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Development of furfuraldehyde formazans as potential antitubercular agents

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ABSTRACT

This article envisages the development of furfuraldehyde formazans as antitubercular agents. A series of furfuraldehyde formazans were synthesized and characterized by spectral analysis. The antitubercular activity of these compounds was assessed against Mycobacterium tuberculosis (MTB) H37Rv. Four compounds, 2b, 2c, 2g and 2i showed moderate to good antitubercular activity against H37Rv. Inactivity of all these compounds against gram positive and gram negative bacteria indicated their specificity against MTB. The synthesized compounds were analyzed for ADME properties and showed potential as good oral candidates. The work identified few lead compounds that can be explored further for the development of potential antitubercular agents.

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Key words: *Mycobacterium tuberculosis*, Furfuraldehyde formazans, Antitubercular activity

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INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (MTB), leading to serious levels of morbidity and mortality [1]. In the year 2015, as per the estimate of WHO, one-third of the world's population, nearly 2 billion people, mostly in the developing countries, has been infected with MTB. There is 14 % increase in people with multi-drug resistant tuberculosis (MDR-TB) as compared to 2014 WHO report [2]. The associated poor patient compliance, extended chemotherapy that relies on drugs developed in the mid-twentieth century, the emergence of drug resistant forms of TB coupled with a strong epidemiological coexistence with HIV/AIDS, highlights the fundamental need for new and more effective drugs to treat the disease [3-5].

Many biologically active molecules contain heteroatoms such as nitrogen, sulphur and oxygen, and were explore by many research groups. Furan and its derivatives have been reported to have various pharmacological and biological activities such as antituberculosis [6], anti-inflammatory [7] and antibacterial [8]. Benzofuran salicylic acid derivative (I-A09) is a lead antitubercular agent, and is currently, in clinical evaluations [9]. Formazans are known for their spectrum of biological activities such as antimycobacterial [10], antibacterial [11], antifungal [12] and anticonvulsant activities [12].

In continuation with our earlier work on design of furfuraldehyde formazans as antitubercular agents [13], synthesis and antitubercular activity of furfuraldehyde formazans, was undertaken. Nine furfuraldehyde formazans were synthesized and evaluated for *in vitro* antitubercular activity against H37Rv. These compounds were also subjected to *in silico* ADME prediction.

MATERIALS AND METHODS

Synthesis

The furfuraldehyde formazans were synthesized by using simple reactions depicted in **Scheme 1**. The key intermediate, furfuraldehyde hydrazone, was prepared in good yield by refluxing furfuraldehye, dissolved in ethanol, with phenylhydrazine containing few drops of glacial acetic acid [14]. This key intermediate was further reacted

with various diazotised primary amines to obtain compounds **2a to 2i**, the furfuraldehyde formazans **(Scheme 1, Table 1)**. These compounds were obtained in good yields and purity, using inexpensive and commonly available reagents. The compounds were fully characterized by spectroscopic analysis. The compounds were further subjected to antitubercular activity, antibacterial activity and *in silico* ADME prediction.

***In vitro* MTB activity studies**

The antitubercular activity of all the compounds **2a-2i** was assessed against sensitive strain of MTB H37Rv, using Microplate Alamar Blue Assay (MABA) [15]. Briefly, 200 µl of sterile deionzed water was added to all outer perimeter wells of sterile 96 wells plate to minimize 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 was directly made on the plate. The concentrations in the range of 50 µg/ml to 0.78 µg/ml, were used for the study. Plates were covered and sealed with parafilm and incubated at 37 ºC for five days. After 5 days, 25 µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10 % tween 80 was added to the plate and incubated for 24 h. The readings were taken in duplicates. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as the lowest drug concentration, which prevented the color change from blue to pink. The MIC values of furfuraldehyde formazans along with the standard drugs are presented in **Table 2**.

Antibacterial activity

Antibacterial activity of all the nine compounds was tested against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* at the concentrations of 50 and 100 µg/ml using Cup-plate agar diffusion method [16].

