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Short communication

Monocarbonyl analogs of curcumin inhibit growth of antibiotic sensitive and resistant strains of *Mycobacterium tuberculosis*

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ABSTRACT

Tuberculosis (TB) is a major public health concern worldwide with over 2 billion people currently infected. The rise of strains of *Mycobacterium tuberculosis* (Mtb) that are resistant to some or all first and second line antibiotics, including multidrug-resistant (MDR), extensively drug resistant (XDR) and totally drug resistant (TDR) strains, is of particular concern and new anti-TB drugs are urgently needed. Curcumin, a natural product used in traditional medicine in India, exhibits anti-microbial activity that includes Mtb, however it is relatively unstable and suffers from poor bioavailability. To improve activity and bioavailability, mono-carbonyl analogs of curcumin were synthesized and screened for their capacity to inhibit the growth of Mtb and the related *Mycobacterium marinum* (Mm). Using disk diffusion and liquid culture assays, we found several analogs that inhibit *in vitro* growth of Mm and Mtb, including rifampicin-resistant strains. Structure activity analysis of the analogs indicated that Michael acceptor properties are critical for inhibitory activity. However, no synergistic effects were evident between the monocarbonyl analogs and rifampicin on inhibiting growth. Together, these data provide a structural basis for the development of analogs of curcumin with pronounced anti-mycobacterial activity and provide a roadmap to develop additional structural analogs that exhibit more favorable interactions with other anti-TB drugs.

1. Introduction

TB is a major public health concern, with over 2 billion people currently infected, 8.6 million new cases per year, and more than 1.3 million deaths per year. The current drug regimen combination for TB consists of isoniazid, rifampicin, ethambutol, and pyrazinamide, administered over six months [1,2]. Although this treatment has a high success rate, the utility of this regimen is limited by compliance issues, which has resulted in the rise of strains that are resistant to some or all of the first and second line antibiotics [3]. These strains, called MDR, XDR, and TDR Mtb, have worse disease outcome [4]. Recent efforts in TB drug development have resulted in the discovery of new therapeutics including bedaquiline, which retain activity against MDR and XDR strains. However, additional drugs are urgently needed.

Natural products and their plant-derived analogs are often a source of drugs or drug templates with limited toxicity, which has the potential to mitigate compliance issues during protracted administration. One natural product candidate of interest is curcumin [1,7-bis(4-hydroxy-3-methoxy phenyl)-1,6-heptadiene-3,5-dione], a phenolic compound originally extracted from the plant *Curcuma longa* and the primary component of the spice turmeric [5,6] (Fig. 1). For centuries, various Asian cultures have used curcumin as a traditional medicine to treat numerous disorders, particularly those associated with the skin and digestive tract. Curcumin has been found to have anti-cancer and anti-inflammatory properties, but the comparative action of monocarbonyl derivatives demonstrates the superior efficacy of this class

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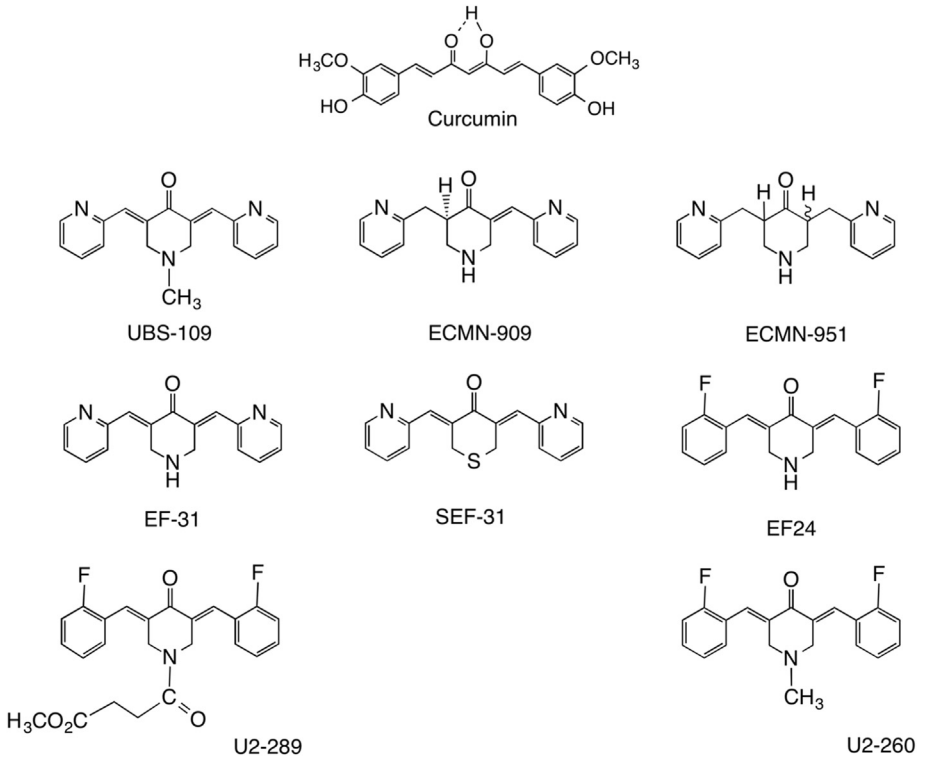

