Graphical Abstract

N-Oxide heterocycles and imidazoles replacing ring D of calanolides against *Mycobacterium Tuberculosis*

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We have explored the chemistry of *N*-Oxide heterocycles and imidazoles replacing ring D of the natural product (+)-Calanolide A, and have synthesized 12 new analogues, Two of which were active against both R *Mtb* and NR *Mtb* with MIC values of 12.5 $\mu\text{g/mL}$, which would lead to further optimization for more potent anti-TB candidates.

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ABSTRACT

We have explored the chemistry of *N*-Oxide heterocycles and imidazoles replacing ring D of the natural product (+)-Calanolide A to develop anti-TB reagents, and have synthesized 12 new analogues. Two of the new bioreductive compounds were active against both R *Mtb* and NR *Mtb* with the same MIC values of 12.5 $\mu\text{g/mL}$, which would lead to further optimization for potent anti-TB candidates.

Keywords:

Mycobacterium tuberculosis

(+)-Calanolide A

N-Oxide heterocycle

Benzoimidazole

Bioreductive drug

1. Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*Mtb*), is one of the world's deadliest communicable diseases. (+)-Calanolide A is known to be an active natural product against *Mtb* [1]. Its analogues including **1a** and **1b**, which we synthesized recently, were demonstrated to be active against both replicating (R) and non-replicating (NR) *Mtb* (Fig. 1) [2].

Bioreductive drugs are prodrugs that are activated by specific reductases followed by conversion into potent cytotoxins, which in turn damage critical cellular functions. This concept has been clinically applied in specifically designed systems to target hypoxic tumor cells for their radiotherapy response [3,4]. Nitroimidazoles, nitrofurans, nitrobenzene and quinoxaline-di-*N*-Oxides represent four new chemical scaffolds of anti-TB reagents with significant bactericidal activity *via* intracellular bioreducing to reactive free radical species, as well as with activity against NR *Mtb* [5,6]. As the quinoxaline-di-*N*-oxides with no nitro group, they might be safer and more druggable candidates comparing to other three scaffolds.

Meanwhile, other *N*-Oxide heterocyclic derivatives *e.g.*, benzoimidazole-*N*-Oxides are active on tumor cell, and benzofuroxan-*N*-Oxides are potent antitrypanosomal reagents [7,8]. Therefore, we designed benzoisoxazole-*N*-Oxides (**7a**, **7b**), benzofuroxan-*N*-Oxides (**15a**, **15b**) and benzoimidazole-*N*-Oxides (**16a**, **16b**, **16f**) incorporated with calanolides, aiming to explore possible new bioreductive anti-TB lead compounds (Fig 2).

Moreover, for the purpose of anti-TB activities of benzoimidazoles [9,10] as well as comparison with bioreductive calanolides, we designed imidazole replacing ring D of calanolides (**17a-e**) following the same synthesis route with benzoimidazole-*N*-Oxide calanolides.

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MIC Value: (+)-Calanolide A :3.1 $\mu\text{g/mL}$ (R), ND (NR); **1a**: <0.1 $\mu\text{g/mL}$ (R), 0.2 $\mu\text{g/mL}$ (NR); **1b**: 0.08 $\mu\text{g/mL}$ (R), 0.08 $\mu\text{g/mL}$ (NR)
 SI: (+)-Calanolide A : 2.4; **1a**: >510; **1b**: 10-19

Fig. 1 (+)-Calanolide A and its analogues, R and NR denote replicating and non-replicating *Mtb*, respectively.

Fig. 2 Designed calanolide analogues

2. Experimental

Analogues **7** were synthesized following the route shown in Scheme 1. Compounds **3a** (or **3b**) were prepared from phloroglucinol (**2**) using the Pechmann reaction which gave the desired product in 96% (or 90%) yield. Compound **3a** (or **3b**) was in turn treated with acetic anhydride and aluminium chloride according to a published procedure to provide the regioselective product **4a** (or **4b**) [11] which was subsequently cyclized to form intermediate **5a** (or **5b**) in 58 (or 60%) yield. Compound **5a** (or **5b**) was then treated with hydroxylamine hydrochloride to provide compound **6a** (or **6b**) in 85% (or 83%) yield. HTIB-catalyzed cyclization of **6a** (or **6b**) gave the anticipated *N*-oxide isoxazole **7a** (or **7b**) in 75% (or 80%) yield. For the last step, we have tried cyclized oxime instead of ketoxime **6**, but with no success (Scheme 1).

Scheme 1. Synthesis of target compounds **7a, b** containing the *N*-Oxide ring moiety. Reagents and conditions: (a) $\text{C}_3\text{H}_7\text{COCH}_2\text{COOEt}$ (or $\text{C}_3\text{H}_7\text{COCH}_2\text{COOEt}$), $\text{BF}_3 \cdot \text{Et}_2\text{O}$, reflux, 5 h, 96% (90%); (b) Ac_2O , AlCl_3 , 100 $^\circ\text{C}$, 1 h; (c) $(\text{CH}_3)_2\text{CCHCH}(\text{OEt})_2$, pyridine, toluene, reflux, 3 h, 58% (60%); (d) $\text{NH}_2\text{OH} \cdot \text{HCl}$, AcONa , $\text{CH}_3\text{CH}_2\text{OH}$, reflux, 3 h, 85% (83%); (e) HTIB, MeOH , r.t., 30 mins, 75% (80%).

