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Research Article

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DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF ANTI-TUBERCULAR AGENTS TARGETING DECAPRENYL PHOSPHORYL RIBOSE D EPIMERASE-2 AND METHOXY MYCOLIC ACID SYNTHASE-2

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ABSTRACT

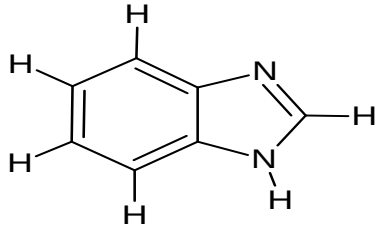
Mycobacterium tuberculosis MTB, or TB (*tubercle bacillus*), also called Phthisis or Phthisis pulmonalis, is a common, and in many cases lethal, infectious disease caused by various strains of mycobacteria, usually *Mycobacterium* tuberculosis. Over the past 200 years, tuberculosis has been responsible 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. Among these, Benzimidazole and Thiophene derivatives are reported as effective compounds for tuberculosis both with respect to their inhibitory activity and their favorable selectivity ratio. So compounds with the benzimidazole and thiophene nucleous were designed and docked against MTB enzyme decaprenyl phosphoryl ribose d-epimerase-2 and methoxy mycolic acid synthase-2 by using Argus lab® software. 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 R2 possesses anti-tubercular activity with an MIC below 25 mcg/mL while R1, K 1, K2 showed moderate anti tubercular activity with an MIC below 50mcg/mL.

KEYWORDS: Benzimidazole, Thiophene, Docking, MABA.

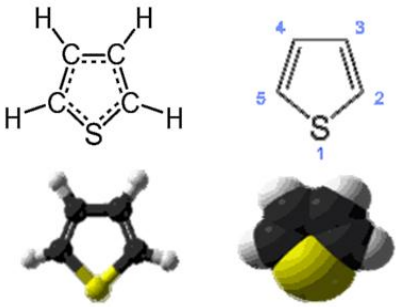
INTRODUCTION

Mycobacterium tuberculosis MTB, or TB (*tubercle bacillus*), also called Phthisis or Phthisis pulmonalis, and colloquially, consumption or tisic, is a common and in many cases lethal, infectious disease caused by various strains of mycobacteria, usually *Mycobacterium tuberculosis.*[1] Tuberculosis typically attacks the lungs, but can also affect other parts of the body. It is the first bacteria (identified by Koch in 1882) recognized as the causative agent for tuberculosis.[2] It is spread through the air when people who have an active TB infection cough, sneeze, or otherwise transmit respiratory fluids through the air.[3] Over the past 200 years, tuberculosis has been responsible for the death of over 100 million people.[4] 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. Benzimidazole is the heterocyclic compound formed from benzene and imidazole. Synthesis of commercially available benzimidazole involves condensation of o-phenylenediamine with formic acid. The benzimidazole and its derivatives possess important pharmacological activities such as antimicrobial, antiviral, anticancer, anti-inflammatory, analgesic, etc.[5] Thiophene, also called as thiofuran, is a heterocyclic compound with the formula C_4H_4S. It consists of a flat five membered ring; it is aromatic as indicated by its extensive substitution reactions.[6]

Basic Nucleolus



Benzimidazole



Thiophene

MATERIALS AND METHODS

Drug Design

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.[7] Drug likeness is a qualitative concept used in drug design for how "druglike" a substance is with respect to factors like bioavailability. The properties are estimated from the molecular structure before the substance is even synthesized and tested.[8] Lipinski's rule: Lipinski's rule is used to predict if a molecule is likely to be orally bio-available or to evaluate drug likeness.[9]

Evaluation of in-silico toxicity

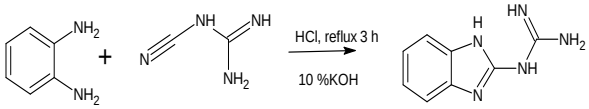
Insilico approaches like OSIRIS® Property explorer predicts the carcinogenicity, mutagenicity, teratogenicity, immune toxicology, irritation, sensitization etc. It let us draw chemical structures and calculates various drug relevant properties whenever a structure is valid. Prediction results are valued 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.

Synthetic methodology

Sheme1

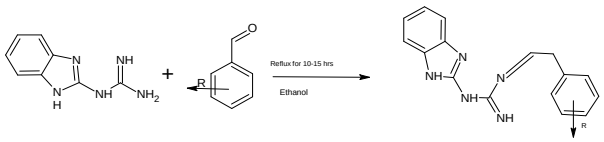
Step1: Synthesis of 2-benzimidazolylguanidine[10]

A mixture of *o*-phenylenediamine (100 mmol), cyanoguanidine (100 mmol) and concentrated Hydrochloric acid (20ml) in water (200 ml) was heated under reflux for 3 hrs. The reaction mixture was cooled at 0°C and Potassium hydroxide (10%; 50 ml) was added slowly. The precipitates of 2-guanidinobenzimidazole was collected by filtration, washed with water, dried and recrystallized using ethanol.



