ANTIMYCOBACTERIAL ACTIVITY OF 4- AND 3,4-SUBSTITUTED COUMARINS

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Summary: The present article describes the evaluation of the in vitro activity against M. tuberculosis H37Rv of eight coumarin-derived aminoalcohols and amidoamines. Seven of the compounds display activity between 10 and 20 times higher than the classicall anti-TB drug ethambutol. The combination of coumarin scaffold with aminoalcohol fragments gave the most promising activities with MICs up to 0.32 μM.

Key words: aminocoumarins, aminoalcohols, amidoamines, tuberculosis

Introduction

Tuberculosis (TB) still remains a global health problem, claiming approximately 1.3 million lives per year [1]. The negative socioeconomic impact and human suffering in the context of diagnosis and treatment of TB and multidrug-resistant (MDR) TB necessitates the search for new anti-mycobacterial agents with novel structures and mode of action [2,3]. Although, many drug candidates are already in clinical development [4], few new anti-TB drugs have been introduced during the last 30 years.

Coumarin is the scaffold of a large class of synthetic and naturally occurring compounds with diverse biological activities [5]. The 2H-1-benzopyran-2-one can be considered as one of the most important heterocyclic structures due to its cell membrane permeability and readily interaction with diversity of enzymes and receptors [5,6]. Additionally, coumarin scaffolds are part of the molecules of licensed for clinical use drugs, such as novobiocin and warfarin [7,8]. In recent studies natural occurring coumarins have been reported to possess potent anti-mycobacterial activity [9,10]. Noteworthy, (+)-Calanolide A was found to be extremely active against M. tuberculosis H37Rv strain and a number of MDR-TB strains [11,12].

Inspired by the anti-tubercular activity of all above mentioned compounds, we have evaluated the antimycobacterial activity of a set of 3- and 3,4-substituted coumarins with aminoalcohol and amidoamine functionalities. This work is a continuation of our efforts on the development of molecular templates as novel anti-mycobacterial agents.

Materials and Methods

The antimycobacterial activity was determined through the proportional method of 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 chemotherapeutics [13-16].

A sterile suspension/solution of each tested compound was added to Löwenstein-Jensen egg based medium before its coagulation (30 min at 85 °C). Each compound was tested at four concentrations – 2 μg/ml, 0.2 μg/ml, 0.1 μg/ml and 0.05 μg/ml in DMSO. Tubes with Löwenstein-Jensen medium (5 ml) containing tested compounds 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

The synthesis and characterization of all tested compounds was reported elsewhere [17]. The procedures are briefly summarized in Scheme 1. In summary, reaction of 4-hydroxycoumarin with aminoalcohols in ethanol resulted in N-substituted 4-aminocoumarins 1 and 2. The same reaction but with
Table 1. Structures and antimycobacterial activity of the tested compounds

<table>
<thead>
<tr>
<th>Cmpd No</th>
<th>Structure</th>
<th>MW</th>
<th>Anti-MTB(^a) MIC (µM)</th>
<th>cLog P(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(\text{NHCH}_2\text{CH}_2\text{OH})</td>
<td>205.21</td>
<td>0.97</td>
<td>1.34</td>
</tr>
<tr>
<td>2</td>
<td>(\text{NHCH}_2\text{CH(OH)}\text{CH}_3)</td>
<td>219.24</td>
<td>0.91</td>
<td>1.65</td>
</tr>
<tr>
<td>3</td>
<td>(\text{NH(CH}_2\text{)}_2\text{NHCOCOH}_3)</td>
<td>246.26</td>
<td>0.81</td>
<td>1.30</td>
</tr>
<tr>
<td>4</td>
<td>(\text{NH(CH}_2\text{)}_3\text{NHCOCOH}_3)</td>
<td>260.29</td>
<td>19.2</td>
<td>1.61</td>
</tr>
<tr>
<td>5</td>
<td>(\text{NH}(_2\text{)}_2\text{OH})</td>
<td>276.29</td>
<td>0.36</td>
<td>-0.50</td>
</tr>
<tr>
<td>6</td>
<td>(\text{NH}(_2\text{)}_3\text{OH})</td>
<td>304.34</td>
<td>0.32</td>
<td>0.39</td>
</tr>
<tr>
<td>7</td>
<td>(\text{NHCH}_2\text{CH(OH)}\text{CH}_3)</td>
<td>247.25</td>
<td>0.80</td>
<td>1.12</td>
</tr>
<tr>
<td>8</td>
<td>(\text{NH}(_2\text{)}_2\text{OH})</td>
<td>233.22</td>
<td>0.85</td>
<td>1.07</td>
</tr>
<tr>
<td>9</td>
<td>EMB.2HCl(^d)</td>
<td>233.22</td>
<td>7.22</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Antimycobacterial activity towards reference strain of *M. tuberculosis* H37Rv; \(^b\) cLog P, calculated octanol-water partitioning coefficient; \(^d\) Ethambutol dihydrochloride (reference compound).
diaminoalkanes in acetic acid gave 4-aminocoumarin acetamides 3 and 4. N'-Acetylation of the second amino group took place in both cases. Vilsmeier-Haack formylation of the starting compound gave 4-chlorocoumarin-3-carbaldehyde, which could be transformed into aminoalcohol functionalized coumarins 5-7. Finally, hydrolysis of 4-chlorocoumarin-3-carbaldehyde afforded 4-hydroxycoumarin-3-carbaldehyde, which was reacted with 2-aminoethanol to give aminoalcohol 8 (Scheme 1).

![Scheme 1. Synthesis of the compounds](image)

All compounds are in agreement with the formal Lipinski’s rule of five. Evident from the calculated Log P values the compounds have acceptable water solubility, a feature which conditions bioavailability and presumably good pharmacokinetic behavior (Table 1). Additionally the molecular mass of the compounds is around 300, they do not possess more than five hydrogen bond donors, and do not possess more than ten hydrogen bond acceptors.

The compounds were evaluated for their in vitro activity against the reference strain *M. tuberculosis* H37Rv using the method of Canetti (Table 1). Except amidoamine 4, which was not active, all other structures displayed profound anti-TB activity. It is known from the structure of ethambutol that the existence of β-aminoalcohol fragment is crucial for its activity. In the case of 4, the two electron donors are separated by three carbon atoms, which can be an explanation for its high minimal inhibitory concentration (MIC). Five of the compounds 1-3, 7, 8 have shown activity of 0.8-0.9 μM, which is around 10 times higher than that of ethambutol 9, used as reference (Table 1). In the case of 1,2,7,8 exists β-aminoalcohol fragment in the structure, which is in analogy with ethambutol. In the case of 3 the amino group is combined with amide functionality, in a way that the two electron donors are in β-position to each other, again analogously to ethambutol. The aminoalcohols 5 and 6 displayed even higher activity with MICs of 0.36 and 0.32 μM, respectively. Obviously, the combination of aminoalcohol fragment on the 4th position and iminoalcohol fragment on the 3rd position of the coumarin scaffold enhanced the activity.

**Conclusion**

A small series of coumarin derivatives was evaluated for anti-mycobacterial activity against *M. tuberculosis* H37Rv. Seven of the compounds have shown excellent activity, between 10 and 20 times higher than the classical anti-TB drug ethambutol. The presence of aminoalcohol fragment in the structure of the tested compounds appeared to be crucial for their activity. The best result was displayed by the compounds bearing aminoalcohol fragment on the 4th positions and iminoalcohol fragment on the 3rd positions of the coumarin structure. The coumarins can be considered as promising scaffolds for the development of novel anti-mycobacterial agents.

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**References**

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