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Laboratory note

A macrolactone from benzo[*a*]phenazine with potent activity against *Mycobacterium tuberculosis*

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Abstract

We report here an alternative to the MCPBA or ozonolysis-based oxidation methods of quinoxaline-featuring compounds prepared from beta-lapachones. The use of peracetic acid allowed a simple preparation of the corresponding macrolactones by cleavage of the ring system. These lactones were evaluated for their antimycobacterial potential and compound **4** turned out to have an MIC of 0.62 μg per mL on *Mycocabteriumtuberculosis* H37Rv. These results justify further research into its value as a potential lead for an original treatment of tuberculosis.

Keywords: Lapachol; Phenazines; Peracetic acid oxidation; Macrolactones; Tuberculosis

1. Introduction

Tuberculosis (from now on abbreviated as TB) is one of the most severe infections affecting human beings due to its high morbidity and mortality, and therefore a threat to over billions of people living in areas of high incidence. This disease, caused by *Mycobacterium tuberculosis*, according to the World Health Organization (WHO), is one of the most deadly infectious diseases of today. Worldwide, there are already 14 million people infected, mainly in third world and developing nations [1,2]. Furthermore, a rising number of people in the developed world are contracting TB because their immune systems are compromised by immunosuppressive drugs, substance abuse, or HIV/AIDS [3,4].

The present work stems from a biological guided screening program aiming at the use of heterocyclic derivatives synthesized from the readily available lapachol **1**. This naturally occurring compound is isolated from *Tabbebuia* sp., a species which is abundant in the Brazilian flora. Previous reports describing our results focusing on other endemic infections such as Chagas disease have already been published [5–8]. We wish to report here an improved preparation of compounds **4**, **7** and **10** we previously synthesized with the use of MCPBA or ozonolysis-based oxidation methods [9–11].

In the course of our research on the valorisation of the naturally occurring lapachol, we have prepared in three steps, via an improved oxidation of the quinoxaline ring system, the corresponding macrolactone which is demonstrating high level of antimycobacterial activity.

2. Chemistry

As shown in Scheme 1, the quinoxaline-featuring compounds **3**, **6**, **9** and **12**, were prepared from the corresponding orthoquinones **2**, **8**, **11** readily obtained from beta-lapachones **1** [12]. Their oxidation using the acetic anhydride, hydrogen peroxide and chloroform mixture gave cleaner reactions which

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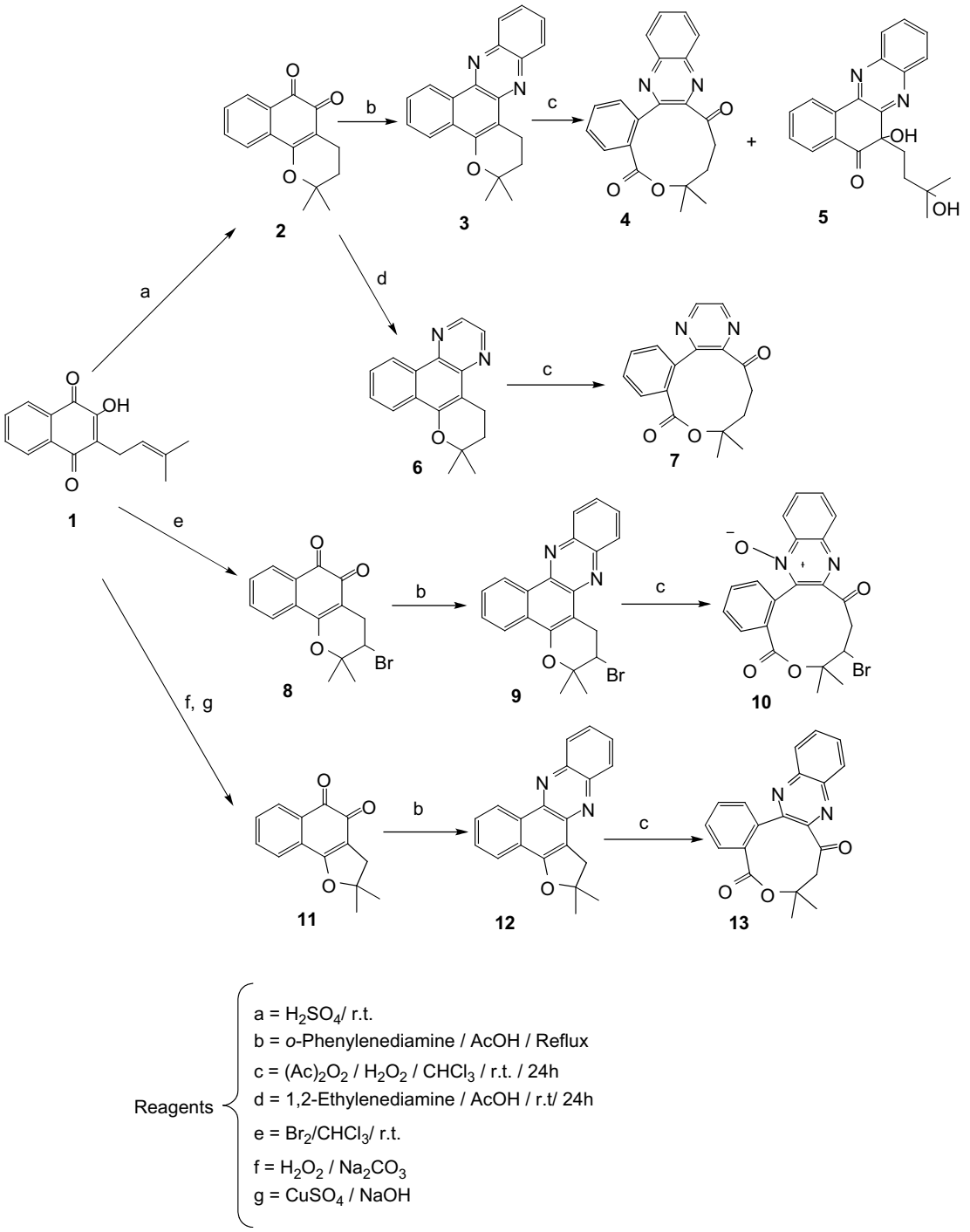

