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Original article

Efficient synthesis of new (*R*)-2-amino-1-butanol derived ureas, thioureas and acylthioureas and *in vitro* evaluation of their antimycobacterial activity

Georgi M. Dobrikov [a,*], Violeta Valcheva [b], Yana Nikolova [a], Iva Ugrinova [c], Evdokia Pasheva [c], Vladimir Dimitrov [a,*]

[a] Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Bl. 9, Acad. G. Bonchev Str., Sofia 1113, Bulgaria
[b] Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Bl. 26, Acad. G. Bonchev Str., Sofia 1113, Bulgaria
[c] Institute of Molecular Biology "Roumen Tsanev", Bulgarian Academy of Sciences, Bl. 21, Acad. G. Bonchev Str., Sofia 1113, Bulgaria

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ABSTRACT

The synthesis of 22 structurally diverse urea, thiourea and acylthiourea derivatives containing the (*R*)-2-amino-1-butanol motif has been performed. The evaluation of their *in vitro* activity against *Mycobacterium tuberculosis* (H_{37}Rv and strain 43) showed promising results in the case of the acylthiourea derivatives (MIC range 0.36–7.46 μM for H_{37}Rv strain).

1. Introduction

Tuberculosis (TB) is one of the most devastating diseases primarily due to several decades of neglect, HIV infection, immigration and globalization [1]. Approximately one-third of the world's population has been infected with the causative organism *Mycobacterium tuberculosis* (MTB), eight million become sick with TB and globally it accounts for approximately two million deaths per year. The spreading of collaborative TB/HIV infections [2] and the reemergence of TB accompanied by an increasing number of drug resistant and multidrug-resistant (MDR) strains MTB (i.e. resistant to at least rifampin [RIF] and isoniazid [INH]) has been noted since the mid-1980s [3–5]. Thus, management of tuberculosis is complicated, which has become a serious health problem worldwide.

The frontline drugs INH, RIF, pyrazinamide (PZA) and ethambutol (EMB) are currently recommended by the World Health Organization (WHO) for the treatment of TB [6]. The problems with current TB treatment are complex and include: a prolonged standard course regimen of six months, which often result in patient noncompliance; emergency of extremely drug-resistant tuberculosis (XDR-TB) strains; lack of effective drugs against the latent state. One approach to decrease treatment time is improvement of potency of currently used anti-tuberculosis drugs [7], mainly through discovery of more effective combinations with newer, more potent and less toxic active compounds [8,9]. There is a clear trend toward gradually increasing partition of new active compounds, including derivatives of known anti TB drugs [10] and natural products [11]. Except of few new chemical entities [12,13] no other anti MDR-TB drugs with proved novel mechanism of action are available in clinical use since last 40 years, but many classes of new potent compounds [13–15] are currently in different steps of their anti TB evaluation.

EMB is a simple (*S*)-2-amino-1-butanol derived 1,2-diamine, clinically used as primarily bacteriostatic anti-tuberculosis agent (Scheme 1) with not fully known mechanism of action. It targets the arabinosyl transferases responsible for arabinogalactan biosynthesis, a key component of the unique mycobacterial cell-wall [16–18]. Despite modest antimycobacterial activity and due to its synergy with other drugs and lower toxicity, EMB is used in combination with more potent frontline antimycobacterial agents. The configuration of

* Corresponding authors. Tel.: +359 2 9606152; fax: +359 2 8700225.
E-mail address: gmdob@orgchm.bas.bg (G.M. Dobrikov).

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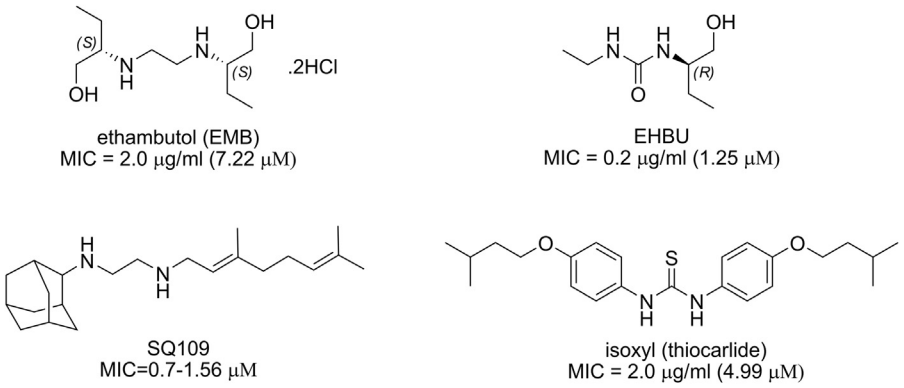

Scheme 1. Representative examples of compounds possessing antimycobacterial activity.

the molecule is decisively important for the activity, since EMB is approximately 200–500 fold more potent than its (*R*,*R*) enantiomer [19].

