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Research paper

Conjugation of a 5-nitrofuran-2-oyl moiety to aminoalkylimidazoles produces non-toxic nitrofurans that are efficacious *in vitro* and *in vivo* against multidrug-resistant *Mycobacterium tuberculosis*

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ABSTRACT

Within the general nitrofuran carboxamide chemotype, chimera derivatives incorporating diversely substituted imidazoles attached via an alkylamino linker were synthesized. Antimycobacterial evaluation against drug-sensitive *M. tuberculosis* H37Rv strain identified five active druglike compounds which were further profiled against patient-derived *M. tuberculosis* strains *in vitro*. One of the compounds displayed promising potent activity (MIC 0.8 μg/mL) against one of such strains otherwise resistant to such first- and second-line TB therapies as streptomycin, isoniazid, rifampicin, ethambutol, kanamycin, ethionamide, capreomycin and amikacin. The compound was shown to possess low toxicity for mice ($LD_{50} = 900.0 \pm 83.96$ mg/kg) and to be similarly efficacious to etambutol, in the mouse model of drug-sensitive tuberculosis, and to neurotoxic cycloserine in mice infected with MDR tuberculosis.

1. Introduction

The constant emergence of drug-resistant microorganisms [1] mandates that new molecular entities are brought up through the drug discovery process into preclinical and clinical development. However, the current situation with serious non-contained infections in developing countries such as tuberculosis (caused by *Mycobacterium tuberculosis* or *MTb*) is such that the demand for new drug candidates in the development pipeline is not met with adequate productivity in the discovery process [2]. The multidrug-resistant (MDR) forms of tuberculosis are particularly hard to treat with the existing therapies, which leads to increased death rates, especially from co-infection with HIV [3]. The latest breakthrough in antitubercular drug development productivity (which manifested itself in the approvals of Janssen's bedaquiline [4] and Otsuka's delamanid [5]) is somewhat reassuring. However, other classes of drugs should be validated for the development, even by revisiting known antimicrobial chemotypes and attempting to optimize them specifically to target *MTb*.

Nitrofurans (along with nitroimidazoles to which delamanid belongs) are the so-called bioreducible compounds that have demonstrated potential to treat various infectious diseases caused by bacterial pathogens [6]. Their mechanism of action is thought to involve reduction, by the bacterial cell wall enzyme, of the nitro-group to produce free radical species that can react with the bacterial biomolecules and are, therefore, lethal to the microorganism [7]. The major concern associated with nitrofurans and possibly hindering their advancement into development is their being also a structural alert from toxicology perspective. Indeed, if it is not

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ensured that a chemotherapeutic agent in question is selectively taken up and metabolized by the bacteria, issues of toxicity and mutagenicity may arise [8,9]. It is perhaps unsurprising that since the introduction of nitrofurazone (**1**) [10] in the 1940s as well as the other, mostly topical, compounds nifuroxazide (**2**) and nitrofurantoin (**3**) [11] examples of nitrofuran compounds as advanced as preclinical phase of development have remained scarce. However, the recent success of nitroimidazoles delamanid (*vide infra*) and pretomanid (which is currently in phase III clinical study [12]) led to a belief that the nitrofuran drug class, too, can deliver efficacious and non-toxic antitubercular drug candidates via a careful optimization of compounds' periphery. The interest to revisiting this well-established class of antimicrobial agents in antitubercular context (i. e. in the area highly prone to drug resistance issues) is additionally fueled by the latest clinical data meta-analysis on furantoin demonstrating that the potential of microbial resistance to nitrofurans is low [13].

Exploration of the nitrofuran periphery for the purposes of selectively increasing antimycobacterial activity and reducing toxicity to the host is potentially a vast and resource-intensive area of research. Here certain guidelines for selecting specific groups and moieties should be considered. While predictive computational models are being developed [14], medicinal chemist often choose a more intuitive drug design strategy whereby the pharmacophoric nitrofuran moiety is conjugated with or embedded into structures that also have some documented antitubercular efficacy associated with them. For instance, nitrofuranylamides discovered by Lee in the form of the early hit (**4**) [15] and optimized into more advanced compounds such as Lee562 (**5**) with demonstrated safety and efficacy *in vivo* [16] attest both to acceptability of the amide linkage and the presence of basic heterocyclic moieties in the candidate compounds' periphery. *N*-Aminolactams conjugated with a nitrofuran motif via a hydrazone linkage (**6**) helped reveal a preferred *N*-cyclohexyl carboxamide periphery [17]. Nitrofuran-containing isostere **7** of antimycobacterial natural product (+)-calanolide A illustrates another productive design strategy [18]. Merging a broadly antimicrobial 2-aminothiazole and antimycobacterial nitrofuranyl moieties in the structure of compound **8** speaks for the power of the chimera design [19].

