ACS Medicinal Chemistry Letters

Letter

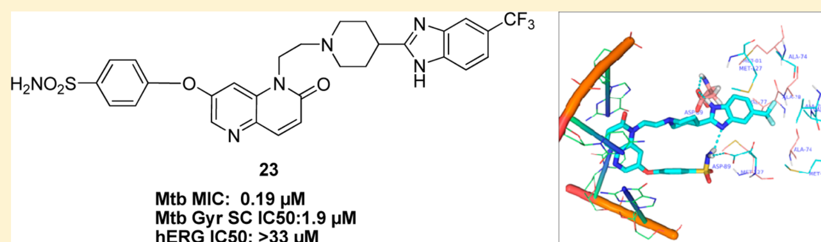
pubs.acs.org/acsmedchemlett

Benzimidazoles: Novel Mycobacterial Gyrase Inhibitors from Scaffold Morphing

Shahul Hameed P,*[,†] Anandkumar Raichurkar,[†] Prashanti Madhavapeddi,[†] Sreenivasaiah Menasinakai,[†] Sreevalli Sharma,[†] Parvinder Kaur,[†] Radha Nandishaiah,[†] Vijender Panduga,[†] Jitendar Reddy,[†] Vasan K. Sambandamurthy,[†] and D. Sriram[‡]

[†]AstraZeneca India Pvt. Ltd., Avishkar, Bellary Road, Bangalore 560024, India

Supporting Information



ABSTRACT: Type II topoisomerases are well conserved across the bacterial species, and inhibition of DNA gyrase by fluoroquinolones has provided an attractive option for treatment of tuberculosis (TB). However, the emergence of fluoroquinolone-resistant strains of *Mycobacterium tuberculosis* (*Mtb*) poses a threat for its sustainability. A scaffold hopping approach using the binding mode of novel bacterial topoisomerase inhibitors (NBTIs) led to the identification of a novel class of benzimidazoles as DNA gyrase inhibitors with potent anti-TB activity. Docking of benzimidazoles to a NBTI bound crystal structure suggested that this class of compound makes key contacts in the enzyme active site similar to the reported NBTIs. This observation was further confirmed through the measurement of DNA gyrase inhibition, and activity against *Mtb* strains harboring mutations that confer resistance to aminopiperidines based NBTIs and *Mtb* strains resistant to moxifloxacin. Structure−activity relationship modification at the C-7 position of the left-hand side ring provided further avenue to improve hERG selectivity for this chemical series that has been the major challenges for NBTIs.

KEYWORDS: *Tuberculosis, type II topoisomerases, DNA gyrase, NBTIs, aminopiperidines, benzimidazoles*

Tuberculosis remains a major public health problem across the world and claims approximately 1.5 million lives each year.[1] The current treatment regimen involves a combination of four drugs administered for six months. The emergence of multidrug resistant (MDR) forms of *Mycobacterium tuberculosis* (*Mtb*) has further aggravated the situation, and individuals with MDR-TB require treatment with a cocktail of 6−8 drugs over an extended period of up to 24 months.[2]

DNA gyrase, a type II toposiomerase responsible for DNA replication and repair, is essential in all bacteria including mycobacteria and absent in eukaryotes. The enzyme catalyzes the interconversion of various topological forms of DNA, an essential process in DNA replication. It introduces negative supercoiling into circular DNA in an ATP-dependent reaction. This enzyme is a heterotetramer comprising of GyrA and GyrB subunits (A2B2). The GyrA subunit contains the DNA breakage-reunion site, while GyrB hydrolyses ATP to generate the energy required for enzyme activity. Unlike most other bacteria, *Mtb* has a functional DNA gyrase but no topoisomerase IV enzyme. There are at least two types of gyrase inhibitors reported in the literature with potent activity against *Mtb*. The first type of inhibitors target the ATP recognition site of GyrB enzyme and block ATP hydrolysis. Examples of this type are aminopyrazinamides, thiazolopyridine ureas, and pyrrolamides.[3,4] The second type of inhibitors target the GyrA subunit thereby inhibiting the DNA breakage−reunion function of the enzyme. This class is exemplified by the fluoroquinolones (FQ), novel bacterial topoisomerase inhibitors (NBTIs) and aminopiperidines.[3,4]

The approval of FQ such as ofloxacin and moxifloxacin to treat several bacterial infections validates DNA gyrase target for the development of newer antibacterial agents. Several studies have shown the clinical benefit of FQs such as ofloxacin and moxifloxacin in the treatment of TB.[5] However, reports of *Mtb* strains resistant to FQs pose a threat to the continued use of this class of drugs to treat TB.[5] Therefore, identification of a novel GyrA inhibitors with unique binding mechanism that is distinctly different from the binding mode of the FQ class is needed. This would lead to the discovery of novel gyrase agents that can act against FQ resistant *Mtb* strains.

