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Synthesis and Anti-mycobacterial evaluation of coumarin derivatives

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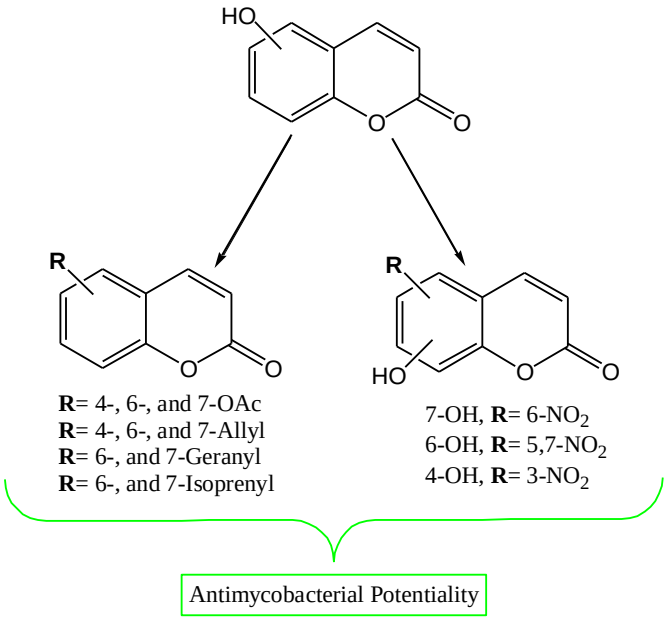
Graphical Abstract



Abstract.

Tuberculosis is one of the oldest known diseases of the world, remaining, within today, with the higher mortality rates caused by a single infectious agent, mainly represented by *Mycobacterium tuberculosis*. It's considered a greater public health problem, for reaching millions of people around the world, mainly in the developing countries, also highlighting their co-infection cases in immunocompromised patients, the example of HIV positive. Their high incidence rates are related to the non-adhesion to, and abandonment of, available treatment, due to its prolonged administration time, which allowed the appearance and increase of resistant strains causing tuberculosis. This evidences the need for the discovery and development of new drugs more efficient and powerful against pathogenic species of *Mycobacterium* genus. Classes of natural products, such as coumarins, have shown themselves to be powerful antimycobacteria agents in their evaluation against

Mycobacterium strains. Thus, this work shows the synthesis, from protocols of *O*-Alkylation, *O*-Acetylation and Nitration well described in the literature, of coumarin derivatives, in good to greater yields, in most cases. This semi-synthetic derivatives, together with some commercial coumarins were evaluated according their antimycobacterial activities against *M. tuberculosis* H37Rv strains, where all compounds demonstrated actives against the tested strains, inhibiting the growth of *M. tuberculosis* after eight weeks of observation (in in 100 μg/mL), being more active than standard-drug used, Isoniazid, which non-inhibiting the growth of pathogens after the fourth week. Showing themselves as therapeutic alternatives in the development of new antimycobacterial active compounds.

Introduction

Historically, tuberculosis is one of the oldest known diseases, remaining, within today, with the higher mortality rates caused by a single infectious agent[1], which is represented by species of the genus *Mycobacterium*, mainly by microorganisms of the *Mycobacterium tuberculosis* specie, and, secondarily, by *M. bovis* and *M. africanum* species[1-5]. This disease is characterized by affecting the airways of infected patients, being responsible for causing the death of about 2 million of people for year, all around the world[6,7], besides 8 million additional cases annually, especially in developing countries[8]. According to World Health Organization (WHO), these numbers should continue to grow in the coming years, if control of this is not strengthened[8].

This high incidence rate of tuberculosis is often related to co-infection in immunocompromised patients, such as individuals afflicted with HIV[9], to the point of being registered, by WHO, in the period from 2000 to 2006, about 700,000 cases of tuberculosis in Brazil, of which 60,000 infected they died, being 20% of these associated to patients co-infected with the HIV virus[2,8].

