Bioorganic & Medicinal Chemistry Letters 26 (2016) 2224–2228

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and docking studies of pyrazine–thiazolidinone hybrid scaffold targeting dormant tuberculosis

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ARTICLE INFO

Article history:
Received 18 December 2015
Revised 10 March 2016
Accepted 15 March 2016
Available online 15 March 2016

Keywords:
Tuberculosis
Dormant
Pyrazine
Thiazolidenone
Hybrid design
Docking

ABSTRACT

The persistence of *Mycobacterium tuberculosis* (MTB) in dormant stage assists the pathogen to develop resistance against current antimycobactrial drugs. To address this issue, we report herein the synthesis of *N*-(4-oxo-2 substituted thiazolidin-3yl) pyrazine-2-carbohydrazide derivatives designed by following the molecular hybridization approach using pyrazine and thiazolidenone scaffolds. The compounds were evaluated against MTB H37Ra and *Mycobacterium bovis* BCG in dormancy model. Most of the compounds had IC_{50} values in 0.3–1 μg/ml range. The active compounds were further tested for anti-proliferative activity against THP-1, Panc-1, A549, and MCF-7 cell lines using MTT assay and exhibited no significant cytotoxicity. We also report molecular docking studies using active analogs and MTB – Decaprenylphosphoryl-β-D-ribose-2′-epimerase (DprE1) to rationalize the biological activity and to provide an insight into the probable mechanism of action and binding mode of hybridized structures. The results obtained validate the use of molecular hybridization approach and also suggest that reported compounds can provide a novel pharmacophore to synthesize lead compounds against dormat MTB.

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (MTB) is prevalent in all parts of the world. It is one of the world's deadliest communicable diseases due to high virulence and ability of MTB to enter into a dormant state which can subsequently undergo reactivation. The long-term persistence of MTB in dormant stage assists the pathogen to develop resistance against current antimycobactrial drugs. Thus, there is need for development of drugs which can target the dormant MTB and help in eradication of the disease.[1–3] *Mycobacterium bovis* (*M. bovis*) is also considered to be a disease causing strain in humans exposed to infected material or immunodeficiency. Hence identification of Bacillus Calmette-Guerin (BCG), an attenuated derivative of virulent strain of *M .bovis* is also considered clinically significant.[4]

Molecular hybridization approach has been an area of interest towards development of agents against MTB. Most important hybridized structures include clinically used drugs such as rifamycin, ethambutol and isoniazid clubbed with other hydrophobic structure such as cinnamyl acid derivatives.[5–11] In this Letter, we report novel chemical structures as antitubercular agents based on the hybridization of pyrazine and thiazolidine derivatives.

Previous studies have indicated promising antitubercular activity for pyrazine and thiazolidine derivatives against active strain.[12–19] Pyrazinamide mannich bases, pyrazine 2-carbohydrazides and pyrazine-2-carboxamide derivatives have been reported to have MIC values in lower micromolar range. Similarly thiazolidinone derivative have been reported to have MIC values in the micromolar range with some compounds having activity in submicromolar range.

Thus, it was of interest to evaluate the activity of hybrids of pyrazine and thiazolidine derivatives against dormant mycobacterium. Since some of our compounds demonstrated promising activity we also investigated their probable mechanism by performing molecular docking study using Decaprenylphosphoryl-β-D-ribose-2′-epimerase (DprE1) as the target receptor.[20]

The starting materials, pyrazine-2-carboxylic acid/3-aminopyrazine-2-carboxylic acid/5-methylpyrazine-2-carboxylic acid were purchased from Spectrochem (Mumbai, India) and their identity was confirmed by IR, ^{1}H NMR and Mass spectra. *N*-(4-Oxo-2 substituted thiazolidin-3yl) pyrazine-2-carbohydrazide derivatives

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http://dx.doi.org/10.1016/j.bmcl.2016.03.055
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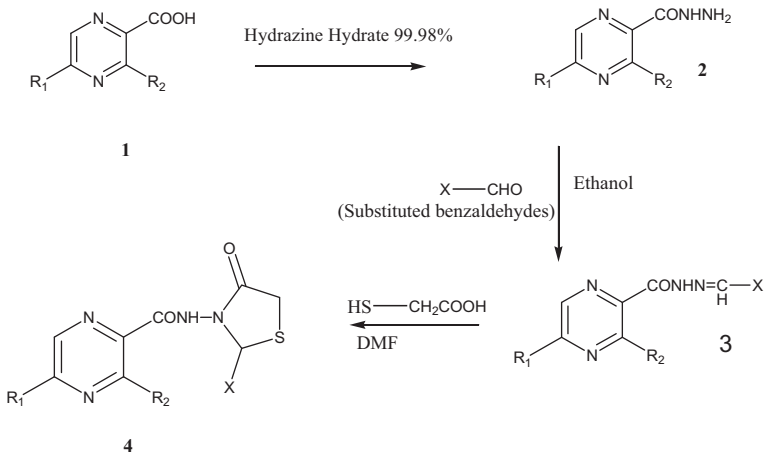
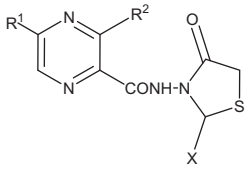


Scheme 1. Synthetic route for *N*-(4-oxo-2 substituted thiazolidin-3yl) pyrazine-2-carbohydrazide derivatives (4a–4w).

