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Biarylmethoxy Nicotinamides As Novel and Specific Inhibitors of *Mycobacterium tuberculosis*

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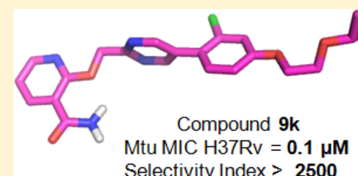
Supporting Information

ABSTRACT: A whole cell based screening effort on a focused library from corporate collection resulted in the identification of biarylmethoxy nicotinamides as novel inhibitors of *M. tuberculosis* (Mtu) H37Rv. The series exhibited tangible structure−activity relationships, and during hit to lead exploration, a cellular potency of 100 nM was achieved, which is an improvement of >200-fold from the starting point. The series is very specific to Mtu and noncytotoxic up to 250 μM as measured in the mammalian cell line THP-1 based cytotoxicity assay. This compound class retains its potency on several drug sensitive and single drug resistant clinical isolates, which indicate that the compounds could be acting through a novel mode of action.



KEYWORDS: *MMIC, biarylmethoxy nicotinamides, anti-TB, clinical isolates*

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (Mtu) is a highly contagious disease and continues to remain the second leading killer of adult population after HIV. In 2010, there were 8.8 million incident cases of TB and 1.45 million deaths. TB primarily affects the lungs, but it can also affect other organs such as the central nervous system, lymphatic system, and circulatory system.[1,2] A deadly synergy between HIV and TB poses new challenges to healthcare system, causing rapid progression and reactivation of latent-TB. Reactivation increases the TB disease burden by an estimated 20% in African and Asian countries where HIV prevalence is high.[3,4] A standard regimen involving a combination of four drugs, Rifampin, Isoniazid, Ethambutol, and Pyrazinamide for 6−8 months developed decades ago[5] needs urgent back-up with better efficacy, reduced toxicity, and effective coverage on latent tuberculosis infection (LTBI).[6] Emergence of resistant strains, multidrug resistant (MDR-TB) and extensively drug resistant (XDR-TB) has taken global proportions with prevalence of MDR-TB ranging from anywhere between 5 and 47% and 9% of the MDR-TB cases leading to XDR-TB. It is estimated that, by 2014, 84 countries would have at least reported one XDR-TB case.[7,8] Significant strides have been made in the past two decades in terms of understanding the biology of this slow-growing pathogen. The complete genome sequence of MtuH37Rv was solved by Stewart Cole et al.[9] This has facilitated the process of identifying novel chemical classes through new druggable targets, understanding their biological role, essentiality, and determination of point mutations associated with drug resistance.

Recently, FDA approved the J&J compound Bedaquiline (SIRTURO) for MDR-TB therapy. Few other advanced molecules such as delamanid, PA824, SQ-109, Moxifloxacin, and AZD5847 are in clinical trials.[10] Despite these advances being made, there are significant challenges associated with the target product profile for new TB-drugs, which involve tackling the emergence of resistance, shortening of treatment period, drug−drug interactions, intolerance, and toxicities, which demand immediate need for new chemical entities for anti-TB with novel mode of action.[11] Various approaches such as modifying existing scaffolds, target based, and whole-cell based screening are followed in TB drug discovery. While the target based approach appears to be lucrative in terms of tracking the mode of action MOA, translation of enzyme potency into cellular activity is the major hurdle. Whole-cell based approach, however, has antimycobacterial activity already embedded, and the advances made in genomics have provided the necessary platform to delayer the MOA.[12,13] In fact, recent success involving Bedaquiline targeting mycobacterial ATP synthase and BTZ targeting cell wall protein DprE1 have originated out of whole-cell based approaches.

In our quest to identify new and novel scaffold with antimycobacterial activity, we ran a whole-cell based screen on a focused library from AstraZeneca compound collection. Biarylmethoxy nicotinamide is one of the promising series that emerged out of this screening. The initial hits were weak in terms of cellular potency, and a subsequent optimization effort resulted in about 200-fold improvement. Herein, we report the chemical optimization of biarylmethoxy nicotinamides.