In the recent past, numerous NBTIs have been reported in the literature as antibacterial and antimycobacterial agents.[3,4]

Received: April 29, 2014
Accepted: June 2, 2014
Published: June 2, 2014

ACS Publications © 2014 American Chemical Society

The crystal structure of a *Staphylococcus aureus* DNA gyrase bound to one of the NBTI provides insight into the unique binding mechanism of this class of compounds and highlights the key interactions involved in the gyrase active site, which are nonoverlapping to the binding site of FQs. This unique interaction in turn confers the ability of NBTIs to retain their activity against FQ-resistant strains of *S. aureus*.[6]

In general, the NBTIs consists of a quinoline or naphthyridine based left-hand side (LHS) ring, a mono- or bicyclic hydrophobic right-hand side (RHS) ring and a linker joining the RHS and LHS in a proper orientation. Many NBTIs with variations in the LHS, linker and RHS with antibacterial and antimycobacterial activity have been reported. This includes the N-linked aminoperidines with both monocyclic and bicyclic RHS (**1a**-**e**),[3,4] *N*-cyclobutyl piperdine-3-carboxylic acid (**1f**),[8] tetrahydropyran (**1g**),[9] and oxabicyclooctane (**1h**) (Figure 1).[10]

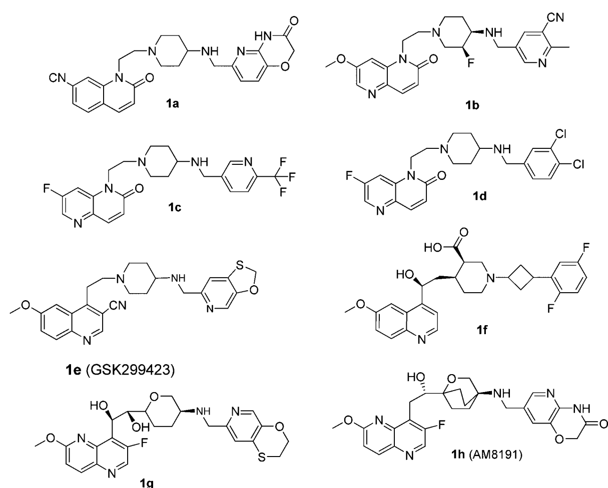


Figure 1. Novel type II topoisomerase inhibitors reported in the literature.

The aminopiperidine-based NBTIs with both monocyclic and bicyclic RHS (**1a**−**1g**) have shown *in vitro* activity against *Mtb* and also been shown to retain their activity against FQ-resistant strains of *Mtb*. In addition, this class of compounds were found to be efficacious in a murine model of tuberculosis.[4,7] However, inhibition of the cardiac ion channel (hERG) remains a serious liability for the aminopiperidine based NBTIs with monocyclic RHS. In order to identify a scaffold with novel RHS group, we embarked on a scaffold hopping approach based on the literature reported amino-piperidines and its bound crystal structure with the *S. aureus* DNA gyrase (Figure 2). Herein, we report the discovery of a novel class of benzimidazoles (**5** and **6**, Figure 2), which inhibits *Mtb* DNA gyrase with potent antimycobacterial activity. These compounds are bactericidal and retain their minimum inhibitory concentration (MIC) against FQ-resistant strains of *Mtb*. The identified structure−activity relationship (SAR) for hERG mitigation provides an attractive opportunity to further optimize the benzimidazole lead toward preclinical candidate selection.

The syntheses of compounds with piperidine linkers are described in Scheme 1.

Alkylation of LHS ring (**2a**−**c**) with 2-bromoethanol in the presence of cesium carbonate as a base provided *N*-alkylated product (**3a**−**d**) as the major isomer. Nucleophilic displace-

