugs. Second-line drugs are classified as cell wall, nucleic acid, energy, or protein synthesis inhibitors; however, no two second-line agents can be used together owing to their nephrotoxicity and ototoxicity [21].

Current front-line therapy consists of two months' treatment with four first-line agents including RIF, INH, and PZA, (with or without EMB), followed by four months' follow-up therapy with INH and RIF. MDR-TB infection requires treatment with second-line drugs such as amikacin, kanamycin, fluoroquinolone, βaminosalicylate, capreomycin, cycloserine, or ethionamide, and this treatment often lasts for up to two years [22].

In addition to the five main antituberculosis drugs, the Mycobacterium bovis BCG vaccine is the current vaccine used to mimic the natural immune response to infection. Although the BCG vaccine has been widely administered for more than eighty years and strongly induces TH 1 cells, its efficacy is highly variable, according to a recent review by Andersen. Drug resistance persists to pose a major health concern. Although drug susceptibility tests are always performed to monitor resistance, previous treatments, not completing treatment, not complying with treatment, and improper or inadequate regimens can confer drug resistance [23].

The literature survey reveals the significance of the research in the area of the development of new drugs for tuberculosis, capable of overcoming MDR and XDRTB. The survey also indicates the importance of the small organic molecules which possess the heterocyclic nucleus such as Pyrimidone and some fused ring systems like indole-pyrimidine as a core active moiety/pharmacophore. Considerable attention has been focused on these heterocyclic ring systems as they were reported to possess various biological activities such as tuberculostatic, anti-cancer, anti-tumour, hypoglycemic, anti-inflammatory, analgesic, anti-bacterial, anti-fungal, anti-coagulant and anti-oxidant activities.

In present study directly aims to design and synthesize some heterocyclic analogues such as pyrimidone and some fused ring systems like indole-pyrimidone which will prove to be effective against *Mycobacterium tuberculosis.*

MATERIALS AND METHODS

Chemicals used

Indole.3. aldehyde, acetophenone, p-chloro phenyl urea, p-amino phenyl urea, p-hydroxy phenyl urea,, p-methoxy phenyl urea,, p-nitro phenyl urea, Ethanol, NaOH, Conc. HCl [24].

Apparatus used

Beakers, Boiling tubes, Test tubes, Thermometer, Round bottom flask, Reflux condenser, Glass rod, Funnel.

Analytical work

Melting points were determined by using melting point apparatus MP-DS TID 2000 V. scientific and were uncorrected Reactions were monitored by TLC on pre-coated silica gel G plates using iodine vapors as visualizing agents.

IR spectra were recorded on JASCO FT/IR-140 spectrophotometer in the Department of Pharmaceutical Analysis, SRIPMS-Coimbatore, Tamilnadu, India.

Step 1: Synthesis of chalcones

To a solution of substituted aldehyde (0.01 mol) Ketones (0.01 mol) in ethanol (25 ml), a solution of NaOH (6 ml 40%) was added. Then the reaction mixture was stirred at a reaction time for a period of 24 hours and acidified with Conc. HCl. The product obtained was filtered, washed with water and recrystallized from ethanol.

Step 2: Synthesis of phenyl urea

Aniline (65 g) and urea (120 g) was dissolved in water (200 ml) followed with HCl (4 ml) in a reflux condenser. The content was boiled for 30 minutes. Fine white crystals appeared within 15 min of refluxing. At the end of reaction time the flask was cooled in ice and the solid was collected. The mixture of phenyl urea and diphenylurea has formed and separated. Phenyl urea dissolved in boiling water while diphenylurea remain undissolved. This was filtered, and the filtrate cooled thoroughly. The phenylurea crystallized out was collected by filtration and dried in steam oven [25].

- Yield: 10 g
- Molecular formula: $C_7H_8N_2O$
- Mobile phase for TLC: Ethyl acetate: n-hexane
- Melting point: 149 Celsius
- Rf value: 0.68

Step 3: Synthesis of pyrimidine

In RBF (0.01 mol) chalcone and add substituted phenyl urea was dissolved in 10 ml of ethanol and refluxed for about 6 h and poured in to the ice water then add catalytic amount of conc. HCl. The solid product obtained was filtered and washed with water, recrystallized from absolute alcohol (Figures 2 and 3) [26].

