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Modified calanolides incorporated with furan-2-nitro mimics against *Mycobacterium tuberculosis*

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ABSTRACT

(+)-Calanolide A was identified as an active agent against both HIV-1 and *Mycobacterium tuberculosis* (*Mtb*). We modified Ring D of calanolide compounds with furan-2-nitro mimics and obtained 15 compounds. After *in vitro* tests, two compounds were shown to be active against replicating (R) *Mtb*, but not against non-replicating (NR) *Mtb*. Further optimization for potent candidates against both R *Mtb* and NR *Mtb* will result from this research.

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Natural products, often derived from plants or microbes, are essential sources for new antibacterial agents, and have helped inspire the development of new structurally diverse molecules that can play a crucial role in drug discovery. Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*Mtb*), is a leading cause of death around the world, killing 1.5 million people in 2013¹. *Mtb* can persist protractedly in terms of slight replication, which is known as non-replicating (NR) *Mtb*, and requires prolonged treatment—more than six months using current drugs. New and effective lead compounds with activity against *Mtb* and the capability to reduce the treatment time of TB would be in great demand²⁻³.

The natural pyranocoumarin compound (+)-calanolide A (**1**, Fig.

Fig 1. Antitubercular lead compounds with a benzofuran moiety. R and NR denote replicating and non-replicating *Mtb*, respectively.

Recently, we have elucidated a comprehensive structure–activity relationship (SAR) of the synthetic calanolides and concluded that Ring D-modified calanolide derivatives bearing a 2-nitrofuran group had markedly improved *in vitro* efficacy and reduced mammalian cell toxicity compared with the parent compound, (+)-calanolide A (**2** and **3**, Fig. 1)¹⁰. Unfortunately, their eukaryotic genotoxicity precluded them from clinical progression. We hypothesized that the nitro group is the key factor inducing this genotoxicity. Herein, we report our new results on the furan moiety replacing Ring D of calanolide A, especially using furan-2-nitro mimics, for the purpose of discovering new lead compounds against human *Mtb*.

When the furan-2-nitro group is replaced by a cyano group (**12a**), then the latter group's weaker electron-withdrawing capability decreases its Michael addition activity (Scheme 1), which may reduce the off-target toxicity of lead compound. It was clarified that the nitro group of the candidate drug PA824 was reduced by nitro reductase to form an unstable nitroso intermediate to produce NO, which acted as a bactericidal for mycobacteria¹¹⁻¹². When we monitored our ring D furan-2-nitro of calanolide A in *Mtb* medium, an ammonia vinyl form of

1), isolated from a tropical rainforest plant of the species *Calophyllum lanigerum*, is active against both HIV and *Mtb*, but with a low selective index⁴. Natural products with the benzofuran moiety display a wide range of biological properties, such as anti-inflammatory, antibacterial, and antitumor activities, and therefore they are promising lead compounds⁵⁻⁷. Their biological properties, especially the antibacterial properties of benzofuran rings substituted at the C-2/C-3 position, have been studied extensively⁸. For example, the natural dibenzofuran based on the secondary metabolite of lichen usnic acid (**4**, Fig. 1), isolated from *Cladonia substellata*, has been shown to have moderate antitubercular activity⁹. The synthetic dibenzofuran analogues **5** and **6** show significantly improved *in vitro* and *in vivo* antitubercular activity against *Mtb* H37Rv⁹.

compound was clearly detected by LC-MS/MS analysis (data not shown). We proposed that this ammonia vinyl group, which could be reduced by nitro reductase from the nitrofuran group, may provide useful lead compounds. However, its instability led us to add an ethoxy carbonyl acyl group, resulting in compound **14**, which is a potential prodrug. Compounds **9**, **10**, and **11** were aimed at improving the solubility of calanolide derivatives via incorporating carboxyl group (**9** and **10**) and acyl hydrazine group (**11**). Calculated ClogP values depicted in Scheme 1 indicated the improved solubility of **10b**, **11a**, **11a'** and **11b** indeed.

