ejpmr, 2016,3(7), 403-406.

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF ANTI-TUBERCULAR AGENTS TARGETING MTFAB D, MALONYL COA - ACYL CARRIER PROTEIN TRANSACYLASE

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Article Received on 11/05/2016 **Article Revised on 01/06/2016** **Article Accepted on 22/06/2016**

ABSTRACT

Mycobacterium tuberculosis MTB, or TB (*tubercle bacillus*), is a common, and in many cases lethal, infectious disease caused by various strains of mycobacteria, usually *Mycobacterium* tuberculosis. Over the past 200 years, tuberculosis has been responsible for the death of over 100 million people. Moreover due to the emergence and reemergence of multi-drug resistant tuberculosis (MDR-TB), extremely - drug resistant tuberculosis (XDR-TB), totally -drug resistant tuberculosis (TDR-TB) and also because of the co-infection of TB with HIV there is an urgent need for new Anti-TB agents. Today hetero cyclic compounds have attained wide attention in the discovery of new drug candidates because of their diverse biological activity. Heterocyclic ring system encompasses the core of the active moiety as pharmacophore. For a period of decade's heterocyclic therapeutic agents plays a pivotal role in chemotherapy. In view of the above facts, this research work deals with the designing of chalcones containing heterocyclic moiety and synthesizing it for potential anti-tubercular activity. So molecules were designed and docked against MTB enzyme mtFab D, malonyl CoA - acyl carrier protein transacylase The screened molecules were synthesized by condensation method, purified by chromatographic techniques, characterized by various spectral analytical techniques and evaluated for in-vitro anti mycobacterial activity against tuberculosis H37RV strain by Microplate Alamar Blue Assay (MABA) method. The experimental results show that Compound Y possesses better anti-tubercular activity with an MIC below 1.6 mcg/mL while NA, and PW showed moderate anti tubercular activity with an MIC below 6.25mcg/Ml, and Compound X exhibited good anti tubercular activity with an MIC below 12..5mcg/Ml.

KEYWORDS: Mycobacterium tuberculosis, Chalcones, Docking, MABA.

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by the bacillus, *Mycobacterium tuberculosis.* Tuberculosis generally affects the lungs (pulmonary TB) but can also affect other sites of the body as well (extra pulmonary TB). Patients with active pulmonary TB are the main source of infection and most of the people infected with *Mycobacterium tuberculosis* contain it as asymptomatic known as latent TB infection (LTBI).[1] TB is spread through the air when people who are affected with Pulmonary TB, expel bacteria, by sneezing or coughing. TB is common among men than women, and affects especially adults.[2] The characteristic symptoms of active tuberculosis infection are chronic cough with blood tinged sputum, night sweats, weight loss and fever.

Despite the availability of extremely efficacious treatments for decades and due to the emergence and reemergence of multi-drug resistant tuberculosis (MDR-TB), extremely - drug resistant tuberculosis (XDR-TB), totally -drug resistant tuberculosis (TDR-TB) and also because of the co-infection of TB with HIV there is an urgent need for new Anti-TB agents the World Health Organization (WHO) declared TB as a global public health emergency.[10] Hence, this research work deals with the designing of chalcones containing heterocyclic moiety and synthesizing it for potential anti-tubercular activity.

MATERIALS AND METHODS

Chemistry

The Compounds were synthesized conventionally by making use of a sequence of Organic Chemical Reactions. Completion of the reactions was monitored by Thin Layer Chromatography (TLC). The synthesized compounds were purified by re-crystallization/repeated recrystallization and by other chromatographic techniques by using suitable solvents. The purity was confirmed by sharp melting points using open capillary tubes. The synthesized compounds were characterized by

the various analytical spectroscopic methods, IR **S**pectroscopy, NMR Spectroscopy and MASS Spectrometry.