In this work, we explored a similar approach with respect to combining, within a single molecule (**9** or **10**), the pharmacophoric 5-nitrofuran-2-oyl moiety and a substituted imidazole motif linked together by a diverse set of aminoalkyl linkers (Fig. 1). This research was particularly motivated by the recently established significance of non-nitrated imidazole motifs in the antitubercular compound design [20] and the validation of imidazole fragments in targeting *MTb* [21]. Herein, we describe the synthesis of compounds **9**–**10** (via $Zn(OTf)_2$-catalyzed amination-cyclization of propargylamides followed by the introduction of the 5-nitrofuran-2-oyl moiety) and the results of their profiling, as potential antitubercular agents *in vitro* and *in vivo*.

2. Results and discussion

2.1. Chemistry

A variety of substituted imidazoles **14** or **15** containing the aminoalkyl side chains required for conjugation to 5-nitrofur-2-yl moiety via an amide linkage in position 2 or 1 of the imidazole nucleus were accessed from the respective propagylamides **12** (prepared, in turn, from carboxylic acids **11** in good to excellent yield, *vide infra*) according to $Zn(OTf)_2$-catalyzed amination-cyclization sequence as described by Beller [22] and us [23]. The initial imidazole cycloadducts **13** (although observed by TLC and ^1H NMR analysis of the reaction mixtures) were not isolated and the crude material obtained in these reactions was taken on to the deprotection step. The latter was brought about by 4 N solution of HCl in 1,4-dioxane and the desired 2- and 1-aminoalkyl substituted imidazoles **14** and **15**, respectively, were obtained as di- and trihydrochloride salts (see Experimental Section) in moderate to good yields over two steps (see Table 1). The amine hydrochlorides **14** or **15**, in presence of triethylamine (required to obtain the free-base amine for acylation) was brought in contact with acyl imidazolide prepared *in situ* from 5-nitrofuran-2-carboxylic acid. The yield in the last acylation step obtained via the use of CDI was surprisingly low throughout the range of amines **14** and **15** employed. However, the employment of an alternative acylation strategy *via in situ* preparation of 5-nitrofur-2-oyl chloride or the use of EDC in lieu of CDI (as was described previously for the same type of acylation reaction by Lee and co-workers [24]) did not improve the yield of the reaction. Overall, the target conjugates of a substituted imidazole moiety with pharmacophoric 5-nitrofur-2-yl moiety via an aminoalkyl linker **9** and **10** were obtained in four chemical operations involving only three isolation or purification steps (Scheme 1).

2.2. In vitro biological activity

The antimycobacterial activity of compounds **9a-e** and **10a-g** was initially evaluated against the drug-sensitive H37Rv strain of *MTb*. As it is evident from the data presented in Table 1, substantial antimycobacterial activity was observed for compounds belonging to either series. It is obvious that, to a large degree, introducing various cyclic motifs as rigidity elements into the structure of the linker appeared to increase the antimycobacterial potency.

Five compounds (**9a-b**, **9d**, **10d** and **10e**) that displayed the lowest values of minimum inhibitory concentration (MIC) were analyzed for the critical characteristics determining druglikeness and prospects for sufficiently high oral bioavailability [25]. As it is evident from the calculated data summarized in Table 2, the compounds which displayed the highest antimycobacterial activity, are well within the limits of druglikeness (as defined by the Lipinski's rule-of-five [26]). The notable feature of the five active leads identified in this work is the narrow range of calculated total polar surface area (TPSA) these compounds fall into. This is in line with the significance of TPSA characteristic for bacterial cell wall permeability noted by Tan and co-workers [27].

The five compound selected based on their antimycobacterial activity against H37Rv strain were further evaluated for the same activity in MDR strains available in the tuberculosis patient-derived mycobacterial strain collection of Saint Petersburg Research Institute of Phthisiopulmonology. In general, the activity of these compounds against the MDR strains was comparable to or significantly lower than that observed on H37Rv strain. The notable exception was compound **9d** which displayed even somewhat higher activity (compared to drug-sensitive strain) against 2712 strain which was isolated from a patient not responding to a number of first- and second-line drugs (Table 3).

It should be noted that the most active compound (**9d**) characterized herein further (*vide infra*) carries a number of attractive features (a rigid achiral azetidine linker, low lipophility) which makes the compound a promising drug development candidate [28]. The same compound did not display any activity (Table 4) against three (*E. faecium, K. pneumoniae and P. aeruginosa*) out of six Gram-positive and Gram-negative bacteria belonging to the so-called ESKAPE panel of pathogens (i. e. the ones most prone to developing resistance to drugs) [29]. The activity against the other three ESKAPE pathogens (*S. aureus*, *A. baumanii* and *E. aerogenes*) can be noted albeit it was lower than the activity of ciprofloxacin (employed as a positive control).