Scheme 1. Synthesis of the macrolides since lapachol.

also allowed the recovery of unreacted substrates. Briefly, the peracid was generated in situ, stirring a mixture of acetic anhydride, hydrogen peroxide and chloroform (1:1:2, v:v:v), 1 mmol of substrate was previously dissolved In the chloroform. This biphasic system was stirred at room temperature for 24 h. This method allowed the preparation of the previously unreported 9-membered lactone **13** in a 70% yield and allowed the preparation of the known compounds **4**, **5**, **7** and **10** in 10, 62, 68 and 33% yield, respectively.

Though the peracetic acid oxidation, here presented, was not selective for the synthesis of **4**, giving it with low yield (10%), we have already published a selective method by ozonolysis for its synthesis with good yield (52%) [10].

3. Results and discussion

Discovery of new types of molecules with antimicrobacterial activity is necessary due to emerging of multidrug-resistant

M. tuberculosis. Recently quinoxalinic and phenazinic compounds had emerged as prototype anti-tubercular drugs [13–16]. The activities against *M. tuberculosis* of the compounds synthesized in the present work are shown in Table 1.

The oxidation of **3** furnished two compounds: macrolide **4** and α-hydroxy-ketone **5**, both already described in the literature, prepared by MCPBA oxidation [9]. Quinoxaline **6** gave **7** with 68% yield. We have already reported the synthesis of **7** by MCPBA oxidation with its corresponding α-hydroxy-ketone, but with a low yield [11]. The bromine-*N*-oxide, macrolactone **10**, was obtained with 33% yield, three times higher than that obtained by MCPBA oxidation [10].

The peracetic acid oxidation allowed to synthesize **13** with good yield (70%). The reaction with MCPBA or ozone did not allow this synthesis. MCBBA oxidation of **12** gave only its phenazine-*N*-oxide [17], while the ozonolysis gave a complex mixture of products. Except for **4**, the peracetic acid oxidation, described here, was a more effective methodology for the synthesis of these macrolides. MCPBA oxidation gave in all cases a complex mixture of products, thus making it very hard to isolate these productions.

Initial assays against *M. tuberculosis* were performed at 100 μg/mL, the MIC 90 was then determined for the most active. The lactone 4 turned out to display an MIC of 0.62 μg/mL, which is better than that the MIC of rifampicin (1.0 μg/mL), one of the standard drugs used in TB chemotherapy.

4. Conclusion

The level of antimycobacterial activity of macrolactone **4** is of interest which justifies laboratory test for citotoxical activity. The current rise of *M. tuberculosis* strains resistant to the current treatments is a major concern. We hope that our initial results may provide a lead toward the design of an original drug which would remain active on these resistant strains.

5. Experimental protocols

The NMR experiments were performed in a Bruker AVANCE DRX-400 instrument, using deuterochloroform as solvent, and TMS as internal standard. Infrared spectra were recorded on a Perkin–Elmer FT-IR Spectrometer. For elemental analysis, a Perkin–Elmer CHN 2400 was used.

The physical and spectroscopic data of compounds **4**, **5**, **7** and **10** are in accordance with the ones described in the literature [6–8]. The new compound **13** had its structure deduced by physical methods (^{1}H, ^{13}C NMR, IR) and elementary analysis.