Drug Design

Drug discovery is the process by which drugs are discovered and/or designed. Current trends in research emphasize, designing organic synthesis of drugs involving green chemistry. Green chemistry would be useful to have an efficient atom economy and minimum hazardous waste production in the synthetic process.[4]

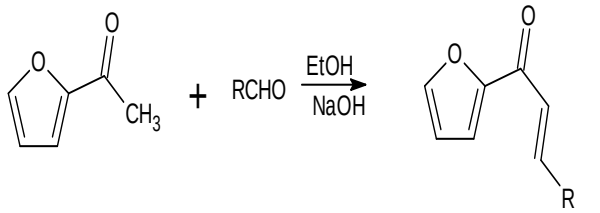
Rational Drug Design (RDD) is the inventive process of finding new molecules based on the knowledge of the biological target. Computer Aided Drug Design (CADD) computational tools and software`s are used to simulate drug receptor interactions. Docking procedure aims to identify the correct binding poses within the binding site of the protein while the scoring function aims to predict binding affinity of ligand for the protein binding region**.**

Evaluation of in-silico toxicity

Insilico approaches like OSIRIS® Property explorer predicts the carcinogenicity, mutagenicity, teratogenicity, immune toxicology etc. It allows us draw chemical structures and calculates various drug relevant properties whenever a structure is valid. Prediction results are valued and color coded. Properties with high risks of undesired effects like mutagenicity indicates red, whereas a green color indicates drug conform behavior. The toxicity predictions of the synthesized compounds are carried out.[5,11]

Synthetic methodology[12]

Synthetic Scheme: Synthesis of *(*substituted aryl aldehyde)-1-(furan-2-yl) prop-2-en-1-one chalcone derivatives.



The starting materials 2-acetyl furan converted in to a series of substituted chalcone were prepared by Claisen-schimidt condensation with substituted aldehydes.

Synthetic Procedure

A mixture of 2-Acetyl furan (0.02mole) and p-hydroxybenzaldehyde (0.02mole) was stirred in ethanol (50 ml) and then aqueous solution of NaOH (40%) (10mL) was added to it portion wise, keeping the temperature below 10°C throughout the addition. The mixture was kept for 36hr and it was acidified with conc. HCl. The reaction mixture was poured into crushed ice and the solid obtained was filtered under vacuum. The solid product was washed, filtered, dried and recrystallized from absolute ethanol.[6]

KETONE USED

1) 4-OH Acetpphenone
2) 2-Acetyl Furan

ALDEHYDE USED

1) Furfuraldehyde.
2) 2-Hydroxynaphthalene-1-Carbaldehyde.
3) 2-Bromo Benzaldehyde.
4) Thionaphthene 3-Carbaldehyde.

SI NO	SAMPLE CODE	KETONE USED	ALDEHYDE (R) USED
1	NA	4-OH ACETPPHENONE	FURFURALDEHYDE
2	PW	2-ACETYL FURAN	2-HYDROXYNAPHTHALENE-1-CARBALDEHYDE
3	X	2-ACETYL FURAN	2-BROMO BENZALDEHYDE
4	Y	2-ACETYL FURAN	THIONAPHTHENE 3-CARBALDEHYDE

Characterization studies IR

(KBr, λmax):3310 cm-1 (Ar-O-H Str), 3058 cm-1(-CH Str. of Ar), 1644 cm-1(C=0 str. of ketone), 1586 cm-1(C=C of chalcone), 1515 cm-1(C = C str. of Ar), 1443 & 1359 cm-1(CH3 def), 1153 & 1167 cm-1(C – O – C str), 830 cm- 1(- CH str.), 747 cm-1(Ar-H opb.);

1H NMR (CDCl3 in δppm): 6.35 (d, 1H, -CO-CH =), 6.95 (d, 1H, C = CH) 7.21 – 8.24 (complex m, 11H, Ar proton), 10.32 (s, 1H, phenolic – OH);

Mass (m/z): 314[M+] 221, 195, 147, 119, 118, 91, 69, 65, 43.

Biological evaluation: Anti-tubercular Activity.[7,8,9]

The anti-mycobacterial activity of compounds were assessed against Mycobacterium tuberculosis using microplate Alamar Blue assay (MABA).This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200µl 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 Middlebrooks 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 37ºC for five days. After this time, 25µl of freshly prepared 1:1 mixture of Alamar 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.