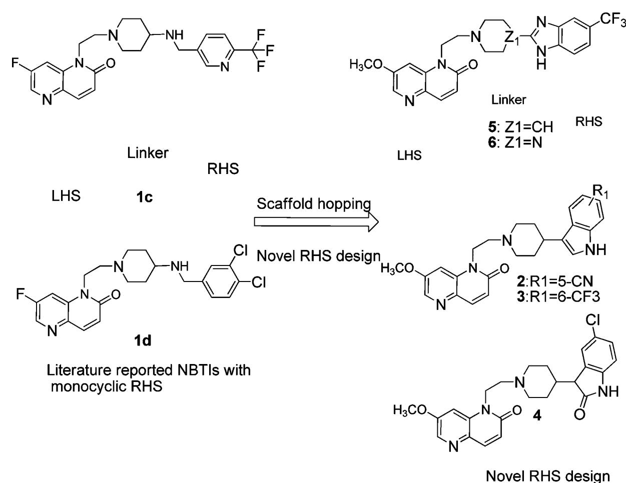
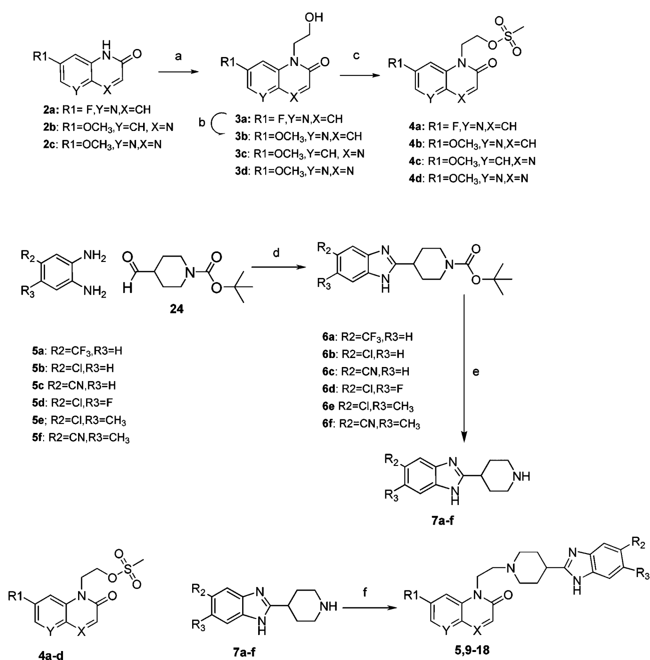


Figure 2. Scaffold hopping of NBTIs.

Scheme 1. Synthesis of Benzimidazoles[a]



[a]Reagents and conditions: (a) $CsCO_3$, DMSO, 90 °C; (b) NaOMe, MeOH, 75 °C; (c) mesyl chloride, DIPIEA, DCM, 0 °C; (d) PTSA, ethanol, 90 °C; (e) 4 N HCl in dioxane, 50 °C; (f) Na_2CO_3, DMF, 130 °C.

ment of **3a** with sodium methoxide in methanol under heating conditions resulted in intermediate **3b**. The mesylates (**4a**−**d**) were obtained by treating **3a**−**d** with mesyl chloride under basic conditions. The mesylates (**4a**−**d**) were freshly prepared and used immediately for the next step due to potential instability reasons. The *N*-BOC protected piperidinyl benzimidazoles (**6a**−**f**) were synthesized by treating corresponding *ortho*-phenylenediamines (**5a**−**f**) with *N*-BOC piperidine-4-carboxaldehyde (**24**) in the presence of *para*-toluene sulfonic acid (catalytic amount) under open air conditions. The secondary piperidines (**7a**−**f**) were obtained upon deprotection of *N*-BOC group under acidic conditions. Alkylation of secondary piperidines (**7a**−**f**) with mesylates (**4a**−**d**) in the presence of Na_2CO_3 as base under thermal heating conditions resulted in the title compounds (**5** and **9**−**18**).

Treatment of mesylate **4a** with *N*-BOC protected piperazine (**8b**) under basic condition and heating provided **9a** (Scheme S2, Supporting Information). Intermediate **9b** was obtained from **9a** using sodium methoxide. Alkylation of secondary piperazines (**7a–g**) with 2-choro benzimidazoles (**11a–c**) in the presence of a base under microwave conditions resulted in the title compounds (**6–8** and **19**, Scheme S2, Supporting Information). Compounds **2**, **3**, and **4** were synthesized as per Schemes S4 and S5 (Supporting Information). Nucleophilic displacement of fluoro naphthyridones (**8** and **10**) with pyridinol (**12a**) or phenol (**12b**) or using cesium carbonate under thermal heating conditions resulted in the title compounds **21–23** (Scheme S3, Supporting Information).

Scaffold Hopping and Hypothesis Generation. Toward identifying a structurally distinct novel mycobacterial gyrase inhibitor, we attempted scaffold hopping of NBTIs. The binding mode of NBTIs from the published crystal structures revealed that the linker –NH group and the RHS part of NBTIs (**GSK299423**, **1e**, Figure 3a)[6] makes a critical interaction in the protein. So we have focused our scaffold morphing efforts toward designing of new compounds with novel RHS (compounds **2–6**, Figure 2). We hypothesized that constraining the NH of amino piperidine based NBTIs as part of a bicyclic ring should provide access to the hydrogen bonding interaction with Asp83 (Asp89 in the case of *Mtb* gyrase) as observed in the NBTI bound crystal structure of *S. aureus* DNA gyrase (PDB ID: 2XCS). To validate this hypothesis, we designed compounds with benzimidzole (**5** and **6**, Table2), indole (**2** and **3**, Table 1), and oxindoles (**4**, Table 1) as novel RHS (Figure 2).

