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Research paper

Hybrid triazoles: Design and synthesis as potential dual inhibitor of growth and efflux inhibition in tuberculosis

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ABSTRACT

Efflux inhibition is proven bacterial machinery responsible for removal of bacterial wastage including antibiotics. Recently, efflux inhibitors (EI) have been tested with encouraging results as an adjuvant therapy for treatment of tuberculosis (TB). Although, EI have emerged as innovative approach of treatment for multi drug resistant (MDR) & extensively drug resistant tuberculosis (XDR-TB), toxicity profile limits their wider use. To address this issue, we have attempted synthesizing hybrid molecules those results by combining known EI and triazole. This synthesis was aimed to arrive at structure that possesses pharmacophore from known EI. Synthesized molecules were evaluated as growth inhibitors (GI) and Efflux inhibitor of TB initially against *Mycobacterium smegmatis* mc^2155. Pharmacologically active compounds were then tested for their cytotoxicity to further narrow down search. Most active compounds 144, 145, 154 and 163 were then tested for their GEI action against *Mycobacterium tuberculosis* (Mtb). Synthesized compounds were also tested for their synergistic action with first line and second line anti-TB drugs and ethidium bromide (EtBr). We arrived at compound 135 as most potent dual inhibitor of tuberculosis.

1. Introduction

Currently, the global armamentarium of antituberculosis drugs is insufficient to address the growing populations of patients with multi-drug resistant (MDR) and extensively drug-resistant (XDR) *Mycobacterium tuberculosis*. According to World Health Organization (WHO), tuberculosis (TB) is the second leading cause of death from an infectious disease. Recent databases suggested 9 million new cases and 1.5 million deaths owing to this infection, including 360,000 deaths among HIV-positive people [1]. It is also stated that one-third of the world's population is infected with latent TB and 10% of which is expected to develop active TB at some point in their lives [2].

Stringent supervision, consistent supply and drug intake fidelity is a must during entire anti-tuberculosis program to be successful and to avoid recrudescence and resistance. Emergence of multi and extensively drug resistant (MDR & XDR) TB has frustrated researchers further.

Above mentioned scenario recommends drug discovery to be synergistic; approach to target newer molecular mechanism and novel NCE which can be combination of pharmacophores of earlier proven entities. Current drug pipeline lacks this innovation [3–5] making "so called" newer NCE vulnerable to mutation based resistance (MBR).

Reports suggests that along with MBR, efflux pump also plays vital role in development of resistance by Mtb [6–8]. Intrinsic resistance of *M. tuberculosis* to standard antimicrobial therapies has been attributed primarily to its lipid-rich cell wall composition which limits drug penetration. Efflux pumps, which reduce the passage of antimicrobials across the bacterial cell wall, have been identified as an additional mechanism of resistance. The recent identification of clinical isolates of resistant *M. tuberculosis* that have an efflux component as part of their resistance phenotype represents an exciting new field in tuberculosis therapeutics. Along-with detoxification of intracellular metabolites and cellular homeostasis [9], efflux pumps does contribute for intrinsic and acquired drug resistance in many bacterial pathogens. Usually they confer low-to-intermediate level of resistance; however, the constant pressure of subinhibitory concentrations of the antibiotic promotes the selection of spontaneous mutants [9]. Based on the literature, we state efflux pumps are crucial in a) low level direct

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drug resistance, b) high level indirect (through effluxing drug from within cell) drug resistance, c) they are effectors of the innate drug-resistance machinery and d) if efflux pumps are inhibited, improvement or restoration of activity of old drug is observed.

Thioridazine [10–15], chlorpromazine [16,17] and Verapamil [18] have shown efflux pumps as area of opportunity in anti-tubercular agents but have not evolved toward clinical usage due to potential toxicities. The development of agents with low toxicity profiles that target drug efflux pumps may be clinically useful in reducing the global threat of MDR and XDR tuberculosis. Based on recent reports [19–22], we have embarked with present series to identify and evaluate novel compounds for their dual inhibitory action; growth and efflux inhibition (GEI).

2. Results and discussion

2.1. Chemistry

Chemical class of triazole is cost effective nucleus which has proven its commercial viability. Thus by undertaking present series, we can state to have a cost effective drug candidate. The synthetic route was followed as reported (Fig. 2) [23].

