SYNTHESIS AND ANTIMYCOBACTERIAL ACTIVITY OF NOVEL MANDELIC ACID DERIVED DIAMIDE

G. Stavrakov1,*, I. Philipova2, V. Valcheva3

1Faculty of Pharmacy, Medical University of Sofia, str. Dunav 2, Sofia 1000, Bulgaria
2Institute of Organic Chemistry, Bulgarian Academy of Sciences, Acad. Bonchev 9, Sofia 1113, Bulgaria
3Institute of Microbiology, Bulgarian Academy of Sciences, Akad. Bonchev 26, Sofia 1113, Bulgaria

Summary: A new mandelic acid derived diamido-diol was synthesised on the base of (1R,2R)-1,2-diaminocyclohexane scaffold and evaluated for its in vitro activity against M. tuberculosis H37Rv. The compound shows activity comparable with the one of the classical anti-TB drug ethambutol.

Key Words: mandelic acid, 1,2-diaminocyclohexane, M. tuberculosis H37Rv

Introduction

Increasing drug resistance and poor activity of existing therapies towards the latent stage of Mycobacterium tuberculosis infection has produced a clear need to develop novel therapeutics to treat tuberculosis [1]. Thus fast-acting drugs with novel mechanisms of action that are not cross resistant to existing drugs are being sought actively.

Wilkinson and coworkers first reported the synthesis and activity of ethambutol (EMB) (Fig. 1. I) [2]. EMB was a useful addition to tuberculosis chemotherapy, despite a relatively modest MIC of 10 μM, in part because of very low toxicity and relatively few side-effects. Based on structure–activity relationship (SAR) studies it appeared that the distance between the two nitrogens, the presence of β-aminoalcohols, and the small side chains were critical for determining activity. The configuration of the molecule is decisively important for the activity, since EMB with (S,S) -configuration is approx. 200-500 fold more potent than its (R,R)-enantiomer. Removal or significant alteration of the basicity of either amino group resulted in a loss of potency, with the exception that the corresponding amides retained activity in some analogues (Fig. 1. II) [3].

Inspired by the two β-amino-alcohol fragments in the molecule of EMB we dedicated our studies towards the development of camphane based structures and evaluation of their antimycobacterial activity towards M. tuberculosis H37Rv. A series of β-amido-alcohol structures were synthesized using 3-exo-aminoisoborneol (Fig. 1. III) and isobornylamine (Fig. 1. IV) as key starting compounds [4]. Some of the new compounds show 25 times higher activity than the classical anti-TB drug ethambutol. Noteworthy, although that the carbon atom at the nitrogen in all camphane structures possesses (R)-configuration, most of the molecules are extremely active. This is opposite to the fact that (S,S)-EMB is approximately 500 fold more active than (R,R)-EMB. In the present study we describe the synthesis and antimycobacterial activity of a novel, mandelic acid derived diamido-diol containing (1R,2R)-1,2-diamidocyclohexane scaffold (Fig. 1. V). Thus, we had the opportunity to investigate both the effect of a second amide function and a different type of aliphatic skeleton.
Materials and Methods

1. Chemistry

Reagents were commercial grade and used without further purification. Thin layer chromatography (TLC) was performed on aluminum sheets pre-coated with Merck Kieselgel 60 F254 0.25 mm (Merck). Flash column chromatography was carried out using Silica Gel 60 230-400 mesh (Fluka). Commercially available solvents for reactions, TLC and column chromatography were used after distillation. The NMR spectra were recorded on a Bruker AVANCE DRX-250 spectrometer (250.1 MHz for 1H, and 60.9 MHz for 13C NMR) with TMS as internal standards for chemical shifts (δ, ppm). 1H and 13C NMR data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants (Hz), integration, identification. The assignment of the 1H and 13C NMR spectra was made on the basis of DEPT, HSQC, and NOESY experiments. Elemental analyses were performed by Microanalytical Service Laboratory of Faculty of Pharmacy, Medical University of Sofia, using Vario EL3 CHNS(O). Dimethyl sulfoxide (DMSO) for testing of bioactivities was commercial (spectroscopic grade) and was used without distillation.

Preparation of (2S,2’S)-N,N’-((1R,2R)-cy clohexane-1,2-diy1)bis(2-hydroxy-2-phenylacetamide) 3:

To a stirred solution of (1R,2R)-diaminocyclohexane (0.2 g, 1.75 mmol) in 10 ml CH2Cl2 was added (+)-(+)-mandelic acid (0.586 g, 3.85 mmol) followed by EDC (0.738 g, 3.85 mmol) and HOBT (0.589 g, 3.85 mmol). The mixture was stirred at r.t. for 6 days, quenched by pouring into water and extracted with CH2Cl2. The combined organic extracts were washed with 2N HCl, sat. aq. NaHCO3 and brine, dried above Na2SO4 and concentrated under vacuum. The product was purified by flash column chromatography on silica gel (EtOAc/CH2Cl2 = 1:1) to give 0.227 g; 34% Yield of 4 as white crystals; m.p. 199-202 °C.