Step 2: Synthesis of Schiff base[11]

An equimolar quantity of 2-benzimidazoylguanidine and substituted aromatic aldehyde was refluxed for 10-15 hours in 20 ml ethanol. Completion of reaction was monitored by TLC. After completion of the reaction the content was poured into ice cold water. The precipitate was collected by filtration, washed with water and dried. Recrystallization is carried out using ethanol.



Scheme 2:

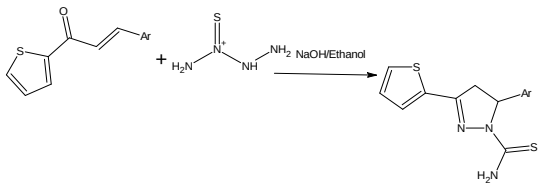
Compound1

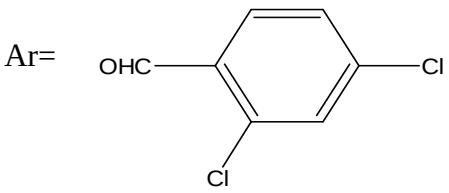
Step1

2-Acetyl thiophene (0.01mol) and appropriately substituted aromatic aldehyde (0.012 mol) were mixed in ethanol (20ml) containing 10% aqueous potassium hydroxide (8ml) and magnetically stirred the solution constantly at room temperature for 10 hours. The whole mixture was transferred in to 100ml ice cold water and acidified with dilute Hydrochloric acid. The solid form was washed, filtered, dried and recrystallized from absolute ethanol.[12]

Step 2

A mixture of chalcone (0.02mol) and thiosemicarbazide (0.02mol) was dissolved in ethanolic sodium hydroxide solution (10ml) and stirred for 3hrs and poured into 400ml of cold water with continuous stirring for 1hour. It was left overnight. The precipitate formed was filtered, washed and recrystallized from ethanol.

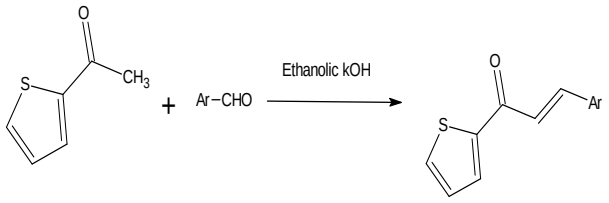


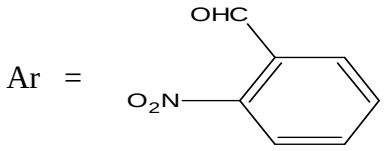


Compound 2

2-Acetyl thiophene (0.01mol) and appropriately substituted aromatic aldehyde (0.012 mol) were mixed in ethanol (20ml) containing 10% aqueous potassium hydroxide (8ml) and magnetically stirred the solution

constantly at room temperature for 10 hours. The whole mixture was transferred in to 100ml ice cold water and acidified with dilute Hydrochloric acid. The solid that was formed was Washed, filtered, dried and recrystallized from absolute ethanol.





Characterization studies

R1: IR- using KBR pellet technique: 3307 *cm^{-1} (-OHstr),1656(-C=Nstr),1542Cm^{-1}(C=Cstr),2929Cm^{-1}(C-Hstr) ^{1}HNMR:δ(5.4 ppm,singlet,1H)(3.4-4.1-ppm,multiplet,3H)),(6.3-7.1ppm,multiplet,8H).MASS: m/z 279(M+).*

R2: IR- using KBR pellet technique: 3307 *cm^{-1} (-OHstr),1656(-C=Nstr),1542Cm^{-1}(C=Cstr),2929Cm^{-1}(C-Hstr) ^{1}HNMR:δ(5.4 ppm,singlet,1H)(3.4-4.1-ppm,multiplet,3H)),(6.3-7.1ppm,multiplet,8H).MASS: m/z 331(M+).*

K1: IR- using KBR pellet technique: 3088.17 *cm^{-1} (Ar C-Hstr),1240.28 cm^{-1}(C=S str),1648.24Cm^{-1}(N=N str),719.48Cm^{-1}(C-Cl str),1480.43Cm^{-1}(C=Cstr) ^{1}HNMR:δ(7.84 ppm,triplet,1H)(7.90-7.97 ppm,multiplet,1H)),(7.33-7.23ppm,multiplet,3H).MASS: m/z 352(M+).*