Table 1
Antimycobacterial activity of the compounds **4**, **5**, **7**, **10** and **13**

Compounds	*M. tuberculosys*, MIC (μg/mL)
4	0.62
7	50
10	25
13	100
5	Resistant
Rifampicin	1.0

5.1. General procedure for the synthesis of *4, 5, 7, 10*

To a solution of 1 mmol of substrates (**3**, **6**, or **9**) in 40 ml of chloroform, 20 ml of solution of hydrogen peroxide (30% v/v) was added, followed by 20 ml of acetic anhydride. The biphasic system has stirred at room temperature for 24 h. The organic phase was separated and consecutively washed with a solution of $Na_2S_2O_3$ 5% (2 × 50 ml), followed by a solution of Na_2CO_3 5% (2 × 50 ml). The organic phase then was dried over anhydrous Na_2SO_4, filtered and evaporated under vacuum. In each case, the solid residues were submitted to column chromatography over silica gel and eluted with mixtures of hexane–ethyl acetate with increasing gradient of polarity. The unreacted substrate was recovered in all cases with a hexane–ethyl acetate mixture at 2%. The macrolactones were eluted from the column with a mixture containing 10% of ethyl acetate. In the oxidative reaction of phenazine **3** polar compound **5** was eluted in a polarity corresponding to 40% of ethyl acetate.

5.2. Synthesis of new compound *13* (7,7-dimethyl-7,8-dihydrobenzo[3,4]oxonino[6,7-b]quinoxaline-5,9-dione)

To a solution of 300 mg (1 mmol) of **12** in 40 ml of chloroform, 20 ml of solution of hydrogen peroxide (30% v/v) was added, followed by 20 ml of acetic anhydride. The biphasic system has stirred at room temperature for 24 h. The organic phase was separated and consecutively washed with a solution of $Na_2S_2O_3$ 5% (2 × 50 ml), followed by a solution of Na_2CO_3 5% (2 × 50 ml). The organic phase then was dried over anhydrous Na_2SO_4, filtered and evaporated under vacuum. The solid residue was submitted to column chromatography over silica gel and eluted with mixtures of hexane/ethyl acetate with increasing gradient of polarity. The unreacted substrate was recovered with a hexane–ethyl acetate mixture at 2%. The macrolactone **13** was eluted from the column with a mixture containing 10% of ethyl acetate, 232 mg yielding 70%.

Coumpound **13**: colorless crystals, mp: 192 °C; ^{1}H NMR 400 MHz [$CDCl_3$, δ (ppm), J (Hz)] δ: 8.22 (m, 1H), 8.13 (m, 1H), 8.08 (dd, $J = 7.8$ Hz, $J = 0.8$ Hz, 1H), 7.93 (dd, $J = 7.7$ Hz, $J = 1.2$ Hz, 1H), 7.86 (m, 2H), 7.73 (td, $J = 12.1$ Hz, $J = 7.5$ Hz, $J = 1.2$ Hz, 1H), 7.56 (td, $J = 7.5$ Hz, $J = 7.5$ Hz, $J = 1.2$ Hz, 1H), 3.91 (d, $J = 14.5$ Hz, 1H), 3.48 (d, $J = 15.5$ Hz, 1H), 1.95 (s, 3H), 1.02 (s, 3H); ^{13}C NMR 100 MHz ($CDCl_3$) δ: 200.05 (s), 168.21 (s), 153.03 (s), 150.52 (s), 142.01 (s), 138.21 (s), 136.52 (s), 133.47 (d), 133.01 (d), 132.98 (s), 131.15 (d), 130.80 (d), 130.31 (d), 129.91 (d), 129.63 (d), 128.79 (d) 82.88 (s), 51.86 (t), 30.55 (q), 30.12 (q); Anal. calcd: $C_{20}H_{16}N_2O_3$: C 72.28, H 4.85, N 8.43; Found: C 72.21, H 4.91, N 8.40.

5.3. Antimicobacterial activity

To test its antimicobacterial activity, the primary screening was conducted at 100.0 μg mL^{-1} against *M. tuberculosis* (ATCC 27294 H_{37}Rv) in BACTEC12B medium using the

Microplate Alamar Blue Assay (MABA) [18]. Compounds exhibiting fluorescence were tested in the BACTEC 460 radiometric system [19]. Compounds showing 90% inhibition in the primary screening were considered active, and then retested at a lower concentration against *M. tuberculosis* (ATCC 27294 $H_{37}Rv$) in order to determine the actual MIC, using MABA. Rifampicin was used as the reference compound (MIC 1.0/μg mL^{-1}).

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