***In silico* ADME prediction**

The ability to detect problematic candidates in the early stage of drug discovery significantly reduces the amount of time and resources being wasted on molecules that are doomed to fail in clinical trials, owing to poor pharmacokinetics (ADME) and toxicity properties.

With this objective, *in silico* ADME prediction was undertaken for the synthesized compounds and drug-likeliness was determined using *QikProp* tool (Schrodinger, LLC., New York), incorporated in Schrodinger molecular modeling suite. This software provides the ranges for comparing the properties of molecules with those of 95 % of known drugs [17]. The descriptors calculated were #stars, logarithm of partition coefficient (Log P), Lipinski's rule of five, % human oral absorption (% HOA), CNS activity (blood-brain barrier partition coefficient) and Caco-2 cell permeability (gut-blood barrier permeability; absorption of orally administered drugs) [17]. The *in silico* ADME prediction data of the compounds was obtained by *QikProp* and is summarized in **Table 3**.

RESULTS AND DISCUSSION

Synthesis

The overall yields of the two-step reaction ranged from 52 to 60 %. Final products were isolated as reddish brown solids.

Biological activity

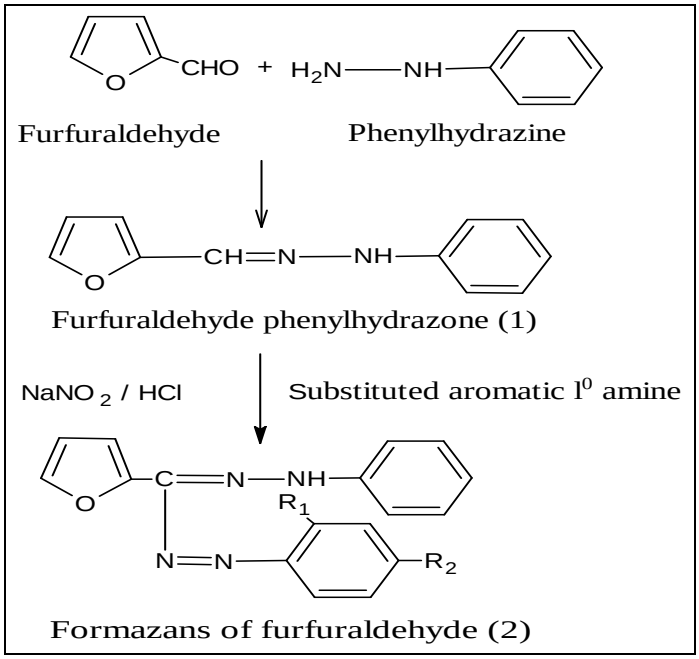
Table 2 summarizes the antitubercular activity of the compounds. Compounds with antitubercular activity >100 µM were considered as inactive against MTB [18]. The compounds **2b** (38.52 µM), **2c** (69.63 µM), **2g** (74.62 µM) and **2i** (81.16 µM) showed good antitubercular activity as compared to the compounds **2a** (172.41 µM), **2d** (156.25 µM), **2e** (156.25 µM), **2f** (164.47 µM) and **2h** (149.25 µM). The compounds with electron withdrawing groups like chloro (**2b, 2c**), nitro (**2g**), fluoro (**2i**) displayed good antitubercular activity as compared to the compounds having phenyl **(2a**) and methyl (**2f**) groups. The *in vitro* antitubercular results obtained are well correlated with our earlier studies in, *in silico* designing of furfuraldehyde formazans [13].

All the compounds showed very poor or no activity against gram positive and gram negative bacteria undertaken in the study. Inactivity of all these compounds against these tested bacteria indicated their specificity toward MTB.

The *in silico* ADME prediction data is summarized in **Table 3**. Lipophilicity is one of the most important physico-chemical properties, which determines the biological activity of molecules, affecting the non-specific diffusion through biological membranes. It is well known that antimycobacterial activity is often enhanced by increased lipophilicity, which facilitates the penetration of compounds through highly lipophilic mycobacterial cell wall. The lipophilicity of all the compounds, as obtained from the software *QikProp*, was in the range of **3.402 to 4.951**. The acceptable range predicted for this parameter is -2.0 to 6.5. The compounds **(2b, 2c, 2g** and **2i)** displayed good lipophilicity and hence, they also exhibited good antitubercular activity.