K2: IR- using KBR pellet technique :1413.88 *cm^{-1} (C=C str),1665.60(-C=Ostr),1576.87Cm^{-1}(C-NO2str),3085.27Cm^{-1}(C-Hstr) ^{1}HNMR:δ(8.05 ppm,doublet,1H)(7.55-7.78-ppm,multiplet,1H)),(7.87ppm,doublet,1H) (7.36ppm,doublet,2H).MASS: m/z 259(M+).*

Biological evaluation: Anti-tubercular Activity[13, 14, 15]

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	Molar refractivity
R1	279	145℃	$C_{15}H_{13}N_5O$	Methanol, ethanol	Reddish **Brown**	79.01 ± 0.5cm
R2	331	118℃	$C_{15}H_{11}Cl_2N_5$	Ethanol, Methaol	Light Brown	87.35 ± 0.5cm^3
K1	356.29	72^{O}C	$C_{14}H_{11}Cl2N3S2$	Ethyl acetate, Ethanol	Yellowishbrown	92.68±0.5cm^3
K2	259.38	67^{0} C	$C_{13}H_9NO_3S$	Ethyl acetate, Ethanol	Light brown	72.03±0.3cm^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	R1	S	S	R	R	R	R	R	R
2	R2	S	S	S	R	R	R	R	R
3	K1	S	S	R	R	R	R	R	R
4	K2	S	S	R	R	R	R	R	R

CONCLUSION

Our research concludes that all the synthesized molecules are effective in inhibiting the target enzyme Decaprenyl Phosphoryl Ribose D Epimerase-2 and Methoxy Mycolic Acid Synthase-2 of Mycobacterium tuberculosis. The synthesized compounds were active at 25mcg – 50mcg/ml. Further refinements to the structure of the synthesized compounds are expected to yield

promising molecules active against Mycobacterium tuberculosis, with minimal toxicity.

REFERENCES

1. Kumar V, Abbas A., Fausto N, Mitchell R.N, (2007). Robbins basic pathology (8th ed.). Saunders elsevier. Isbn 978-1-4160-2973-1. Pp. 516–522.
2. Greenblatt cl, editor. Digging for pathogens: ancient emerging diseases: their evolutionary, anthropological and archeological context. Jerusalem: balaban publishers, 1998.
3. Konstantinos at al (2010). Tuberculosis. Australian prescriber, 33(1): 12–18.
4. "Tuberculosis". World health organization. 2010
5. Walba, H. & Isensee, R.W. Acidity constants of some arylimidazoles and their cations. J. Org. Chem. 1961; 26: 2789-2791.
6. Jonathan swanston "Thiophene" in ullmann's Encyclopedia of industrial chemistry Wiley-VCH, Weinheim, 2006. doi:10.1002/14356007, 726-793
7. Cryan JF, Markou A and Lucki I. Assessing antidepressant activity in rodents: recent developments and future needs. Trends Pharmacol. Sci. 2002; 23: 238-245.
8. http:// www.molinspiration.com/cgi-bin/properties. Retrieved on 7-03 2014.
9. http: // en.wikipedia.org/wiki/Lipinski -27s-rulu -of – five. Retrieved on 2-1-2014.
10. Ahmed M. Soliman, Shaaban K. Mohamedb, Mahmoud A.A. El Remaily. Synthesis and biological activity of dihydroimidazole and 3,4-dihydrobenzo[4,5]imidazo[1,2-a][1,3,5]triazins.
11. Maria C. S. Lourenço,a Marcus V. N. de Souza,b Alessandra C. Pinheiro,b. Evaluation of anti-tubercular activity of nicotinic and isoniazid analogues. 2008.
12. Mohamed J. Elarfi and Hussniya A. Al-Difar., (2012). Synthesis of heterocyclic compounds derived from chalcones. Sci. Revs. Chem. Commun, 2(2): 103-107 ISSN 2277-2669.
13. Bijo Mathew, A. Jerad suresh and S. Anbazhagan., (2012). Microwave assisted synthesis physicochemical properties and antimicrobial activities of sme novel barbitone moiety linked by Benzimidazole chalcones. FSJ Pharm Res/Vol/No3.
14. Accelrys. Adme-tox component collection. Http://accelrys.com/products/pipeline-pilot/Component-collections/adme-tox.html.
15. Namrata Singh, Annamalai Pandurangan, Kavita Rana, Preeti Anand. Benzimidazole: A short review of their antimicrobial activities, International Current Pharmaceutical Journal. 2012; 1(5): 119-127.