The newly designed compounds **2**, **3**, **4**, and **5** were docked into 2XCS and a *Mtb* DNA gyrase subunit A (*Mtb* GyrA) homology model to understand the binding interactions. Figure 3 depicts the possible binding modes of compounds **2**, **3**, **4**, and **5** in the *S. aureus* DNA bound GyrA subunit and in the *Mtb* GyrA model (Figure S7 in Supporting Infromation). The H-bonding contacts with Asp83 (Asp89 in the case of *Mtb* GyrA model) and hydrophobic pocket occupied by Ala68, Val71, Gly72, and Met121 of *S. aureus* DNA bound GyrA (Ala74, Val77, Ala78, and Met127 in *Mtb* GyrA, respectively) were key interactions in the NBTIs binding site. The binding mode for benzimidazole derivative **5** suggests that the hydrogen of N-1 atom of benzimidazole ring is engaged in H-bonding interaction with the carboxylate of Asp83 (Asp89 in the case of *Mtb* GyrA model) and CF_3 group attached to C-5 position point toward the hydrophobic pocket. This is similar to the interactions observed for NBTIs. Thus, compound **5** is likely to be active against *Mtb* gyrase. However, the ring NH group in compounds **2**, **3**, and **4** is not suitably placed to make the desired hydrogen bonding interaction with Asp 83 (Asp89 in the case of *Mtb* GyrA model) due to the adaptation of a different conformational orientation of indole (**2** and **3**) and oxindole (**4**) rings in comparison to benimidazole ring (**5**). Furthermore, the substituent attached to C-5 or C-6 position of bicylic rings in compounds **2** and **4** is likely to be pointing away from the hydrophobic pocket constituting Ala68, Val71, Gly72, and Met121 of *S. aureus* GyrA (Ala74, Val77, Ala78, and Met127 in *Mtb* GyrA, respectively). The CF_3 group attached to C-6 in compound **3** is likely to pick up the hydrophobic interaction. In all cases, the naphthyridone ring assembled between the two base pairs of DNA, similar to the reported binding mode of NBTIs.