In recent years diverse derivatives of (*S*)-2-amino-1-butanol and 1,2-ethylenediamine have been synthesized and evaluated in respect of their antimycobacterial activities and mechanisms of action [14,20–23]. One of the most active (up to 35 fold more active *in vitro*) analogues of EMB of late years is 1,2-diamine SQ109 [14,15,21,24] (Scheme 1). This compound possesses low cytotoxicity, excellent pharmacokinetic properties and selectivity. It demonstrates synergistic interactions with some "classic" anti TB drugs, as well. SQ109 and its analogues are currently under the procedures of their clinical evaluation [24,25].

In our recent study [26] the significant role of the chirality has been demonstrated on the example of a series of (*R*)-2-amino-1-butanol derivatives, some being up to 11 times more active than the *S*,*S*-configured EMB. One of the urea derivatives obtained, namely (*R*)-1-ethyl-3-(1-hydroxybutan-2-yl)urea (EHBU, Scheme 1), showed very promising activity and low toxicity together with good water solubility.

A variety of structurally related thioureas (*N*-aryl-*N*′-alkyl and *N*,*N*′-diaryl substituted) have been extensively evaluated against different strains of MTB [27–32] showing valuable activities, sometimes greatly exceeding EMB. One representative example is isoxyl (thiocarlide; 4,4-diisoamyloxydiphenylthiourea; Scheme 1), efficiently clinically used drug since 1960 [33,34]. Recently, the subclass of acylthioureas have been also an object of interest showing promising anti-TB activity, as summarized by Ananthan et al. [35,36].

Taking into account the above presented results, we were encouraged to perform the synthesis of new series of ureas, thioureas and acylthioureas incorporating the (*R*)-2-amino-1-butanol motif and to evaluate their *in vitro* antimycobacterial activity.

2. Results and discussion

2.1. Chemistry

We have optimized efficient pathways to obtain small libraries of chiral ureas, thioureas and acylthioureas. All compounds described have been isolated in pure form and have been characterized by means of NMR, spectroscopy, mass spectrometry and elemental analysis. Detailed description of the experimental procedures and the data obtained are available in Supplementary data.

2.1.1. Synthesis of ureas *15*–*27*, *30*–*31* and thioureas *34*–*35*

The synthesis of compounds **15**–**27** was performed by mixing **1** and the corresponding isocyanates **2**–**14**, respectively, in THF or dichloromethane (DCM) as a solvent (Scheme 2). They were obtained in very high yields and excellent purity. The preparation of **21**, **22** [37], and **24** [38,39] has been described previously, however as racemic mixtures and without evaluation of their bioactivity.

The urea derivative **30** was synthesized from **1** and **28** (Scheme 3) using a published solvent-free procedure [40]. Compound **31** was obtained by reacting **1** with **29** under standard acylation conditions (0 °C and Et_3N in dry DCM). The thioureas **34** and **35** were obtained in high yields in the same manner as ureas **15**–**27**, by mixing **1** and the isothiocyanates **32** and **33**, respectively (Scheme 3). The preparation of **34** has been mentioned earlier [41].

2.1.2. Synthesis of acylthioureas *41*–*45*

The synthesis of compounds **41**–**45** (Scheme 4) was performed by recently published procedure [42] by using one-pot reaction of acylchlorides **36**–**40** with NH_4SCN in the presence of catalytic amounts of PEG-400, followed by addition of **1** to the reaction mixture. The yields were moderate, however the application of this method was easy to perform and convenient in respect of the purification of the desired products (simple filtration through a pad of silica provided excellent pure products).

2.2. Biology

There are no data regarding the antimycobacterial and cytotoxic activity of the 22 newly synthesized compounds.

2.2.1. In vitro antimycobacterial activity

The synthesized compounds were evaluated for their *in vitro* activity against *M. tuberculosis* $H_{37}Rv$ and MDR strain 43 (Table 1; results are recalculated in μM) using the method of Canetti (see Section 4.2). All the compounds synthesized are in agreement with the formal Lipinski's rule of five. The first 17 derivatives of (*R*)-2-amino-1-butanol – ureas **15**–**27**, **30**–**31** and thioureas **34**–**35** (Schemes 2 and 3) were inactive against *M. tuberculosis* $H_{37}Rv$ even at concentrations of 5 μg/ml (100% growth of the bacteria was observed). The only observed exception was compound **19**, showing activity close to EMB. It is interesting to point out, that even a small structural change in the molecule EHBU [26] (Scheme 1) induce lack of activity. For example, its inactive near homologues possess propyl (**16**), *n*-butyl (**17**) and *t*-butyl (**15**) groups. Replacement of the carbonyl group with thiocarbonyl (thioureas **34**–**35**) leads to the same consequences. Similar negative trend was observed for many derivatives of EMB [7,19].

Other series of five new acylthioureas **41**–**45** (Scheme 4) was designed to contain important pharmacophore groups (discovered in our previous study [26]), attached to acylthioureas containing (*R*)-2-amino-1-butanol moiety. Compounds **41** and **43**–**45** showed