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To understand the structure−activity relationship (SAR), the scaffold was broadly divided into rings A, B, and C (Figure 1).

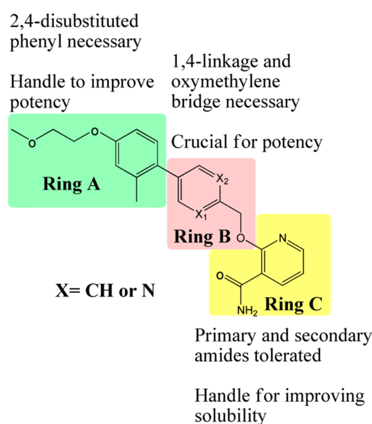


Figure 1. SAR of biarylmethoxy nicotinamides.

Compounds involving ring A and ring B modifications were synthesized starting from their corresponding boronic acids using Suzuki coupling protocol. The synthetic route followed is represented in Scheme 1.

Scheme 1. Synthesis of Biarylmethoxy Nicotinamide: Ring A and B Modifications[a]

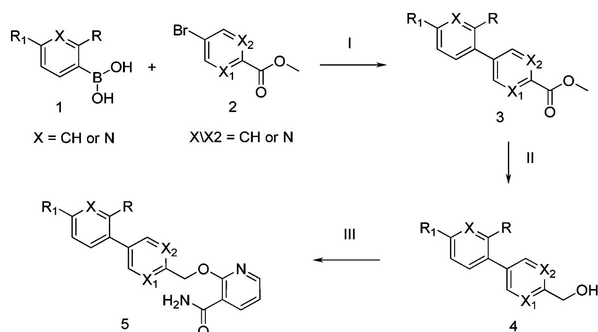


Table 1, entry 5a-g, 16-22

[a]Reagents and conditions: (I) Ar−B(OH)$_2$, PdCl$_2$(dppf)−CH$_2$Cl$_2$, CsF, MeOH, MW at 120 °C, 30 min, yield 55−70%; (II) LiAlH$_4$, THF, 0−25 °C, 30 min, yield 35−50%; (III) 2-chloronicotinamide, NaH (60% suspension in paraffin oil), DMF, 100 °C, 2 h, yield 60−80%.

Optimum results were obtained when the reactions were performed under microwave conditions using PdCl$_2$(dppf)−CH$_2$Cl$_2$ and CsF. Reduction of the biaryl carboxylates **3** by careful addition of lithium aluminum hydride resulted in intermediate **4**. The yield of **4** was moderate and was improved when the reducing agent was added at 0 °C. Nucleophilic displacement of commercially available 2-chloro nicotinamide with **4** using sodium hydride resulted in title compounds **5a−e** (Table 1).

However, the synthetic route mentioned in Scheme 1 was not feasible for the synthesis of compounds **9a−k** (Table 1). These compounds were synthesized using an alternative protocol as shown in Scheme 2. Nucleophilic displacement of 2-chloronicotinamide with corresponding alcohol **6** resulted in intermediate **7**. Alcohol **6** was prepared by reducing the corresponding carboxylate esters **2** with lithium aluminum hydride (LAH). Debromination was one of the major side products formed during the synthesis of **6**. However, debromination could be kept at minimum when the reduction was carried out at 0 °C. This reaction was closely monitored and was quenched immediately after completion. Suzuki coupling of intermediate **7** with commercially available boronic acids following standard protocol resulted in intermediate **8**. We found that the phenolic hydroxyl group does not need protection during the reaction sequence and can be substituted readily with various alkyl bromides to give the title compounds **9a−k** (Table 1).