Their high incidence numbers made with that tuberculosis to be treated as a great public health problem, mainly by high rates of non-adherence to, and abandonment of, available treatments and/or by the appearance of multi-drug resistant strains of tuberculosis (MDR-TB)[6]. Failure to realization or complete treatment, considered as prolonged administration, appear as the main causes of the onset of mycobacterial resistance against the drugs used, as Isoniazid and Rifampicin, or combination of these with Ethambutol or Pyrazinamide, for example[10,11].

This facts reinforce the great need of development of potent new anti-tuberculosis agents more efficient and secure and with therapies of shorter duration when related at drugs currently utilized[7,12].

In this aspect, natural compounds and their derivatives has been shown as potent against *Mycobacterium sp.* Strains[7,12], such as coumarins, which are characterized for being formed by the fusion between benzene and α-pyrone rings[13], and are widely used according to their biological

potentialities, highlighting as antibacterial[14], antifungal[15], anti-inflammatory[16], antitumor[17], anticoagulant[18], antimycobacterial[19], among others.

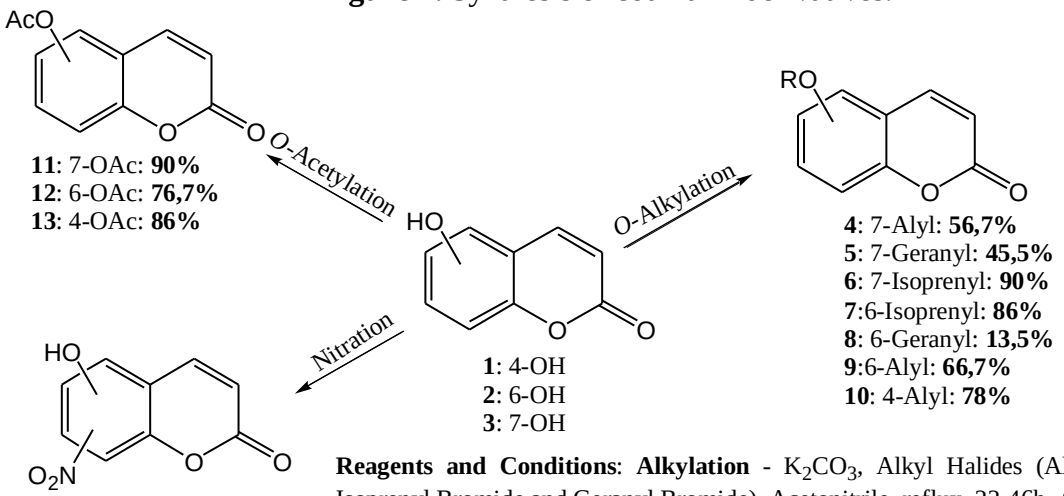
In view of the greater need in the discovery of new compounds with antimycobacterial potentiality, and the greater variety of biological activities of coumarin compounds, the aim of this work was the synthesis and evaluation of the antimycobacterial potential of coumarin derivatives against *M. tuberculosis* strains.

Materials and Methods

Synthesis of coumarin derivatives

The semi-synthetic coumarin derivatives was obtained through of standard procedures for *O*-alkylation, *O*-acetylation and Nitration of commercial coumarins (4-hydroxy-2*H*-1-benzopyran-2-one (**1**), 5-hydroxy-2*H*-1-benzopyran-2-one (**2**) and 7-hydroxy-2*H*-1-benzopyran-2-one (**3**)) (Figure 1), and previously described[15].

Figure 1. Synthesis of coumarin derivatives.