1H NMR (CDCl3, 250 MHz) δ = 1.15-1.30 (m, 4H, Cy-CH2); 1.69-1.72 (m, 2H, Cy-CH2); 1.86-1.91 (m, 2H, Cy-CH2); 2.30 (brs, 2H, -OH); 3.60-3.63 (m, 2H, -CH-N); 4.62 (s, 2H, -CH-OH); 7.25-7.37 (m, 10H, Ar-H) ppm. 13C NMR (CDCl3, 62.9 MHz) δ = 28.38 (Cy-CH3); 35.86 (Cy-CH3); 57.14 (Cy-CH2); 77.52 (CH-OH), 130.57 (CH-Arom); 132.36 (CH-Arom); 132.58 (CH-Arom); 143.47 (CH-Arom); 177.11 (C=O) ppm. C21H23N2O5 (382.45) calecd. C 69.09, H 6.85, N 7.32; found C 70.81, H 6.67, N 7.37.

2. Antimycobacterial activity

The antimycobacterial activity was determined through the proportion method by Canetti towards reference strain M. Tuberculosis H37Rv. This method, recommended by the WHO, is the most commonly used one worldwide for exploration of sensitivity/resistance of tuberculosis strains towards chemotherapy [5-9]. It allows precise determination of the proportion of resistant mutants to a certain drug.

A sterile suspension/solution of the tested compound was added to Löwenstein Jensen egg based medium before its coagulation (30 min at 85 °C). The compound was tested at four concentrations – 5 mg/ml, 2 mg/ml, 0.2 mg/ml and 0.1 mg/ml (in DMSO). Tubes with Löwenstein-Jensen medium (5 ml) containing the tested compound and those without them (controls) were inoculated with a suspension of M. tuberculosis H37Rv (105 cells/ml) and incubated for 45 days at 37 °C. The ratio between the number of colonies of M. tuberculosis grown in medium containing compounds and the number of colonies in control medium were calculated and expressed as percentage of inhibition. The MIC is defined as the minimum concentration of compound required to inhibit bacterial growth completely (0% growth). The MIC values are calculated and given as μM.

Results and Discussion

Since the trans-1,2-diaminocyclohexane 1 is the backbone of many useful chiral ligands, we became interested in using it as a key starting material for the planned chiral linkage. The commercially available racemic trans diamine [1:1 mixture of (1R,2R)-1,2-diaminocyclohexane and (1S,2S)-1,2-diaminocyclohexane] was separated into the two enantiomers using enantiomerically pure tartaric acid as the resolving agent [10].

Scheme 1.

Mandelic acid 2 was chosen as a building block, since it is a useful precursor to various drugs. This α-hydroxy acid has a long history of use in the medical community as an antibacterial, particularly in the treatment of urinary tract infections [11]. It has also been used as an oral antibiotic, and as a component of ‘chemical face peels’, along with other α-hydroxy acids.
The formation of the amide linkage between (1R,2R)-1,2-diaminocyclohexane 1 and mandelic acid 2 was accomplished by procedure developed for peptide synthesis. The reaction was performed in methylene chloride in the presence of N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole hydrate (HOBT) as coupling reagents to yield 3. The product was obtained in moderate yield and excellent purity after flash column chromatography.

The synthesized compound was evaluated for its in vitro activity against *M. tuberculosis* H37Rv using the method of Canetti. The compound is in agreement with the formal Lipinski’s rule of five. The molecule has low aqueous solubility and high lipophilicity (cLogP = 3.43). The diamide exhibited activity with MIC of 13.07 μM, which is comparable with the one of the reference compound ethambutol dihydrochloride with MIC of 10 μM. In comparison the amide derived from isobornylamine and mandelic acid (Fig. 1. IV) has MIC of 0.17 μM.

Obviously, the replacement of the highly aliphatic camphane skeleton with cyclohexane lead to a drop of the activity. Additionally, although that the carbon atoms at the nitrogens possesses (R,R)-configuration, the molecule is active, which is opposite to the fact that (R,R)-EMB possesses extremely low activity [3]. Therefore, the direct comparison of the structure presented in this paper with EMB should be handled with care.

**Conclusion**

In summary, we have synthesized a new mandelic acid derived diamido-diol on the base of (1R,2R)-1,2-diaminocyclohexane scaffold. The compound was screened for antimycobacterial activity against the H37Rv Strain of *Mycobacterium tuberculosis*. The molecule shows activity comparable with the one of the classical anti-TB drug ethambutol. The data presented in this study suggests that the amido-alcohols are novel promising antimycobacterial agents.

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**References:**


