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Short communication

Extending the N-linked aminopiperidine class to the mycobacterial gyrase domain: Pharmacophore mapping from known antibacterial leads

Karyakulam Andrews Bobesh, Janupally Renuka, Variam Ullas Jeankumar, Singh Kakan Shruti, Jonnalagadda Padma Sridevi, Perumal Yogeeswari, Dharmarajan Sriram*

Department of Pharmacy, Birla Institute of Technology & Science-Pilani, Hyderabad Campus, Shameerpet, Jawaharnagar, RangaReddy District, Hyderabad 500 078, India

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ABSTRACT

Bacterial DNA gyrase is a well-established and clinically validated target to develop novel antibacterial. Our effort was designated to search for synthetically better compounds with possibility of hit to lead development. With this as objective, a series of 1-(2-(4-aminopiperidin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one derivatives were designed by molecular hybridization strategy and synthesized following nine step reaction to yield activity in low nanomolar range and commendable antibacterial activities. Compound 1-(4-fluorophenyl)-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl) urea (**35**) emerged as the most promising inhibitor with an IC_{50} of 78 nM against *Mycobacterium tuberculosis* DNA gyrase enzyme, with MTB MIC of 0.62 μM, and not cytotoxic at 50 μM in eukaryotic cell line.

1. Introduction

Human negligence and lack of novel therapeutic agents have allowed *Mycobacterium tuberculosis* (*Mtb*), the etiological agents of Tuberculosis (TB) to resurge in more deadly drug resistant forms, with the recently identified totally drug resistant strains rendering complete resistance to currently available drug regime [1].The current TB drug discovery pipeline, though presently insufficient to address the unmet need of treatment, has few promising reports on leads that have the potential to become future drug candidate. The ones in the most advanced stages include the fluoroquinolones (FQ), specifically gatifloxacin and moxifloxacin, which are currently being evaluated in phase 2 and 3 clinical trials respectively [2]. This class of drugs acts by inhibiting DNA gyrase, the sole topoisomerase in *Mtb* that introduce negative supercoils into DNA and regulate the super helical state of the bacterial chromosomes [3]. These drugs predominately bind to the GyrA subunit of DNA gyrase, thereby trapping the gyrase-DNA complex which further resulted in oxidative damage that ultimately led to the bacterial cell death and has been found effective against both replicating and non-replicating, persistent mycobacterium strains [4]. The reports on moxifloxacin, also suggested that DNA gyrase may be a good target for reducing the length of TB treatment regimens. However, in spite of the quite successful progress made by the FQ class of analogues, the emerging resistance to the FQ leads to grave concern and drives the quest for newer, safer, and more effective TB treatment options [5]. Novel bacterial type II topoisomerase inhibitors (NBTIs) are promising class of topoisomerase inhibitor that are structurally and mechanistically different from the FQ class and hence may not be impacted by target mutations that cause resistance to FQ [6]. These classes of drugs have made significant progress in the recent years exhibiting broad spectrum antibacterial potency with many promising candidates being evaluated at various stages of drug discovery program. Their overall chemical structure, topology incorporates an N-linked '4-aminopiperidine' linker fused between a bicyclic left hand nucleus (LHS) and an aryl/heteroaryl right-hand-core (RHS). NBTIs, though well explored as a probable class for developing newer antibacterial leads; scarcely anything have been

* Corresponding author.
E-mail addresses: dsriram@hyderabad.bits-pilani.ac.in, drdsriram@yahoo.com (D. Sriram).

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achieved with regard to exploiting this class as a potential lead for developing novel antimycobacterial agents [7]. Recent success in the design of many mycobacterial gyrase inhibitors in the earlier reports on antibacterial analogues of the same chemical class encouraged us to evaluate the inhibitory potential of NBTIs towards the mycobacterial gyrase domain. This is in consideration to the fact that these compounds act on a clinically proven target through a novel mechanism of inhibition with potency against fluoroquinolone-resistant isolates. In this study we disclose a new class of mycobacterial DNA gyrase inhibitor developed *via* molecular hybridization of previously reported antibacterial leads [6–7]. The designed molecules were subsequently synthesized and evaluated *in vitro* for their ability to inhibit DNA gyrase enzyme and whole cell MTB as important steps towards the derivation of structure–activity relationship.