Fig. 1. Monoketone curcumin analogs evaluated for anti-mycobacteria properties.

of molecules [7–10]. Moreover, curcumin inhibits the growth of various microorganisms including *Escherichia coli*, *Bacillus subtilis*, *Helicobacter pylori*, and Mtb [11–13]. In addition, curcumin acts synergistically with co-administered antibiotics to suppress growth of *Staphylococcus aureus in vitro* [14]. While curcumin inhibits growth of various bacterial species, this effect requires a relatively high inhibitory concentration [IC] compared to other antimicrobial agents, which is not achievable *in vivo* due to limited bioavailability [15] and chemical instability [16–18].

One approach to overcome these limitations and improve the inhibitory activity of curcumin is to develop structural analogs [10,19,20]. Curcumin has three sectors that can be targeted for modification: the β-diketone moiety, the aromatic rings, and the flanking double bonds conjugated to the β-diketone moiety. We evaluated a series of monocarbonyl analogs (Fig. 1) for their capacity to inhibit growth of the pathogenic mycobacteria Mtb and Mm.

2. Experimental procedures

2.1. Chemicals

All compounds were prepared by previously described methodology [20,21]. Briefly, the monocarbonyl analogs were generated by allowing the ketones to react with a variety of aromatic aldehydes under basic aldol condensation conditions. The structures of the final products (>95% pure, Fig. 1) have been characterized by standard analytical techniques as previously reported: UBS-109 [22–25], ECMN-909 [25], ECMN-951 [25], EF-31 [21,23,26], SEF-31 [21,27], EF-24 [20,21,28], U2-289 [29] and U2-260 [30]. Curcumin (≥94% curcuminoid content, ≥80% curcumin), Rifampicin and dimethyl sulfoxide (DMSO) were purchased from Sigma (St. Louis, MI).

2.2. Bacterial strains, and growth conditions

Mycobacterium tuberculosis strain H37Rv, H37Rv-RifR, and Beijing F2, and *Mycobacterium marinum* strain 1218R were grown in Difco Middlebrook 7H9 broth (Becton, Dickinson, and Company, Sparks, MD) supplemented with BBL Middlebrook ADC Enrichment (Becton, Dickinson, and Company, Sparks, MD) and 0.5% Tween 80 (*Mtb*) or 0.025% Tween 80 (*Mm*) (7H9-ADC broth). Difco Middlebrook 7H10 agar (Becton, Dickinson, and Company, Sparks, MD), supplemented with 10% oleic acid-albumin-dextrose-catalase (7H10-OADC) was used for Mm. Stocks of Mm were grown at 30 °C in 5% CO_2 until OD_{600} of 0.8, centrifuged, the supernatant removed, and the bacteria resuspended in fresh 7H9 broth. Aliquots were stored at −80 °C. For some experiments, strains with mutations that rendered *M. marinum* (1218R^{rifR}) or Mtb (H37Rv$^{rpoB\ H526Y}$) resistant to rifampicin were used [31].

2.3. Disk diffusion assay

Mm was grown in 7H9 broth until it reached an OD_{600} of 0.35. The culture was diluted to OD_{600} of 0.04, and 100 μL of the diluted stock was spread on 7H10 plates. The plates were allowed to air dry for 10 min. Next, sterilized round filter paper (6 mm diameter BBL disks; Becton, Dickinson, Sparks, MD) was placed in the center of each plate, to which 10 μl of a particular curcumin analog (100 mM in DMSO or sterilized water) was added. Three plates were assessed for each analog. The plates were then incubated at 30 °C in 5% CO_2 for 7 days, after which time the extent of the zone lacking bacteria around the filter was measured ("zone of inhibition").

2.4. Determination of 50% inhibitory concentration (IC_{50}) for curcumin analogs against Mm and Mtb

Mm was grown in 7H9-ADC broth until OD_{600} of 0.35 and then