A series of amide analogues **15a−h** were synthesized (Table 2) to investigate the function of amide on antimycobacterial activity and aqueous solubility. The route followed to synthesize these compounds is illustrated in Scheme 3. Synthesis starts with alkylation of 4-hydroxy-2-methyl bromobenzene with 1-bromo-2-methoxy ethane. Quantitative yield was obtained for intermediate **11** when the coupling was carried out at 50 °C in the presence of potassium carbonate. Intermediate **12** was synthesized following Suzuki protocol using intermediate **11** and 6-(hydroxymethyl)pyridin-3-ylboronic acid in about 36% yield. The biaryl methanol **12** was subjected to nucleophilic displacement of chloride group on methyl 2-chloro nicotinate, and the resulting ester intermediate **13** was hydrolyzed using lithium hydroxide in dioxane to result in **14**. Title compounds **15a−h** (Table 2) was obtained in about 30−50% yield by simple amide coupling with the corresponding amine using commonly employed coupling agent HATU.

The effect of various substitutions on ring A and the optimal substitutions at the X1 and X2 positions were examined. The SAR of these modifications is summarized in Table 1. The results indicate that the presence of 2, 4-disubstituted phenyl ring is essential for cellular potency. Heterocyclic rings such as imidazolyl **5f** and pyridyl **5g** are inactive as compared to their match pair **5b**. A greater than 10-fold improvement in potency could be achieved by extending 4-methoxy of **5b** to two carbon 4-(2-methoxy) ethyl side chain **9a**. An interesting observation could be made out of compounds **9c−e**, the *S*-isomer appears to be slightly more potent than the *R*-isomer. Lipophilic, 2-chloro substituent is an ideal replacement for 2-methyl (**9i−k**) and a combination of 2-chloro and 4-(3-fluoro) propyl side chain drastically improved the potency by about 10-fold, resulting in the potent compounds of the series, **9j** and **9k**.

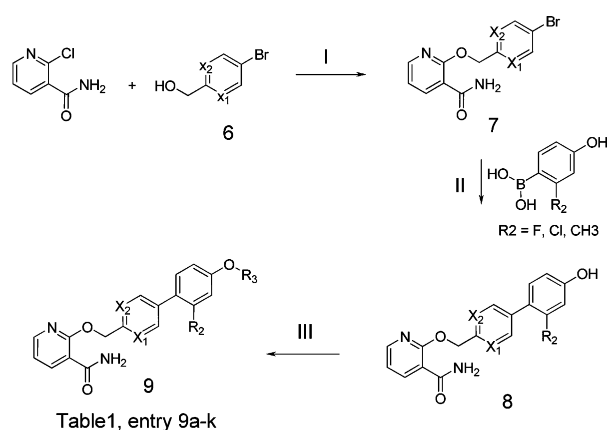
The details of structural modification carried out on ring C are shown in Table 2. Throughout this study, 4-(2-methoxyethoxy)-2-methylphenyl (ring A) and pyridyl (ring B) were kept constant, and modifications were made only on ring C. Preliminary changes were directed toward understanding the requirement of primary amide. In the process, secondary amide **15a** and tertiary amides **15b,c** using methyl amines, dimethyl amines, and 3-hydroxypyrolidine, respectively, were synthesized. The results indicate that the donor−acceptor pair of the amide functionality is very crucial for mycobacterial activity, and the compounds **15b,c** that lacks this were inactive. Other modifications such as sulphonamides **15f** and pyrazine **15g** are also tolerated albeit with less cellular potency. Ring C can also be utilized for improving solubility of the series. Introduction of secondary amide such as **15a** increased the solubility by more than 35-fold without compromising the cellular potency.