As detailed literature discussing SAR on efflux inhibitors as anti-TB is lacking, we initiated our synthesis based on Ligand-based Drug Design approach. Two of most studied efflux inhibitors were identified for drug designing, Thioridazine (TZ) and Verapamil (VP) wherein TZ is also explored in depth for its enhancer activity along-with other anti-TB agents [24,25]. Phenothiazine ring is crucial in conferring neuroleptic activity for TZ while efflux systems are known to affine to basic amino group. Given these premises, we embarked at a structure which reflects parts of these two pharmacophores with couple of variations. The triazole core is well known privileged nucleus in recent drug discovery as it's a nifty heterocycle with a wide range of activities like antibacterial, antifungal, antiviral and also antimycobacterial. Many reports are available confirming fused or linked triazole emerging as novel antitubercular agent [26–29]. Most of the studies have also suggested amino and mercapto position's availability for extensive variation for modulation of activity. Thus combination of hybrid design linked with triazole was finalized to arrive at dual inhibitors. (Fig. 1)

Target structure we have designed was combination of 3 precursors viz., protected triazole (4), a chalcone (7) and heterocycle. In brief, initially hydrazide (1) was synthesized by reacting carbon disulfide with hydrazine hydrates. Bromo acetic acid was then treated with hydrazine (2) to produce 4-amino-3-bromomethyl-5-mercapto-(1,2,4)-triazole (3). This conversion did not yield products for several time at the addition was tricky. After several trial and error we identified root cause is the way and speed of addition of reactants. Slow, drop wise addition of bromo acetic acid over 30 min to hydrazine provided compound 3. Amino and mercapto groups which served as reactive terminals were then protected by Boc anhydride and benzyl bromide respectively to furnish protected triazole (4). Second precursor, chalcone (7) was prepared by reacting 2-hydroxy acetophenone (5) with 2,4-dichlorobenzaldehyde (6). These triazolyl-chalocones (8) were then cyclized using hydroxylamine and further deprotected at amino and thio function by Pd/C and then TFA/DCM to furnish compound PDST121. This compound has shown good inhibition against primary screening. This compound is under further screening at our lab and currently out of scope of this manuscript. Meanwhile, it was thought worthwhile to develop SAR related to PDST121.

In attempt to do so, we first reacted free amino group and converted to substituted amide (136–138). On observing promising activity of amide compounds, it was further decided to alter reactive thiol group. As initial transformation, esterification was performed to produce PDST139-141. As these compounds shown detrimental effects on inhibitory action, program for ester linkage was scrapped and instead chain elongation strategy was considered. Starting substitutions with smallest linkage, methyl group we extended to ethyl, propyl and n-butyl which have shown betterment of activity. We also synthesized compounds having *a*. appendage with double bond, *b*. branching instead of elongation and *c*. ring insertion but were shown diminishing activity. Thus to develop a concluding SAR for this series, we synthesized PDST142-168 and were evaluated. Most of the conversion were smooth except mentioned ones and gave products with high purity.

2.2. Pharmacology

An urgent need for effective alternative anti-TB treatment, especially for the severe destructive and disseminated forms of TB initiated attempts to design and develop compounds with anti-mycobacterial activity. Current manuscript reveals first report wherein a hybrid molecule is coupled with triazole that are aimed as potent and rationally designed TB-GEI (growth and efflux inhibitor). Total of 33 compounds were synthesized in this experiment and all underwent inhibitory assay. All the compounds were initially tested against *Mycobacterium smegmatis* mc^2155 reference strain to achieve faster and cheaper results. Molecules, which have shown inhibitory activity better than TZ were further tested for cytotoxicity. Compounds with better inhibition and lesser cytotoxicity were then assessed on *M. tuberculosis* H37rv and further for synergistic profile with first and second line anti-tubercular agents.

2.2.1. Preliminary inhibitory assessment

2.2.1.1. Growth inhibition of M. smegmatis mc^2155. As mentioned earlier, we have observed promising dual inhibitory activity of PDST121 from our in-house cluster of compounds. To further explore SAR of this compound, we initiated current synthetic

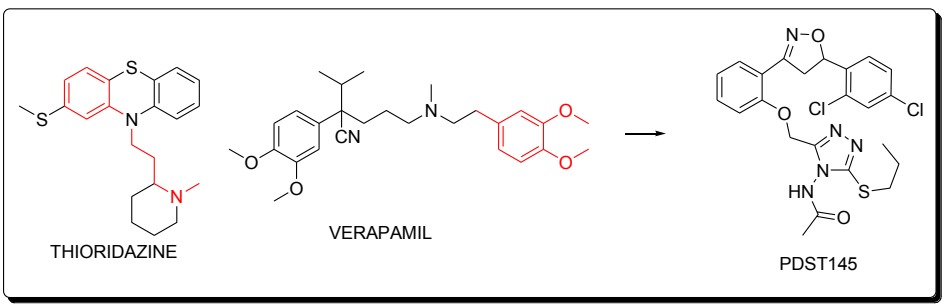

Fig. 1. Identification of molecular fragments in generating hybrid molecule.