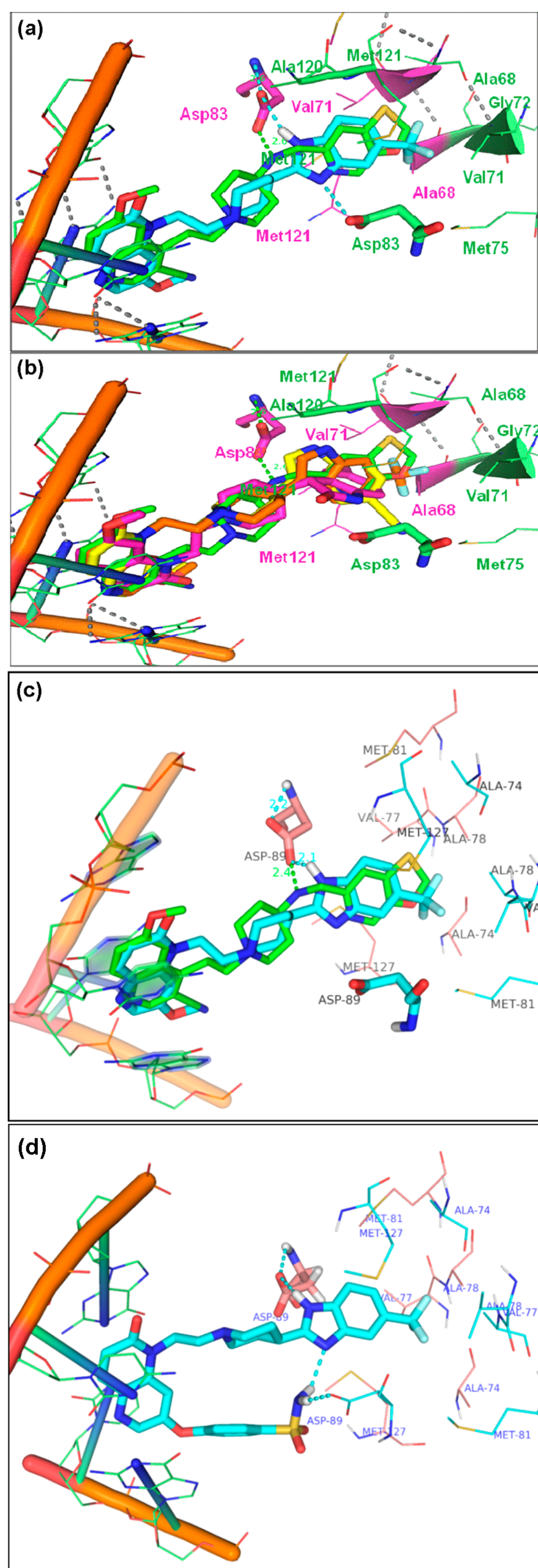


Figure 3. (a,b) Docking pose of compounds **5** (cyan), **4** (pink), **3** (orange), and **2** (yellow) on to the crystal bound structure of GSK299423 (green) to *S. aureus* DNA gyrase.[6] (c) Docking pose of compound **5** on to the homology model of *Mtb* DNA gyrase subunit A. (d) Docking pose of compound **23** on to the homology model of Mtb DNA gyrase subunit A.

Table 1. Profile of Compounds with Novel RHS

entry	MIC *Mtb* H37Rv (μM)	*Mtb* gyrase SC IC_{50} (μM)	Msm GyrB ATPase IC_{50} (μM)	cytotoxicity THP-1 IC_{50} (μM)	hERG IC_{50} (μM)	logD pH 7.4
2	100	>50	>100	>50	2	3.3
3	50	>50	>100	>50	1.8	4
4	100	>50	>100	>50	4.8	2.8
5	0.19	1.0	>100	>50	1.0	4

On the basis of these observations, we synthesized compounds **2**–**5** with different RHS and tested for *Mtb* gyrase inhibition. The biological activities, *in vitro* safety, and logD of these novel leads are shown in Table 1.

Compound **5** with benzimidazole RHS inhibited *Mtb* DNA gyrase supercoiling in the single-digit micromolar range and displayed potent MIC against *Mtb*. Compounds **2**, **3**, and **4** with indole and oxindole RHS, were inactive against the enzyme (IC_{50} > 50 μM) and showed weak *Mtb* MIC (50–100 μM). As predicted by docking studies, compounds **2**–**4** are

Table 2. SAR Modifications around the RHS and LHS Part of Benzimidazoles

Entry	R1	X,Y,Z	R_2 & R_3	MIC *Mtb* (μM)	LogD pH7.4	hERG IC_{50} (μM)
5	OCH_3	X=Z=CH, Y= N	R_2=CF_3 R_3=H	0.19	4	1
6	OCH_3	X=CH, Y=Z=N	R_2=CF_3 R_3=H	3.13	2.8	10
7	OCH_3	X=CH, Y=Z=N	R_2=H R_3=H	100	2.4	7
8	F	X=CH, Y=Z=N	R_2=CF_3 R_3=H	15	3.7	1
9	F	X=Z=CH, Y=N	R_2=CF_3 R_3=H	0.78	ND[a]	ND[a]
10	F	X=Z=CH, Y=N	R_2=CN R_3=H	50	ND[a]	ND[a]
11	OCH_3	X=Z=CH, Y=N	R_2=Cl R_3=H	0.25	3.6	1
12	OCH_3	X=Z=CH, Y=N	R_2=CN R_3=H	3.15	2.4	4.5
13	OCH_3	X=Z=CH, Y=N	R_2=Cl R_3=F	0.06	4	1.2
14	OCH_3	X=Z=CH, Y=N	R_2=Cl R_3=CH_3	0.13	3.8	1.4
15	F	X=Z=CH, Y=N	R_2=Cl R_3=CH_3	1.56	3.7	1.2
16	OCH_3	Y=Z=CH, X=N	R_2=Cl R_3=CH_3	0.13	ND[a]	ND[a]
17	OCH_3	X=Y=N, Z=CH	R_2=Cl R_3=CH_3	0.39	3.5	10
18	F	X=Z=CH, Y=N	R_2=CN R3=CH_3	3.15	ND[a]	ND[a]
19	OCH_3	X=Z=CH, Y=N	R_2=CN R_3=CH_3	0.19	2.8	10
20	OCH_3	X=CH, Y=Z=N	R_2=CN R_3=CH_3	0.78	3.2	>33
21		X=Z=CH, Y=N	R_2=CN R_3=H	3.13	2.9	1.4
22		X=Z=CH, Y=N	R_2=CN R_3=H	0.78	2.2	>33
23		X=Z=CH, Y=N	R_2=CF_3	0.19	2.4	>33

[a]Not determined.