Other modifications involving aminoethanol **15d** and basic side chain such as the amino piperidine (**15e**) followed similar

Table 1. Ring A Modifications and SAR

Entry	Ring A substitution	X1/X2	MIC Mtu H37Rv (μM)	MMIC THP-1 (μM)
5a		CH/N	>50.0	ND
5b		CH/N	22.0	>50
5c		CH/N	16.9	>50
5d		CH/N	5.4	>50
5e		CH/N	39.7	ND
9a		CH/N	0.6	>50
9b		N/N	1.5	>50
9c		CH/N	4.7	>50
9d		CH/N	1.2	>50
9e		CH/N	4.7	>50
9f		CH/N	1.1	>50
9g		CH/N	>100	ND
9h		CH/N	8.9	>50
9i		N/N	0.47	>250
9j		N/N	0.12	>250
9k		N/N	0.12	>250
5f		CH/CH	>100	>50
5g		CH/CH	>100	>50

Scheme 2. Synthesis of Alkoxy Nicotinamide: Ring A Modification at the 2nd and 4th Positions[a]



[a]Reagents and conditions: (I) NaH (60% suspension in paraffin oil), DMF, 110 °C, 2 h, yield 60−85%; (II) $PdCl_2$ (dppf)−CH_2Cl_2 (5 mol %), CsF, MeOH, MW at 120 °C, 30 min, yield 60−70%; (III) R_3-Br, K_2CO_3, DMF, 110 °C, 2 h, yield 40−79%.

trends, although with marginal decrease in potency (for solubility data, see Supporting Information Table S7).

In order to understand the requirement of ring B and oxymethylene linker, key compounds were synthesized, and the summary of those changes are given in Table S4 (Supporting Information). The results suggest that the six-membered ring containing 1,4 linkers are necessary to retain the cellular potency, and any deviation from this symmetry results in inactive compounds **20** and **22**. The flexible oxymethylene bridge appears to be equally important for cellular potency. An attempt to swap hydrogen bond acceptor with donor **17** and restricting the rotation of flexible methylene bridge by introducing a methyl group **21** resulted into loss of cellular potency.

It is important to assess the potential toxicity of these compounds given the long duration of TB treatment. TB being mainly an intracellular infection involving human macrophages, the THP-1 cell line was selected for the in vitro cytotoxicity studies. Further, THP-1 is also being used as a macrophage model to examine intracellular growth rates of Mtu.[14] The data in Tables 1 and 2 indicate good selectivity between Mtu and eukaryotic THP-1 cell line. Some of the best compounds in the series did not show cytotoxicity up to 250 μM (**9i−k**), thus demonstrating excellent selectivity.

Compounds **9b**, **9f**, **9j**, and **9k** were tested on a broad spectrum panel involving gram positive, gram negative, and eukaryotic pathogens. The results show that these compounds do not have MIC up to 60 μM, indicating specificity to Mtu (Table 3). This also suggests that these compounds may be inhibiting the target, which is specific to Mtu.

The representative potent compounds were profiled for their in vitro drug metabolism and pharmacokinetics (DMPK)

Table 2. Ring C Modifications and SAR

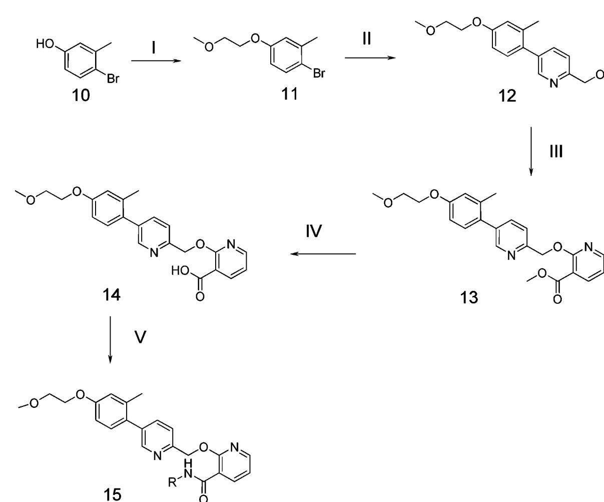
entry	Ring C substitution	MIC Mtu H37Rv (μM)	MMIC THP-1 (μM)
9a		0.6	>50
15a		1.2	>50
15b		>75	>50
15c		>75	>50
15d		4.5	>50
15e		8.1	>50
15f		50	>50
15g		40.5	>50
15h		6.2	>50