Analogues **15**, **16** and **17** were synthesized following the route shown in Scheme 2. We converted **8**, which was obtained starting from **3a** using a reported method [12], to **9a** through methylation of phenol or used the Mitsunobu reaction to introduce 5-morpholinoethyl to obtain crude product **9b**. Deprotection of the 7-OTs on **9a** (or **9b**) provided **10a** (or **10b**) which was then reacted with TiF_2O in pyridine to yield **11a** (or **11b**). Treatment of **11a** (or **11b**) with diphenylmethanimine followed by hydrolysis with aqueous HCl gave intermediate **13a** (or **13b**) in 77% (or 73%) yield. Selective nitration on **13a** (or **13b**) provided **14a** (or **14b**) that contain the *o*-Nitroaniline moiety. **14a** (or **14b**) were cyclized to provide **15a** (or **15b**) catalyzed by $\text{PhI}(\text{OAc})_2$ in a yield of 75% (or 66%), which in turn reacted with 1,3-dicarbonyl compounds to produce the anticipated *N*-Oxide imidazoles **16a**, **16b** and **16f** in yields of 60%, 55% and 60%, respectively. By reducing the nitro group of **14** and cyclization with different aldehydes, we obtained the final products **17a-e**

in yields of 60%, 58%, 59%, 55% and 58%, respectively (Scheme 2).

Scheme 2. Synthesis of target compounds **15** and **16** containing the *N*-Oxide ring moiety. Reagents and conditions: (a) For **9a**: Me₂SO₄, K₂CO₃, acetone, reflux, 3 h, 92%; for **9b**, *N*-hydroxyethyl morpholine, DEAD, PPh₃, THF, r.t., 3 h; (b) 3 equiv. TBAF, THF, r.t., 3 h, 88% (or 85%); (c) Tf₂O, pyridine, CH₂Cl₂, 0 °C, 30 mins, 79% (or 66%); (d) diphenylmethanimine, Pd(OAc)₂, BINAP, Cs₂CO₃, Ar, dioxane, 100 °C, 5 h; (e) 2 mol/L HCl, THF, r.t., 12 h, 77% (or 73%); (f) nitrocyclohexadienone, CF₃COOH, 0 °C, 2 h; (g) PhI(OAc)₂, acetone, r.t., 3 h, 75% (or 66%); (h) for **16a**, **16b**, *N,N*-dimethylacetamide, 2-aminoethanol, CaCl₂, MeOH, r.t., 3 h, 60% (or 55%); for **16f**, ethyl acetoacetate, Et₃N, CaCl₂, EtOH, microwave, 1 h, 60%; (i) 1) Pd/C, HCOONH₄, THF/MeOH, r.t., 3 h, 2) aldehyde, AcOH, r.t., 3 h, 55-60%.

3. Results and discussion

In the process of synthesizing compounds **15**, **16** and **17**, nitration of **13** to obtain intermediates **14** is the key step. Because the alkoxy and amino groups supply the coumarins with an electronic rich region, the nitration is not practical using the traditional nitric acid/sulfuric acid condition, indicating that a mild and weakly oxidative nitration reagent should be used. Alternatively, we chose nitrocyclohexadienones [13,14] and successfully synthesized **14**. For the next step, because of the larger coumarin scaffold of the substrates, the products **15** would be *N*-Oxide imidazoles rather than quinoxaline-di-*N*-Oxides from the Beirut reaction [15]. We confirmed the nitration site at 8-coumarin using the NOE test on **17a** (See Supporting information).

All of the final 12 synthesized compounds were used in an anti-*Mtb* whole-cell screening with both R *Mtb* and NR *Mtb*. NR *Mtb* was tested under a combination of four physiologic conditions: hypoxia (1% O₂), mild acidity (pH 5.5), nitrosative stress (0.5 mmol/L NaNO₂), and restriction of the major carbon source to a fatty acid (butyrate) that was published in our previous papers [2,10]. The cutoff value was defined as 50 $\mu\text{g/mL}$ that **15a** and **15b** were active against both R *Mtb* and NR *Mtb* with the same MIC values of 12.5 $\mu\text{g/mL}$ (Fig.3), indicating that 8-oxo-8H-chromeno [7, 8-c] [1, 2, 5] oxadiazole is a pharmacophore core structure against R *Mtb* and NR *Mtb* rather than *N*-oxide isoxazole and *N*-oxide imidazole.

NR *Mtb*, which can persist for a long time in a non- or slowly replicating state, is the cause of the necessity for prolonged treatment of TB in clinics, and contributes to a high incidence of side effects and drug-resistant *Mtb* strains. Most current TB drugs are less effective against NR *Mtb* [16]. Therefore, seeking and discovering hit compounds with new scaffolds against NR *Mtb* are urgently needed. This work reports a new scaffold, 8-oxo-8H-chromeno[7, 8-c] [1, 2, 5] oxadiazole, with the ability to kill R *Mtb* and NR *Mtb*. However the current compounds obtained are weakly potent. Research is continuously ongoing to develop more potent antagonist to R *Mtb* and NR *Mtb*.

Fig. 3 Compounds **15a** and **15b** turned out to have activities against *Mtb*.

4. Conclusion

In summary, we have explored the chemistry of *N*-Oxide heterocycles and imidazoles replacing ring D of the natural product (+)-Calanolide A, and have synthesized 12 new analogues. New scaffolds of compounds were developed and two of the 8-oxo-8*H*-chromeno [7, 8-*c*] [1, 2, 5]oxadiazole 1-oxides were active against both R *Mtb* and NR *Mtb* with MIC values of 12.5 $\mu\text{g/mL}$, which would lead to further optimization for more potent anti-TB candidates.

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