dx.doi.org/10.1021/ml5001728 | ACS Med. Chem. Lett. 2014, 5, 820–825

expected to be inactive against the *Mtb* gyrase, as these compounds are unable to pick up the critical interaction with Asp89. Hence, the observed lack of *Mtb* gyrase inhibition for compounds **2**−**4** is in agreement with the results from the docking studies. In order to rule out any contribution of *Mtb* GyrB inhibition to the observed *Mtb* MICs, compounds were screened against *Mycobacterium smegmatis* (*Msm*) GyrB as a surrogate for *Mtb* GyrB enzyme.[3] The lack of inhibitory activity of compounds **2**, **3**, **4**, and **5** against the *Msm* GyrB clearly indicated that these compounds were inhibitors of either the holoenzyme (GyrA2B2 complex) or GyrA subunit. In addition, these compounds showed no signs of cellular cytotoxicity (IC_{50} > 50 μM) against a human monocytic cell line (THP1), thereby demonstrating excellent selectivity. However, these compounds showed potent hERG inhibition at single digit micromolar range (hERG IC_{50} 1 to 4.8 μM, Table1), likely due to higher lipophilicity (logD = 2.8 to 4) observed with the initial set of compounds synthesized in this study.

Encouraged by the potent *Mtb* activity observed for compound **5**, we envisaged the SAR requirements for the newly identified benzimidazole RHS and we used MIC as an activity indicator to track the SAR. We assumed that the mechanism of gyrase inhibition was similar across the newly synthesized compounds with benzimidazole RHS. Piperidine-based linker was found to be optimal for *Mtb* MIC. A substitution of piperidine with piperazine led to the weakening of *Mtb* MIC by 15-fold (compound **5** versus **6**, Table 2). Removing the R2 substitution at the C-5 position of benzimidazole resulted in the loss of Mtb MIC by 25-fold, thereby proving the essentiality of hydrophobic group at the C-5 position (compound **6** versus 7, Table 2). Similarly, changing OCH_3 to F at R1 position of LHS resulted in the loss of *Mtb* MIC by 4−8-fold, suggesting that a bulky substitution may be essential for the MIC activity (**7** versus **8**, **5** versus **9**, **10** versus **12**, and **14** versus **15** as match pairs, Table 2). As suggested by modeling studies, the RHS benzimidazole-binding region was more hydrophobic in nature; additionally the electron withdrawing hydrophobic groups such as CF_3 strengthens the H-bonding interaction of ring NH with Asp 89. In order to expand the hydrophobic pocket SAR further, we varied R2 substitutions at the C-5 and introduced an R3 substitution at C-6 position of benzimidazole RHS. Replacement of CF_3 group by Cl at the C-5 position retained the *Mtb* MIC (compound **5** versus **11**), whereas CN substitution weakened MIC by 20-fold (compound **5** versus **12**). This data suggests that the electron withdrawing hydrophobic group at the C-5 position is essential for conferring potent *Mtb* MIC for this series. Introduction of other hydrophobic substituents such as methyl or fluorine enhanced the potency by 4-fold (compound **11** versus **13** and **14**, Table 2). The addition of a nitrogen at the 8 position of LHS ring as pyrido[2,3-*b*]pyrazin-2(1*H*)-one (compound **16**) or moving nitrogen to 4-position of LHS as 1,4-quinoxazolinone (compound **17**) was broadly tolerated for potency. Compound **17** showed moderate improvement in reducing hERG inhibition (hERG IC_{50} 10 μM against 1.4 μM for compound **14**). This could be due to a slight reduction in logD or disturbance of pi-stacking of the LHS group to the hERG channel. Furthermore, changing Cl to CN at the C-5 position of benzimidazole RHS with both piperidine and piperazine linker retained the *Mtb* MIC but showed >4-fold improvement in mitigation of hERG (**14** versus **19** and **20**, Table 2). The mitigation of hERG liability observed for compounds **19** and **20** may be due to lowered logDs and disturbing the pi-stacking interaction of the RHS group to the hERG channel.

To expand the SAR scope of R1 group at LHS and improve hERG selectivity, we explored suitably substituted aryl rings at R1 of LHS that can lower logD. Initially, introduction of 3-pyridyloxy at R1 instead of OCH_3 retained *Mtb* MIC but did not show any improvement in reducing hERG liability. Replacement of 3-pyridyloxy by 4-phenoxy sulphonamide at R1 position of LHS showed excellent improvement in *Mtb* MIC and hERG mitigation (compound **21** versus **22** and **23**, Table 2). This suggested that polar bulky substitution at R1 position may not disturb the binding orientation of NBTIs as evident from the docking pose of **23** in *Mtb* GyrA homology model (Figure 3d). Further exploration of this trend with various combinations of RHS will be presented. In order to link whole cell potency to *Mtb* gyrase inhibition, representative compounds (**5**, **6**, **13**, **14**, **19**, and **23**) were tested in the *Mtb* DNA gyrase supercoiling assay (Table 3). Most of the compounds with MIC against *Mtb* also inhibited supercoiling activity of *Mtb* gyrase thus confirming their mode of inhibition.