properties (see Supporting Information Table S5). The log *D* of these compounds ranged from 2.8 to 3.4. The intrinsic clearance determined from mouse microsomes showed low to moderate values. Among the representatives tested for human plasma protein binding (PPB), **9a** had the best free fraction at 4.2%. These compounds were also subjected to screening against a panel of cytochrome P450 isozymes (CYP) to understand the possible drug−drug interaction issues. The most potent compounds **9j** and **9k** did not inhibitthe majority of the isozymes tested except CYP2C19 and CYP2C9, which had an IC_{50} of 18 and19 μM, respectively. Compound **9a**, which is marginally less potent, did not inhibit any of the isozymes tested.

These compounds were further tested on a panel of drug sensitive and single drug resistant clinical isolates (see Supporting Information Table S6). The compounds **9a**, **9j**, and **9k** retain their cellular potency against all drug-sensitive strains and were found to be within acceptable 4-fold variation in MIC values. In the case of single drug resistant strains, the compounds retain their potency across all the five strains. The significance of potency variation of greater than 4-fold with certain resistant strains will only be confirmed after target link is established. However, the results indicate this class of molecules is likely to be effective against a wide range of clinical isolates and is likely to act through a novel mode of action.

In conclusion, we have shown biarylmethoxy nicotinamides as an attractive and novel chemotype with antimycobacterial activity. The medicinal chemistry optimization resulted in compounds with excellent potency and good selectivity index toward eukaryotic cell lines. The series exhibited tangible SAR and was very specific to Mtu. We believe that this class of molecules could be acting through a novel mode of action and would be more likely to be effective against a wide range of clinical isolates. Studies are underway to identify the target and decipher the mechanism of action of this class of compounds. In vivo profiling efforts are in progress to optimize pharmacokinetic properties for demonstrating efficacy in an animal model.

Scheme 3. Synthesis of Alkoxypyridine Nicotinamide: Ring C Modifications[a]



[a]Reagents and conditions: (I) 1-bromo-2-methoxyethane, K_2CO_3, DMF, 50 °C, 30 min, yield 95%; (II) 6-(hydroxymethyl)pyridin-3-ylboronic acid $PdCl_2(dppf)-CH_2Cl_2$ (5 mol %), $CsCO_3$, MeOH, MW at 120 °C, 45 min, yield 36%; (III) methyl, 2-chloronicotinate, NaH (60% suspension in paraffin oil), DMF, 120 °C, 2 h, yield 54%; (IV) LiOH, 1,4-dioxane, r.t., 18 h, yield 94%; (V) HATU, DIEA, DMF, r.t., 2 h, yield 30−50%.

Table 3. Studies on Broad Spectrum Panel

species	**9b** MIC (μM)	**9f** MIC (μM)	**9j** MIC (μM)	**9k** MIC (μM)
M. tuberculosis H37Rv	1.5	1.1	0.1	0.1
E. coli	>60	>60	>60	>60
H. influenzae	>60	>60	>60	>60
S. aureus	>60	>60	>60	>60
S. pneumoniae	>60	>60	>60	>60
P. aeruginosa	>60	>60	>60	>60
K. pneumoniae	>60	>60	>60	>60
S. pyogenes	>60	>60	>60	>60
C. albicans	>60	>60	>60	>60

ASSOCIATED CONTENT

Supporting Information

Experimental procedures and compound characterizations. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

S.J.T., M.P., S.R., and V.R. drafted/corrected the manuscript and participated in the design and execution of this study. N.R.C. and C.K. performed the chemical syntheses. S.S., P.K., and S.G. performed and interpreted microbiological data. K.M. was the lead biologist. B.B. contributed in scientific discussions.

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Notes

The authors declare no competing financial interest.

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