Based on our previous SARs experiments, Ring C of (+)-calanolide A substituted by a 2-(morpholine-4)-ethyloxy group exhibited MIC values of 0.08 and 0.2 µg/mL toward R and NR *Mtb*, respectively (Fig. 1)¹⁰. This result significantly promotes the synthetic design of new compounds. Compound **12b** was identified for the same reasons given above of **12a**. Compound **12b** was further converted to **15** and **16**, because urea is an important pharmacophore in drugs. Compounds **17**, **18** and **18'** are bioisosteres of urea.

Scheme 1. The design of furan-2-nitro mimic compounds.

Analogs **8–11** were synthesized following the routes shown in Scheme 2. We converted intermediate **7**, which has previously been synthesized according to a published method^{13–14}, to **8a** by annulation with 1, 1-diethoxy-3-methylbut-2-ene under reflux in a yield of 60%. And for **8b**, compound **7** underwent the Mitsunobu reaction in a yield of 77%. Compounds **9a** and **9b** were obtained from the cyclization of compounds **8a** and **8b** with ethyl bromoacetate in yields of 70% and 66%, respectively.

Subsequent hydrolysis of the esters catalyzed by potassium carbonate afforded the corresponding carboxylic acids **10a** and **10b** in yields of 75% and 80%, respectively. Compounds **10a** and **10b** reacted with hydrazine hydrate *via* the active ester to form **11a** and **11b** in yields of 70% and 65%, respectively. When hydrazine hydrate was replaced by isoniazid, compound **11a'** was obtained from **10a** in a yield of 73%.

Scheme 2. Reagents and conditions: (a) $(\text{CH}_3)_2\text{CCHCH}(\text{OEt})_2$, pyridine, toluene, reflux, 3 h, 60% yield; (b) N-hydroxyethyl morpholin, DEAD, PPh_3 , THF, rt, 3 h, 77% yield; (c) ethyl bromoacetate, Cs_2CO_3 , CH_3CN , reflux, 5 h, 70% (or 66%) yield; (d) K_2CO_3 , EtOH, H_2O , reflux, 3 h, 75% (or 80%) yield; (e) (i) DIC, HOSu, THF, rt, overnight, (ii) for **11a** and **11b**, $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$, 30 min, 70% and 65% yield, respectively, and for **11a'**, isoniazid, 1 h, 73% yield.

Analogues **12–18** were prepared using the routes shown in Scheme 3. Compounds **8a** and **8b** were cyclized with bromoacetonitrile to provide **12a** and **12b** in yields of 66% and 70%, respectively. Cyano group in **12b** underwent addition with hydroxylamine hydrochloride, which converted **12b** to **15** in a yield of 70%, which was further reduced to amidine **16**

under a condition of Pd/C, HCOONH₄, AcOH in a yield of 65% or cyclized with CDI or anhydride to form compounds **17** and **18** (or **18'**) in yields of 70% and 65% (or 70%), respectively. Furan 2-carboxylic acid **10a** reacted with DPPA to form the acyl azide **13**, and was then converted to **14** by a “Curtis rearrangement” in a yield of 76%.

Scheme 3. Reagents and conditions: (a) bromoacetonitrile, K₂CO₃, DMF, 120 °C, 66% (or 70%) yield; (b) DPPA, Et₃N, 1,4-dioxane, 40 °C, overnight; (c) EtOH, reflux, 4 h, 76% yield; (d) NH₂OH·HCl, Et₃N, EtOH, reflux, 8 h, 70% yield; (e) Pd/C, HCOONH₄, AcOH, 100 °C, 3 h, 65% yield; (f) CDI, DBU, 1,4-dioxane, reflux, 2 h, 70% yield; and (g) acid anhydride (or trifluoroacetic anhydride), pyridine, 110 °C, 4 h, 65% (or 70%) yield.

The reaction of salicylaldehyde with α-halogenated acid esters or α-halogenated cyanides to prepare benzofuran is known as the Rap–Stoermer reaction, and it was important choosing the base, halide, and solvent. We optimized the reaction conditions

for the coumarin compounds. Table 1 shows that BrCH₂CO₂Et gave an acceptable yield of **9a** (70%) in the presence of Cs₂CO₃ in CH₃CN, but K₂CO₃ in DMF was required to form **12a** (66%).