Table 3. Activity of Benzimidazoles against Mutants Resistant to Compounds 1a, 1d, or Moxifloxacin

		MIC (μM)			
entry	*Mtb* gyrase SC IC_{50} (μM)	*Mtb* H37Rv wild-type	Compd **1a**^{R} mutant (A74V)	Compd **1b**^{R} mutant (D89N)	Moxi^R mutant (G88N)
1a	0.11	0.19	4	>100	<0.19
1c	0.25	0.19	2	>100	<0.19
5	1.0	0.19	50	25	0.78
13	2	0.03	100	25	0.06
19	4.3	0.19	>100	100	0.78
20	ND^a	0.78	>100	100	1.56
23	1.9	0.19	25	12.5	0.39
moxifloxacin	12.5	0.13	0.25	2	4
ciprofloxacin	ND^a	0.5	0.5	16	8
isoniazid	ND^a	0.06	0.06	0.03	0.06
rifampicin	ND^a	0.015	0.008	0.015	0.008

^{a}Not determined.

In order to differentiate benzimidazoles from FQs and NBTIs, compounds (**5**, **6**, **13**, **14**, **19**, and **23**) were screened against moxifloxacin and NBTI-resistant mutant of *Mtb* (**1a** and **1b** mutants).[4] These compounds retained their MIC against lab derived moxifloxacin-resistant mutant, but lost their activity against a NBTI-resistant mutant by >25-fold. This result indicates that this class of molecules is likely to be effective against FQ-resistant strains of *Mtb* and that the mechanism of inhibition is similar to that reported for other NBTIs.

The representative compounds (**19** and **20**) were evaluated for their activity against a panel of cytochrome P450 isozymes (CYP) and *in vivo* pharmacokinetics profile in rats (Table S4, Supporting Information). Compounds **19** and **20** did not inhibit CYP isozymes up to 20 μM, except against CYP3A4 enzyme (IC_{50} of 9.5 μM for **19**).

Compounds **19** and **20** exhibited low to moderate *in vivo* clearance, low volume of distribution, and short half-life in rats. Oral bioavailability was found to be very low (1 to 3%) for both compounds despite low to moderate clearance (Table S4, Supporting Information). This could be due to poor oral

absorption associated with low solubility and poor intestinal permeability of these compounds.

In conclusion, we have described the discovery of benzimidazoles as novel gyrase inhibitors with potent *Mtb* MIC and with a similar mechanism as that of NBTIs. Introduction of polar groups at the C-7 position of LHS provided avenues for building selectivity SARs against hERG. Further efforts are required to optimize PK parameters and to assess *in vivo* safety. We believe that this class of compounds has potential to be developed as anti-TB drug candidate.

■ ASSOCIATED CONTENT

Supporting Information

Computational methods; determining the antimycobacterial properties; determination of IC_{50} for the supercoiling activity of *M. tuberculosis* H37Rv gyrase holoenzyme; determination of resistance frequency; genetic mapping of mutations conferring resistance to N-linked aminopeperidinyl alkyl quinolones and naphthyridones; assay procedures for log D and hERG measurement; DMPK profile for benzimidazoles; sythetic schemes and procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(S.H.P.) Tel: + 91 080 23621212. Fax: + 91 080 23611214. E-mail: shahulmehar0615@gmail.com or shahulmehar@yahoo.com.

Present Address

‡(D.S.) BITS-Pilani, Hyderabad Campus, Shameerpet, Hyderabad 500 078, India.

Author Contributions

S.H.P., V.S., A.R., P.M., and S.D. wrote the manuscript and participated in the design and execution of this study. S.H.P. performed the chemical syntheses. S.S., R.N., J.R., P.V., and P.K. performed and interpreted biological data. S.M. performed the analytical experiments to assign the structure of newly synthesized compounds.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Our sincere thanks to Drs. T. Balganesh, B. Ugarkar, Pravin Iyer Balasubramanian, and Shridhar Narayanan for their valuable suggestions and encouragement during the course of this work. We aslo thank CMG Bangalore, AZ Global DMPK, Safety, Biosciences (AZ Boston, USA), and Discovery Sciences (AZ, Alderly Park, UK) for technical support in various assays

■ REFERENCES

(1) World Health Organization. Global tuberculosis control, 2012. http://www.who.int/mediacentre/news/releases/2011/tb_20111011/en/index.html.