(4a–4w) were synthesized in three steps as shown in Scheme 1. Briefly, substituted pyrazine-2-carboxylic acid derivative were converted to carbohydrazides using hydrazine hydrate. Substituted pyrazine-2-carbohydrazides were then converted to their respective hydrazine using substituted benzaldehydes. Lastly, carbohydrazones were reacted with with thioglycolic acid in the presence of catalytic amount of $ZnCl_2$ to obtain final hybrid compounds.[14,19,21] In total 23 compounds were synthesized (4a–4w; Fig. 1). Final compounds were purified using column chromatography and structures of all the compounds were confirmed by IR, ^{1}H NMR and mass spectroscopy (Supplementary Data). Purity of the compounds was determined by TLC/HPLC. The melting points were found in the range of 145–167 °**C**.



N-(4-oxo-2 substituted thiazolidin-3yl) pyrazine-2-carbohydrazide derivatives were screened for in vitro antitubercular activity against MTB H37Ra (ATCC 25177) and *M. bovis* BCG (ATCC 35743). Preliminary antitubercular screening was performed at concentrations, 30, 10 and 3 μg/ml (Supplementary Material). The XTT Reduction Menadione assay (XRMA) which is a well established anti-tubercular screening protocol (for dormancy model) was used for screening the compounds, against MTB H37Ra.[22] For *M. bovis* BCG, Nitrate reductase assay protocol was used.[3] The antitubercular drugs, rifampicin, pyrazinamide and isoniazid were used as reference standards. The MIC and IC_{50} are presented only for compounds which showed more than 90% inhibition at 30 μg/ml concentration (Table 1) in dormant assay for MTB H37*Ra* and *M. bovis* BCG.[3] To evaluate the selectivity towards mycobacterium, the derivatives were tested (Supplementary Data Table 2) against *Mycobacterium smegmatitis* as well as antibacterial screening using two Gram Positive (*Staphylococcus aureus* and *Bacillus subtillus*) and two Gram Negative bacteria (*Pseudomonas fluorescens* and *Escherichia coli*) was carried out.[23] The compounds, 4a, 4g, 4h, 4m, 4n, 4p,4s, 4t, 4v and 4w showed more than 90% inhibition of MTB H37Ra and *M. bovis* BCG. Compound 4p, displayed MIC = 26.56 μg/ml and IC50 value of 0.337 μg/ml against MTB H37Ra, whereas 4v showed, MIC = 28.82 μg/ml and IC50 value of 3.26 μg/ml against *M. bovis* BCG respectively. None of the derivatives showed inhibition of *M. smegmatitis.* According to the data, substitution in phenyl ring with hydroxyl and ethoxy groups has favored activity against MTB H37Ra and presence of 4-Chloro has shown more activity against *M. bovis* BCG. In general presence of 4-Chloro, 4-Nitro and substitution with hydroxyl and ethoxy/methoxy groups in ortho positions on phenyl ring have seem to give better activity. Remaining 13 compounds have shown% inhibition in a range of around 50–80% and hence were not studied further. The mentioned 10 compounds were then further examined for toxicity in a non adherent, human monocytic (THP-1) cell line, in adherent human pancreas epithelioid (Panc-1), adherent human

Sr. No	Comp code	X	R^1	R^2	Sr. No	Comp code	X	R^1	R^2
1	4a	$-C_6H_5$	–H	$-NH_2$	13	4m	$3\text{-}NO_2C_6H_5$	$-CH_3$	–H
2	4b	$4\text{-}ClC_6H_5$	–H	$-NH_2$	14	4n	$4\text{-}NO_2C_6H_5$	$-CH_3$	–H
3	4c	$2,4\text{-}ClC_6H_5$	–H	$-NH_2$	15	4o	$4\text{-}OHC_6H_5$	$-CH_3$	–H
4	4d	$3\text{-}NO_2C_6H_5$	–H	$-NH_2$	16	4p	$3\text{-}OC_2H_5$, $4\text{-}OHC_6H_5$	$-CH_3$	–H
5	4e	$4\text{-}NO_2C_6H_5$	–H	$-NH_2$	17	4q	$4\text{-}OCH_3C_6H_5$	$-CH_3$	–H
6	4f	$4\text{-}OHC_6H_5$	–H	$-NH_2$	18	4r	$3,4\text{-}OCH_3C_6H_5$	$-CH_3$	–H
7	4g	$3\text{-}OC_2H_5$, $4\text{-}OHC_6H_5$	–H	$-NH_2$	19	4s	$3\text{-}OCH_3$, $4\text{-}OHC_6H_5$	$-CH_3$	–H
8	4h	$3,4\text{-}OCH_3C_6H_5$	–H	$-NH_2$	20	4t	$4\text{-}N(CH_3)_2C_6H_5$	$-CH_3$	–H
9	4i	$4\text{-}FC_6H_5$	–H	$-NH_2$	21	4u	$4\text{-}OHC_6H_5$	–H	–H
10	4j	$4\text{-}N(CH_3)_2C_6H_5$	–H	$-NH_2$	22	4v	$4\text{-}ClC_6H_5$	–H	–H
11	4k	$-C_6H_5$	$-CH_3$	–H	23	4w	$4\text{-}NO_2C_6H_5$	–H	–H
12	4l	$4\text{-}ClC_6H_5$	$-CH_3$	–H					