Table 1. Optimization of the synthesis of compounds **9a** and **12a**.

Compound	Halide	Solvent	Base	Yield
9a	ClCH ₂ CO ₂ Et	DMF	Cs ₂ CO ₃	20%
		CH ₃ CN	Cs ₂ CO ₃	40%
	BrCH ₂ CO ₂ Et	CH ₃ CN	Cs ₂ CO ₃	70%
		Acetone	Cs ₂ CO ₃	30%
12a	BrCH ₂ CN	DMF	Cs ₂ CO ₃	45%
		DMF	K ₂ CO ₃	66%

All of the final fifteen synthesized compounds were subjected to anti-*Mtb* whole-cell screening using both R *Mtb* and NR *Mtb*. For replicating (R) conditions, *Mtb* was propagated at 37 °C with 20% O₂ and 5% CO₂. For nonreplicating (NR) conditions, *Mtb* were incubated at 37 °C with 1% O₂ and 5% CO₂ in a Sauton's based medium ("NR medium") consisting of 0.5 g of MgSO₄, 0.05 g of ferric ammonium citrate, 0.5 g of KH₂PO₄, 0.5% BSA, 0.085% NaCl, 0.02% tyloxapol, 50 μM butyrate, and 0.5 mM NaNO₂ at pH 5.5 to mimic an activated macrophage condition^{10,15}. The cut off value was defined as 50 μg/mL that compounds **11a** and **11a'** turned out active against R *Mtb* with MIC values of 50 and 25 μg/mL,

respectively (Fig.2), although they were inactive toward NR *Mtb*. A primary SAR indicated that among calanolides incorporated with furan-2-nitro mimics in this letter, furan-2-hydrazides were active against R *Mtb*, which provides a new hit scaffold instead of furan-2-nitro group of ring D-modified calanolide.

In summary, we have explored the chemistry of furan-2-nitro mimics replacing Ring D of the natural product (+)-calanolide A, and have synthesized 15 new analogues to obtain two compounds that were active against R *Mtb* but inactive against NR *Mtb*. Further optimization for potent candidates against both R *Mtb* and NR *Mtb* will result from this research.

Fig 2. Compounds **11a** and **11a'** turned out to have activities against replicating *Mtb*.

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References and notes

1. Tuberculosis, WHO, Global tuberculosis report 2014, http://www.who.int/tb/publications/global_report/en/
2. Xu, Z.-Q. *et al. Bioorganic & Medicinal Chemistry*, **2004**, 12, 1199.
3. Xu, Z.-Q. *et al. Bioorganic & Medicinal Chemistry*, **2006**, 14, 4610.
4. Y. Kashman. *et al. J. Med. Chem.* **1992**, 35, 2735.
5. Urzúa, A. *et al. Molecules*, **2008**, 13, 882.
6. Hayakawa, I. *et al. Bioorganic & medicinal chemistry letters*, **2004**, 14, 455.
7. Galal, S. A. *et al. Bioorganic & medicinal chemistry letters*, **2009**, 19, 2420.
8. Manna, K. & Agrawal, Y. K. *Bioorganic & medicinal chemistry letters*, **2009**, 19, 2688.
9. Patpi, S. R. *et al. Journal of Medicinal Chemistry*, **2012**, 55, 3911.
10. Puring Zheng. *et al. Journal of Medicinal Chemistry*, **2014**, 57, 3755.
11. Manjunatha U. *et al. Communicative & Integrative Biology*, **2009**, 2, 215.
12. Singh R. *et al. Science*, **2008**, 322, 1392.
13. Xiao Yong Guo. *et al. Chinese Chemical Letters*, **2013**, 24, 295.
14. P.P. Deshpande. *et al. J. Org. Chem.* **1995**, 60, 2964.
15. Yaling Gong. *et al. European Journal of Medicinal Chemistry*, **2014**, 75, 336.

Graphical Abstract

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