(2) World Health Organization. Multidrug and extensively drug-resistant TB (M/XDR-TB); WHO 2010 global report on surveillance and response.

(3) Mayer, C.; Janin, Y. L. Non-quinolone inhibitors of bacterial type IIA topoisomerases: a feat of bioisosterism. *Chem. Rev.* **2014**, *114*, 2313−2342.

(4) Hameed, P. S.; Patil, V.; Solapure, S. M.; Sharma, U.; Madhavapeddi, P.; Raichurkar, A.; Murugan Chinnapattu, M.; Manjrekar, P.; Shanbhag, G.; Puttur, J.; Shinde, V.; Menasinakai, S.; Rudrapatana, S.; Achar, V.; Awasthy, D.; Nandishaiah, R.; Hmnabadkar, V.; Ghosh, A.; Narayan, C.; Ramya, V. K.; Kaur, P.; Sharma, S.; Wirngren, J.; Hoffner, H.; Panduga, V.; Naveen Kumar, C. N.; Reddy, J.; Mahesh Kumar, K. N.; Ganguly, S.; Bharath, S.; Mukherjee, K.; Arora, U.; Gaonkar, S.; Coulson, M.; Waterson, D.; Sambandamurthy, V. K.; de Sousa, S. M. Novel N-linked aminopiperidine based gyrase inhibitors with improved hERG and in vivo efficacy against *Mycobacterium tuberculosis*. *J. Med. Chem.* **2014**, DOI: 10.1021/jm500432n.

(5) Duong, D. A.; Nguyen, T. H.; Nguyen, T. N.; Dai, V. H.; Dang, T. M.; Vo, S. K.; Do, D. A.; Nguyen, V. V.; Nguyen, H. D.; Dinh, N. S.; Farrar, J.; Caws, M. Beijing genotype of *Mycobacterium tuberculosis* is significantly associated with high-level fluoroquinolone resistance in Vietnam. *Antimicrob. Agents Chemother.* **2009**, *53*, 4835−4839.

(6) Bax, B. D.; Chan, P. F.; Eggleston, D. S.; Fosberry, A.; Gentry, D. R.; Gorrec, F.; Giordano, I.; Hann, M. M.; Hennessy, A.; Hibbs, M.; Huang, J.; Jones, E.; Jones, J.; Brown, K. K.; Lewis, C. J.; May, E. W.; Saunders, M. R.; Singh, O.; Spitzfaden, C. E.; Shen, C.; Shillings, A.; Theobald, A. J.; Wohlkonig, A.; Pearson, N. D.; Gwynn, M. N. Type IIA topoisomerase inhibition by a new class of antibacterial agents. *Nature* **2010**, *466*, 935−940.

(7) Barrows, D. Recent Advances in TB Drug Development. 40th IUATLD Meeting, Cancun, Mexico, Dec 5, 2009. http://www.newtbdrugs.org/eventfiles/p4/Novel%20Mtb%20DNA%20Gyrase%20inhibitors_The%20Union_40th.pdf.

(8) Mitton-Fry, M. J.; Brickner, S. J.; Hamel, J. C.; Brennan, L.; Casavant, J. M.; Chen, M.; Chen, T.; Ding, X.; Driscoll, J.; Hardink, J.; Hoang, T.; Hua, E.; Huband, M. D.; Maloney, M.; Marfat, A.; McCurdy, S. P.; McLeod, D.; Plotkin, M.; Reilly, U.; Robinson, S.; Schafer, J.; Shepard, R. M.; Smith, J. F.; Stone, G. G.; Subramanyam, C.; Yoon, K.; Yuan, W.; Zaniewski, R. P.; Zook, C. Novel quinoline derivatives as inhibitors of bacterial DNA gyrase and topoisomerase IV. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 2955−2961.

(9) Surivet, J. P.; Zumbrunn, C.; Rueedi, G.; Hubschwerlen, C.; Bur, D.; Bruyère, T.; Locher, H.; Ritz, D.; Keck, W.; Seiler, P.; Kohl, C.; Gauvin, J. C.; Mirre, A.; Kaegi, V.; Dos Santos, M.; Gaertner, M.; Delers, J.; Enderlin-Paput, M.; Boehme, M. Design, synthesis, and characterization of novel tetrahydropyran-based bacterial topoisomerase inhibitors with potent anti-Gram-positive activity. *J. Med. Chem.* **2013**, *56*, 7396−7415.

(10) Singh, S. B.; et al. Oxabicyclooctane-linked novel bacterial topoisomerase inhibitors as broad spectrum antibacterial agents. *ACS. Med. Chem. Lett.* **2014**, *5*, 609−614.