All the synthesized compounds were screened for their in-vitro anti mycobacterial activity by means of micro plate alamar blue assay. The compounds were tested in the concentration range of 100 to 0.8 µg/ml against *M.tuberculosis* H37Rv strain grown in Middlebrook 7H9 broth in 96 well titre plates. Pyrazinamide- 3.125µg/ml and Streptomycin- 6.25µg/ml were used as standards for comparison.

RESULT AND DISCUSSION

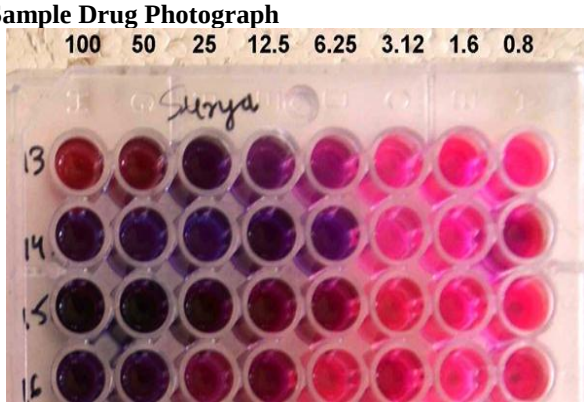
Table 1: product profile

S.l No	Mol Wt	Melting point	M.Formula	Solubility	Colour	YIELD	Molar refractivity
NA	214.22	66ºC -70ºC-	$C_{14}H_{12}O$	Methanol, $CHCl_3$	Reddish Brown	94%	61.28 ± 0.3 cm^3
PW	264.27	75ºC -78ºC-	$C_{17}H_{14}O_3$	Methanol, $CHCl_3$	Brown to Black	88%	79.13 ± 0.3 cm^3
X	277.11	58ºC-60ºC	$C_{13}H_9BrO_2$	Methanol, $CHCl_3$	Brown	80%	67.09 ± 0.3 cm^3
Y	254.30	80ºC-85ºc	$C_{15}H_{10}O_2S$	Methanol, $CHCl_3$	Yellowish powder	84%	75.63 ± 0.3 cm^3

Table 2: MABA Report of the Synthesized Compounds

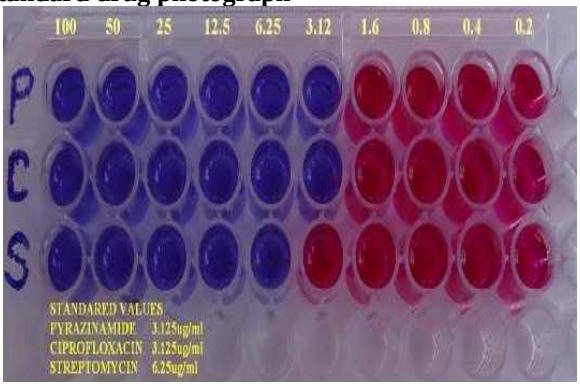
S. No	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1	NA	S	S	S	S	S	R	R	R
2	PW	S	S	S	S	S	R	R	R
3	X	S	S	S	S	R	R	R	R
4	Y	S	S	S	S	S	S	S	R

Sample Drug Photograph



NOTE: S-sensitive, R-Resistant, Strain Used- M.tuberculosis (H37 RV strain).

Standard drug photograph



CONCLUSION

This research concludes that all the synthesized molecules are effective in inhibiting the target enzyme Mtfab D, Malonyl COA - Acyl Carrier Protein Transacylase screened molecules were synthesized by condensation method, purified by chromatographic techniques, characterized by various spectral analytical techniques and evaluated for in-vitro anti mycobacterial activity against tuberculosis H37RV strain by Microplate Alamar Blue Assay (MABA) method. The experimental results show that Compound Y possesses better anti-tubercular activity with an MIC below 1.6mcg/mL while NA, and PW showed moderate anti tubercular activity with an MIC below 6.25mcg/Ml, and Compound X exhibited good anti tubercular activity with an MIC below 12..5mcg/Ml.

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