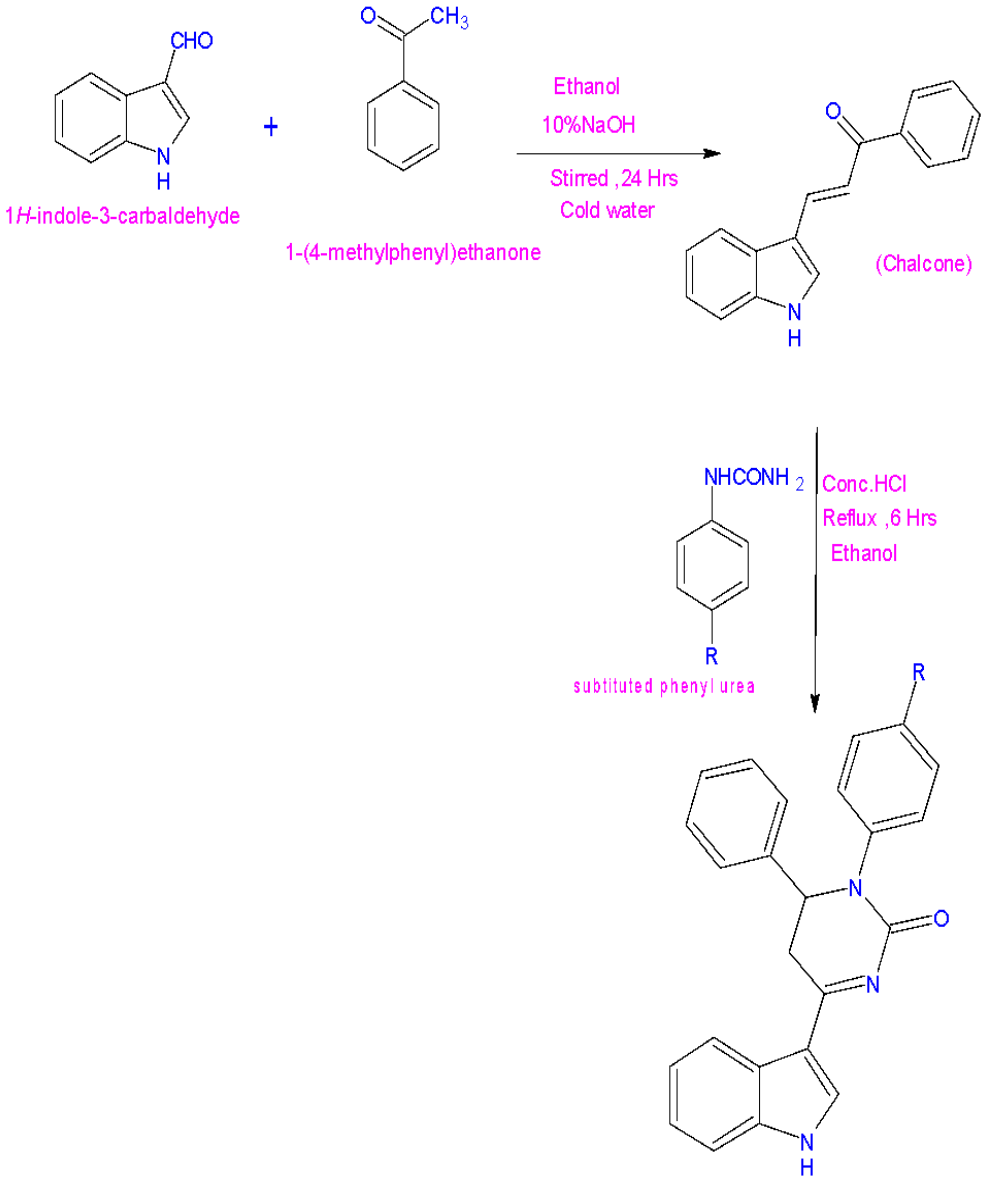


Figure 2: R= (-Cl, NO_2, F, Di-methyl) Physical characterization.

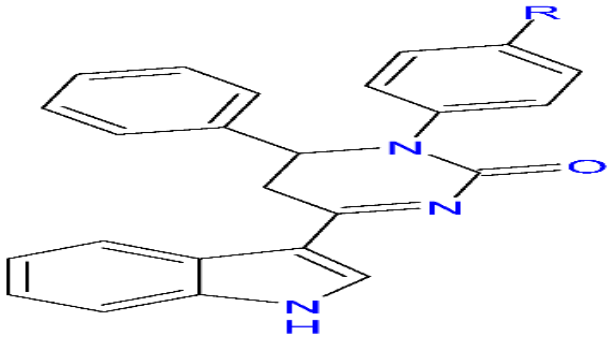


Figure 3: General structure.