2. Resultsand discussion

2.1. Design and synthesis

With target-based/phenotypic screening approaches offering few tangible successes in discovering novel antitubercular drugs, the concept of molecular hybridization could be of significant use to generate newer scaffold as potential antimycobacterial leads [8]. Molecular hybridization approach is an emerging structural modification tool involving adequate fusion of the two or more pharmacophoric units derived from previously reported leads/drugs in the design of new hybrid architecture that could maintain preselected characteristics of the original template [9]. We therefore envisaged that re-engineering the previously reported NBTIs could deliver a new scaffold/lead with better antimycobacterial activity *via* inhibition of the gyrase domain. The design strategy utilized for developing the inhibitor has been sketched in Fig. 1. It was decided to retain the 1-(2-(4-aminopiperidin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one scaffold in our initial structure-activity exploration as it was understood to be an important requisite in retaining the gyrase inhibitory potential. Since fluoro and methoxy groups at 7th position of the 1,5-naphthyridin-2(1H)-one core significantly improved the gyrase inhibition of the previously reported antibacterial NBTIs, these were also retained in our studies. Various carbamide/thiocarbamide derivatives were introduced as right hand core to increase stability and also to evaluate the steric and electronic effects on the antimycobacterial potency. The N-linked aminopiperidine based analogues have made significant progress in recent years as potential antibacterial leads. Research group from GSK have also found success by extending the above antibacterial aminopiperidine class to the antimicrobial target as well, with many compounds exhibiting promising inhibition of MTB [10].

Synthesis of the compounds started with the construction of 7-fluoro1-5-naphthyridin-2(1H)-one *via* a Heck coupling reaction of 2-chloro-5-fluoropyridin-3-amine (**5**) with butyl acrylate. Though various literatures have explored a variety of conditions and reagents to afford the Heck product, the protocol reported by Voight et al. was the most beneficial as it underwent an in-situ cyclization of the so obtained Heck product butyl 3-(3-amino-5-fluoropyridin-2-yl)acrylate to give the desired 7-fluoro1,5-naphthyridin-2(1H)-one (**6**) in good yield [11]. The ethyl bridge that connected the naphthyridinone core to the aminopiperidine linker was introduced at N-1 position by alkylating the so obtained 7-fluoro-1,5-naphthyridin-2(1H)-one (**6**) with bromoethanol in presence of Cs_2CO_3. Small amounts of the O-alkylated product obtained were removed by column chromatography. Compound **7** on further treatment with trifluoromethanesulfonic anhydride and pyridine afforded the corresponding triflate (**8**) in good yield. This was further condensed with 4-N-Boc-aminopiperidine *via* a SnAr displacement to obtain the nitrogen-linked analog (**9**). Subsequent Boc deprotection afforded the scaffold **11** in good yields. Concordantly, the so obtained *tert*-butyl (1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)carbamate (**9**) was also treated with sodium methoxide in methanol under reflux to introduce the methoxy substituent at the 7th position of 1,5-naphthyridin-2(1H)-one core *via* nucleophilic displacement of the fluoro group. This was subsequently subjected to Boc deprotection in a similar fashion to the fluoro analogue to give the desired product (**12**). The final library was then assembled by treating the obtained scaffolds **11**and **12** with the desired isocyanates/isothiocyanates to afford compounds **13**–**52** in excellent yields.

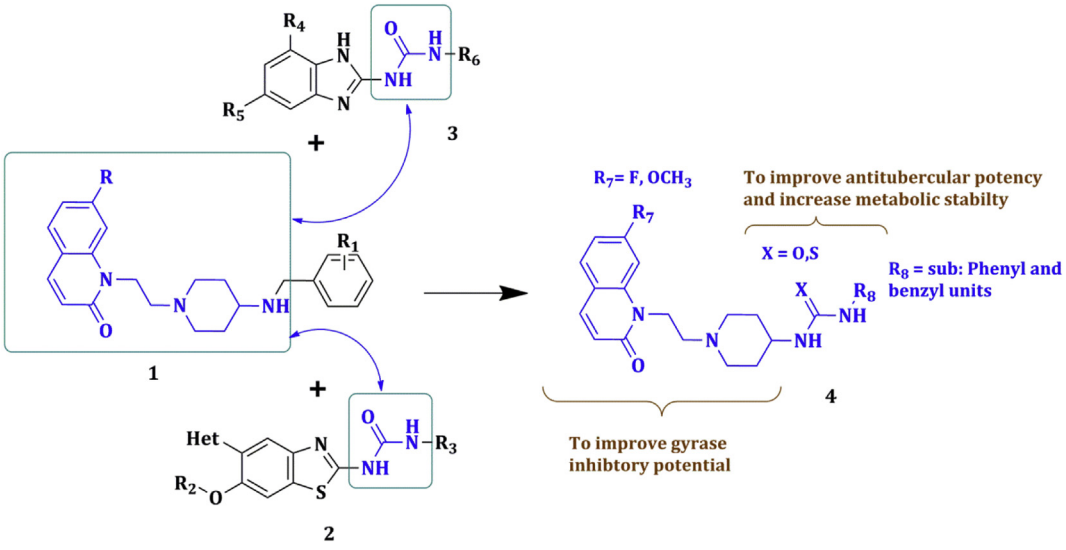

Fig. 1. Strategy employed for designing the lead. Chemical structure of previously reported synthetic inhibitors of DNA gyrasebearing1,5-naphthyridin-2(1H)-one core (**1**), carbamide side chain (**2**–**3**) and the inhibitor designed through molecular hybridization (**4**).