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ARTICLE

Formulation and Evaluation of β-Cyclodextrin-mediated Inclusion Complexes of Isoniazid Scaffolds: Molecular Docking and *In Vitro* Assessment of Antitubercular Properties

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Poor aqueous solubility is the major problem encountered with formulation of new bioactive chemical entities. In the present study, we report the synthesis and evaluation of inclusion complexes between two poorly water soluble antitubercular agents (*p*-hydroxybenzaldehydeisonicotinylhydrazone (HBIH) and 2,3-butanedionebisisonicotinylhydrazone (BDIH)) and *β*-cyclodextrin. Solubility of these compounds has been enhanced by inclusion complexation and the solid complexes were characterized using FTIR, PXRD, NMR and SEM. Phase-solubility studies indicated that HBIH/BDIH formed a 1:1 stoichiometric inclusion complex with *β*-CD. Moreover, molecular docking analysis identified the most favorable host-guest interactions in the inclusion complexes. The complexes were evaluated against *Mycobacterium tuberculosis* strains and exhibited more than 95% growth inhibition. The complexes were devoid of cytotoxicity when tested against L929 fibroblast cell lines. Docking studies were carried out on DprE1 and Thymidine Monophosphate Kinase protein enzymes to provide some understanding into the mechanism of action of these compounds.

1. Introduction

Mycobacterium tuberculosis (Mtb), an infectious bacillus, is the causative agent of many cases of tuberculosis and is the deadliest communicable disease prevalent in all parts of the world.[1] Epidemiological studies have indicated that 10 million people, as much as one third of the world's population, are infected by tuberculosis every year.[2] Mycobacteria can evade the host immune system and remain dormant for a long period of time. This allows the Mtb to reactivate to a virulent form under immune-compromised conditions of the host.[3] The enduring persistence of Mtb in dormant stage helps the pathogen to establish resistance against current anti-mycobacterial drugs.[4] Almost all of the antibiotics that can be used to treat TB work when the bacteria are actively dividing. However, in order to kill the persistent or slow growing strains of Mtb, the continuation phase of the treatment is essential. Thus, there is an urgent need for the development of new drugs and strategies which can tackle the dormant Mtb and for the eradication of the disease.

TB can be effectively treated with first-line drugs isoniazid, pyrazinamide, rifampin and streptomycin.[5-8] However, this first-line therapy often fails to cure the TB due to the emergence of drug resistant bacteria. The rise of multidrug resistant TB (MDR-TB), i.e. which is resistant to at least isoniazid (INH) and rifampicin (RIF) requires the use of second-line drugs that are difficult to procure and are either less effective or more toxic with serious side-effects.[9-10] Resistance to MDR-TB was followed by emergence of extensively-drug resistant (XDR) and totally drug resistant strains of Mycobacterium tuberculosis.[11] Therefore, the detection and treatment of drug susceptible or single drug resistant TB is an important strategy for preventing the emergence of MDR and XDR-TB. Hence, it is quite essential to develop new strategies for the safe and cost-effective new antitubercular agents.

Molecular hybridization of potential pharmacophore scaffold has been an area of interest towards the design of new prototypes.[12-14] These molecules effectually address resistance problem and have an improved efficacy. Bearing in mind the hybrid concept along with the antitubercular potential of different moieties such as rifampin, ethambutol and isoniazid, it was planned to synthesize hybrid compounds comprising isoniazid moiety clubbed with other organic components and evaluate its anti-mycobacterial activity. Schiff bases of isoniazid have been reported for their potent antitubercular activity.[15-17] The reports suggest that the substitution at amine position as well as preparation of isoniazid derived Schiff bases may enhance antitubercular activity. Herein, we have synthesized two such isoniazid scaffolds by covalent addition on an aldehyde/diketone support (Fig.1a and 1b). The diketone derivative which contains two active isoniazid subunits and aldehyde conjugate with single isoniazid unit were evaluated for antitubercular activity. However, the clinical application of these compounds has been greatly limited due to its poor water solubility (practically insoluble in water) and stability, which severely reduces its bioavailability.[18,19]

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