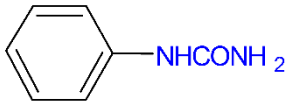
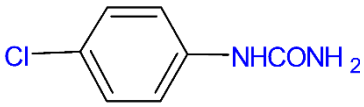
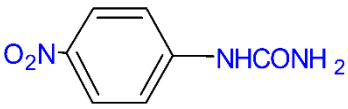
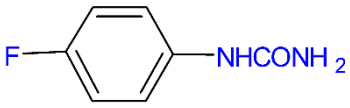
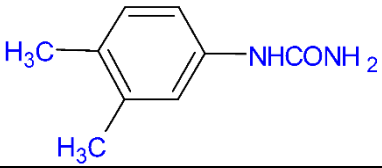
Compound	R	Molecular Formula	Molecular Weight	Melting Point (^{0}C)	Rf value
IP1		$C_{24}H_{18}N_3O$	364.41	162	0.58
IP2		$C_{24}H_{18}ClN_3O$	399.87	156	0.62
IP3		$C_{24}H_{18}N_4O_3$	410.42	168	0.74
IP4		$C_{24}H_{18}FN_3O$	383.41	172	0.64
IP5		$C_{26}H_{23}N_3O$	393.48	164	0.76

Table 1: Spectral studies of compounds.

Spectral studies of compounds

IR spectral data (KBr Pellet Method)

Spectral studies of compound IP-2: CH (str) Aromatic-3209 cm^{-1} C=C (str)-1635 cm^{-1}, C=N (str)-1435 cm^{-1}, C=CH (str) -2362.30 cm-N-N (Indole)-3501.52 cm^{-1} , C-H (-CH2-)-788.89 cm^{-1} C-C (str)-1635.64 cm^{-1} C=O (str)-1725.14 cm^{-1} Ar-Cl (C-Cl-Str)-813 cm^{-1}. (Table 1)

Spectral studies of compound IP-3: CH (str) Aromatic-3167 cm^{-1} C=C (str)-1635 cm^{-1}, C=N (str)-1446 cm^{-1}, C=CH (str) -2605.83 cm-N-N (Indole)-3500.74 cm^{-1}, C-H (-CH2-)-788.89 cm^{-1} C-C (str)-1394 cm^{-1} C=O (str)-1725.35 cm^{-1} N=O (Str)-1521 cm^{-1} C-N (Str)-1446.61 (Tables 2 and 3).

Anti-TB activity using Alomar blue dye

Ingredients	**g/litre**
Ammonium sulphate	0.50
Disodium phosphate	2.50
Monopotassium phosphate	1.00
Sodium citrate	0.10
Magnesium sulphate	0.05
Calcium chloride	0.00
Zinc sulphate	0.00
Copper sulphate	0.00
Ferric ammonium citrate	0.04
L-Glutamic acid	0.50
Pyridoxine	0.00
Biotin	0.0005
Final pH (at 25°C) 6.6+/-0.2	

Table 2: Middlebrook 7h9 broth base.

Bovine albumin fraction V	2.50 g
Dextrose	1.00 g
Catalase	0.002 g
Oleic acid	0.025 g
Sodium chloride	0.425 g
Distilled water	50.00 ml

Table 3: Middlebrook OADC growth supplement.

Anti-TB activity using Alomar blue dye

The anti-mycobacterial activity of compounds was assessed against M. tuberculosis using microplate Lamar Blue assay (MABA). This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200 µl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37°C for five days. After this time, 25 µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink (Table 4, Figures 4 and 5).

S. no	Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1	IP-1	S	S	S	R	R	R	R	R
2	IP-2	S	S	S	R	R	R	R	R
3	IP-3	S	S	S	R	R	R	R	R
4	IP-4	S	S	S	R	R	R	R	R
5	IP-5	S	S	S	R	R	R	R	R

Table 4: Anti-TB results.