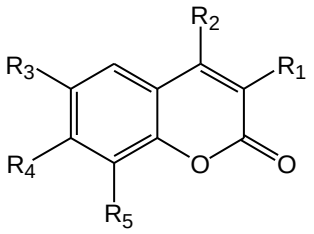


Reagents and Conditions: **Alkylation** - K_2CO_3, Alkyl Halides (Alyl Bromide, Isoprenyl Bromide and Geranyl Bromide), Acetonitrile, reflux, 22-46h. **Acetylation** - Acetic Anhydride, Pyridine, ultradound, room temperature, 15-30 min. **Nitration** - Nitric/Acetic Acids, Ice bath - room temperature, 120 min.

All final compounds had their structures proven by ^{1}H and ^{13}C NMR and physicochemical characteristics described[15].

Commercial coumarin derivatives also were analyzed according to their antimycobacterial activity (Figure 2).

Figure 2. Commercial coumarin derivatives analyzed.



17: $R_1=R_2=R_3=R_4=R_5=H$
18: $R_1=OH$, $R_2=R_3=R_4=R_5=H$
19: $R_1=R_2=R_5=H$, $R_3=R_4=OH$
20: $R_1=COOH$, $R_2=R_3=R_4=R_5=H$
21: R_1=Bis-4-OH-Coumarin, $R_2=OH$,$R_3=R_4=H$
22: $R_1=R_2=R_5=H$, $R_3=OMe$, $R_4=OH$
23: $R_1=R_2=H$, $R_3=OMe$, $R_4=R_5=OH$

Antimycobacterial Activity Assays

The antimycobacterial activity assays were performed using a concentration of 100 µg/mL of the test drugs in Löwenstein-Jensen medium. The H37Rv *Mycobacterium tuberculosis* strains were diluted in distilled water (Mc Farland scale – $3x10^8$ microrganisms/mL) and inoculated in the media containing the drugs to be tested. The controls were composed of simple and in the presence of

isoniazide in the concentration to 0.2 µg/mL Löwenstein-Jensen medium. The containers were placed in stove at 37°C, where were observed once a week until the 8th week for the analysis of bacterial growth or not. The reference drug used was isoniazid.

Results and Discussion

The semi-synthetic coumarin derivatives were obtained from *O*-alkylation, *O*-acetylation and Nitration procedures since 4-, 6- and 7-hydroxylated commercial coumarins, as previously described[15] in good to greater yields, in most cases, as demonstrated in Figure 1.

As demonstrated in the Table 1, all coumarin derivatives analyzed were able of inhibit the growth of pathogenic strains of *M. tuberculosis* after the observation period of eight weeks, demonstrating values more active than the standard-drug used, Isoniazid, which did not inhibit the *M. tuberculosis* growth after the 4th week.

Table 1. Inhibition of *M. tuberculosis* growth in the presence of the coumarin derivatives analyzed.

Drug/Week	1ª	2ª	3ª	4ª	5ª	6ª	7ª	8ª
1	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
2	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
3	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
4	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
5	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
6	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
7	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
8	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
9	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
10	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
11	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
12	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
13	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
14	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
15	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
16	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
17	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
18	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
19	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
20	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
21	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
22	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
23	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Isoniazid	(-)	(-)	(-)	(+)	(+)	(+)	(+)	(+)

(-) - Inhibition of the pathogen growth; (+) - Non-inhibition of the pathogen growth

This results demonstrated the antimycobacterial potential of coumarin derivatives as growth inhibitors of *M. tuberculosis*, main etiological agent causing tuberculosis in humans, can present themselves as an alternative for the treatment of infections by this pathogen. Confirming the potentiality of coumarins against *Mycobacterium* pathogen strains results showed in the literature.

Conclusions

These results allowed the synthesis of semi-synthetic coumarin derivatives by *O*-Alkylation, *O*-Acetylation and Nitration in good to excellent yields, in most cases, which were evaluated according to their antimycobacterial activity against *Mycobacterium tuberculosis*, where all semi-synthetic and

commercial derivatives to be proved potential inhibitors of the *M. tuberculosis* growth. Can be an important alternative in the search of new antimycobacterial drugs.

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