The compounds investigated herein displayed very low susceptibility to acid hydrolysis in stomach, as reflected from the % human oral absorption data **(Table 3)** (value of absorption >80% is considered good and <25% is considered poor). The predicted values of apparent Caco-2 cell permeability which predicts absorption of orally administered drugs, further supports these findings (<25 is considered poor and >500 is considered excellent).

Drugs targeting the central nervous system (CNS) are expected to cross the blood brain barrier in order to reach their destination, while drugs with peripheral site of actions are expected to have no brain penetration to avoid related side effects. The predicted blood-brain barrier partition coefficient for the active compounds **(2b, 2c, 2g** and **2i)**, was in the range of -1 to 0 (acceptable range is -2 to 0 for inactive compounds and 0 to 1 for active compounds) **(Table 3)**, signify that these molecules have very low propensity to cross the blood-brain barrier, thereby eliminating the chance of CNS related toxicity.



Scheme 1: Chemical reactions for the synthesis of furfuraldehyde formazans (2a – 2i)

Table 1: Various substituent's on the ring

Compound code	R_1	R_2
2a	H	H
2b	H	CI
2c	CI	CI
2d	OCH_3	H
2e	H	OCH_3
2f	CH_3	H
2g	NO_2	H
2h	H	NO_2
2i	H	F

Table 2: Antitubercular activity of furfuraldehyde formazans (2a-2i)

Compound code	MIC (µM)
2a	172.41
2b	**38.52**
2c	**69.63**
2d	156.25
2e	156.25
2f	164.47
2g	**74.62**
2h	149.25
2i	**81.16**
Isonaizid	0.72

Table 3: QikProp analysis of furfuraldehyde formazans (2a-2i)

Compound code	#Stars	CNS	QP log Po/w	%HOA	QPP Caco	Lipinski's Rule of Five
2a	1	0	4.108	100	>500	0
2b	1	-1	4.601	100	>500	0
2c	1	-1	4.951	100	>500	0
2d	1	0	4.203	100	>500	0
2e	1	0	4.199	100	>500	0
2f	1	0	4.365	100	>500	0
2g	1	-1	3.536	93.70	>500	0
2h	2	-2	3.402	96.08	>500	0
2i	1	0	4.343	96.34	>500	0

Stars property indicates the number of property or descriptor values that fall outside the 95 % range of similar values for known drugs. A large number of stars that a molecule has, less is its drug-likeliness than the molecules with few stars. The range predicted for this parameter is 0-5, where 0 indicates no violation or best candidate. Almost all of the compounds exhibited physicochemical properties, which fall in the range of known drugs (**Table 3**).

All the compounds followed the Lipinski's rule of five (**Table 3**). Therefore, the overall *in silico* ADME prediction appeared to be interesting.

Though compounds had shown moderate activity as compared to the standard, isoniazid, taking these compounds as lead analogues, with the help of *in silico* study further lead optimization can be done, in an attempt to develop more effective antitubercular agents.

CONCLUSION

In this article, synthesis, antitubercular activity of furfuraldehyde formazans against MTB H37Rv and *in silico* ADME data are reported, in an attempt to develop effective and safe antitubercular agents. The simple, furfuraldehyde formazans were obtained in good yields and purity, using inexpensive commonly available reagents. Few compounds showed good antitubercular activity. The compounds with electron withdrawing groups like Cl and NO_2 displayed higher activity as compared to the other compounds with groups like phenyl and methyl, along with the toxophoric, azomethine – NHN=CH- proton. Inactivity of all these compounds against tested bacteria indicated their specificity toward MTB. All the compounds displayed good *in silico* ADME predictions and also excellent drug likeliness. Further structural modifications can be done on these compounds, to make them valid leads, which would possess better activity than the existing antitubercular drugs.

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