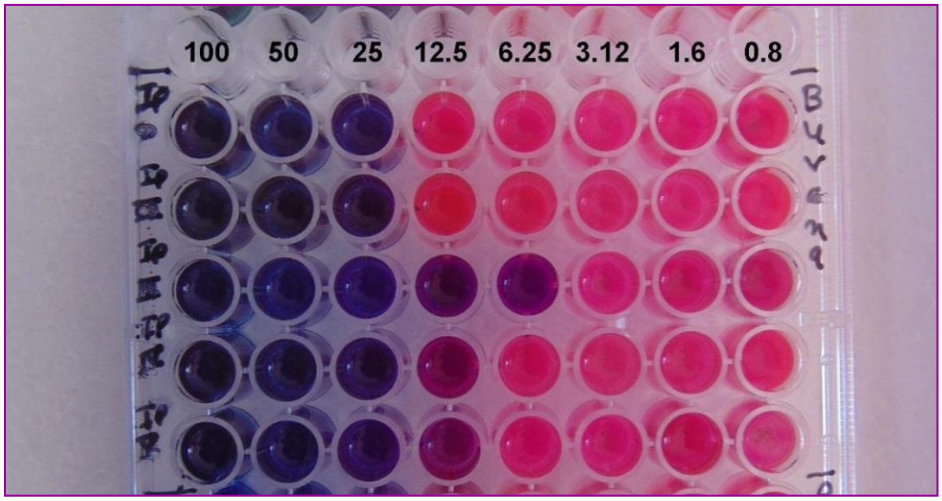


Figure 4: Experimental drug.

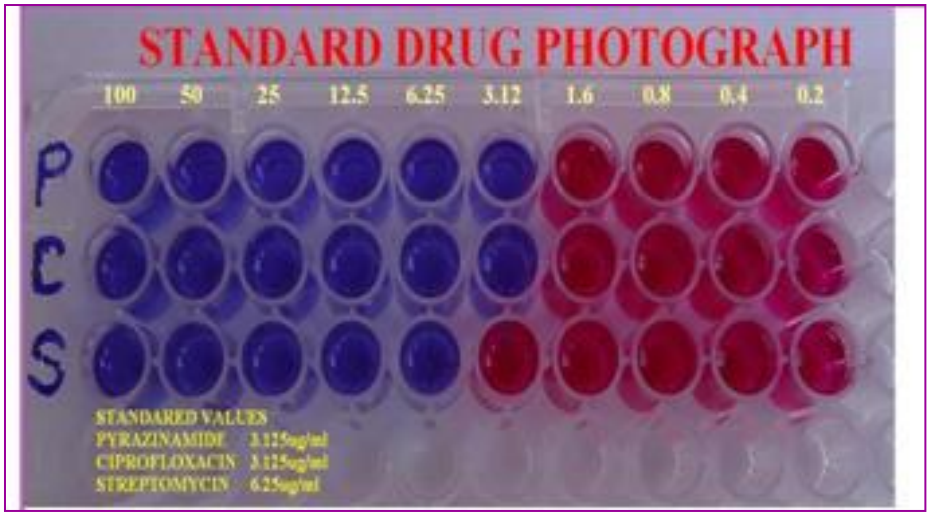


Figure 5: Standard drug.

CONCLUSIONS

- A series of chalcones from indole-3-carbaldehyde and substituted aromatic ketone was synthesized and cyclo condensed with phenyl urea to give indoyl-pyrimidone derivatives. Further these synthetic compounds were characterized by their physicochemical and spectral means as discussed in experimental.
- Compounds were evaluated for their antituberculosis activity against mycobacterium tuberculosis (H37RV Strain). Among the synthesised compounds IP-2 and IP-3 exhibited 6.25 µg/ml and found to be significant in activity when compared with standard drug (3.12 µg/ml).
- Newly synthesised compounds were evaluated for their Anti-TB activity was evaluated by using Alamar Blue Dye method. The synthesised compounds showed significant Anti-TB activity found comparable with standard drug pyrazinamide at the same dose.
- The anti-tubercular activity of the designed scaffold showed in the table compounds IP-2& IP-3 showed significant activity with MIC 6.25 µg/ml. The promising activity of the designed molecule is mainly attributed with the presence of electron withdrawing group such as nitro and chord in the phenyl system.
- Among the synthesized compounds IP-2 & IP-3 exhibited 6.25 µg/ml and found to be significant in activity when compared with standard drug (3.12 µg/ml).

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