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Synthesis and in vitro antimycobacterial activity of B-ring modified diaryl ether InhA inhibitors

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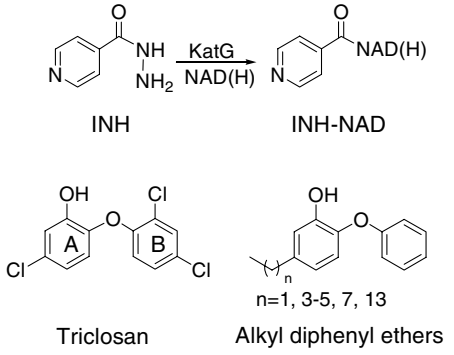
Abstract—Previous structure-based design studies resulted in the discovery of alkyl substituted diphenyl ether inhibitors of InhA, the enoyl reductase from *Mycobacterium tuberculosis*. Compounds such as 5-hexyl-2-phenoxyphenol **19** are nM inhibitors of InhA and inhibit the growth of both sensitive and isoniazid-resistant strains of *Mycobacterium tuberculosis* with MIC_{90} values of 1–2 μg/mL. However, despite their promising in vitro activity, these compounds have *C*log *P* values of over 5. In efforts to reduce the lipophilicity of the compounds, and potentially enhance compound bioavailability, a series of B ring analogues of **19** were synthesized that contained either heterocylic nitrogen rings or phenyl rings having amino, nitro, amide, or piperazine functionalities. Compounds **3c**, **3e**, and **14a** show comparable MIC_{90} values to that of **19**, but have improved *C*log *P* values.

Tuberculosis (TB) is responsible for more than 1.6 million deaths per annum with 8.8 million new cases being reported each year. These numbers make TB one of the leading infectious causes of death, eclipsed only by AIDS. In addition, according to the World Health Organization, the number of multi-drug-resistant and extensively drug-resistant TB cases is growing with almost a half million new cases being reported each year. Therefore, there is an urgent need to develop novel TB chemotherapeutic agents.[1]

The current front-line treatment strategy utilizes isoniazid (INH), a pro-drug which inhibits the synthesis of mycolic acids that are essential components required for the integrity of the bacterial cell wall.[2] INH inhibits InhA, the FabI enoyl reductase (ENR) in the fatty acid synthesis (FAS-II) pathway. However, before INH can inhibit InhA, it must be activated by KatG, a catalase-peroxidase enzyme. The activated form of INH then reacts with NAD^+ to form the INH-NAD adduct (Scheme 1).[3–7] A significant number of the strains resistant to INH arise from mutations in KatG.[8–11] Therefore, the development of an InhA inhibitor which can bypass this



Scheme 1. Enoyl reductase inhibitors.

Keywords: Tuberculosis; Diaryl ether; Enoyl reductase; Mycolic acids; InhA; Isoniazid; Antitubercular drug; Diphenyl ether; FabI; Lipinski parameter; Bioavailability.
* Corresponding authors. Tel.: +1 970 491 1925 (R.A.S.), tel.: +1 631 632 7907; fax: +1 631 632 7934 (P.J.T.); e-mail addresses: richard.slayden@colostate.edu; peter.tonge@sunysb.edu

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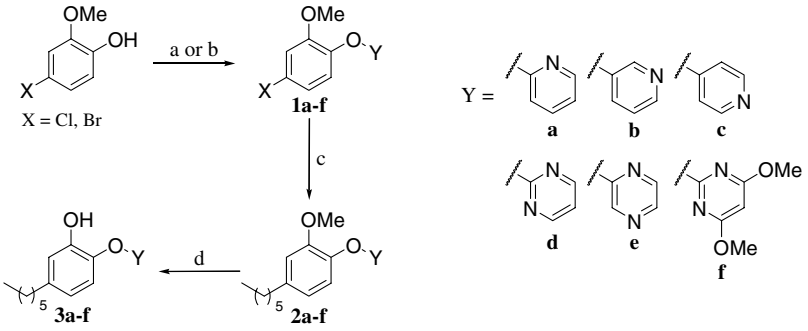
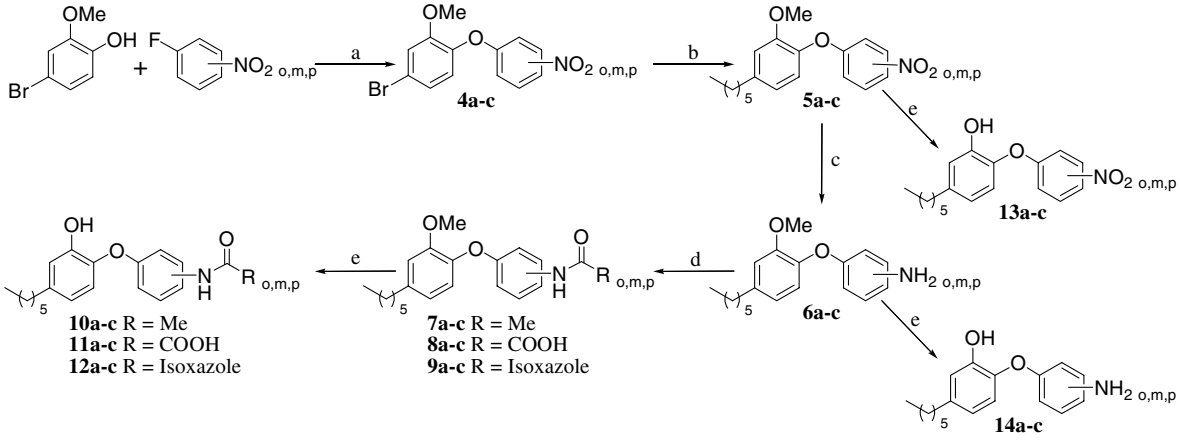
initial activation step should have activity against INH-resistant strains of *Mycobacterium tuberculosis* (MTB).

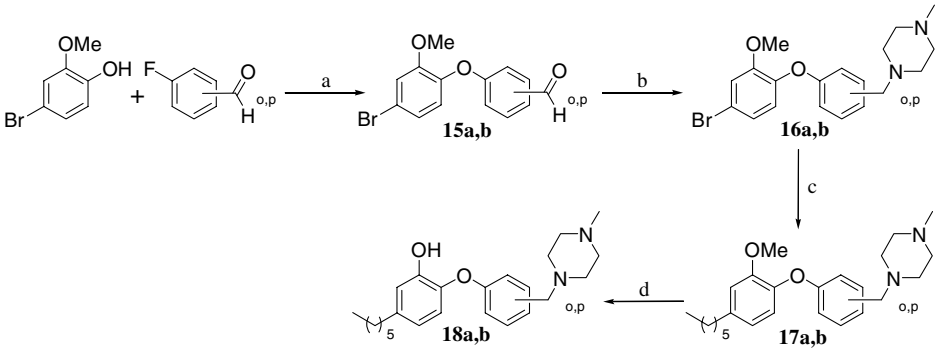
The diphenyl ether triclosan (Scheme 1) is a potent inhibitor of ENR's from many organisms including *Escherichia coli* and *Plasmodium falciparum*.[12–20] However, this compound only inhibits InhA with a K_i value of 0.2 μM.[21] Using structure-based drug design we developed a series of alkyl diphenyl ethers that are potent inhibitors of InhA, with K_i values as low as 1 nM and MIC_{90} values of 1–2 μg/mL against *M. tuberculosis* $H37_{Rv}$.[22] Importantly the alkyl diphenyl ethers display similar MIC values against INH-resistant strains of MTB.[22] However, despite their promising in vitro activity, these compounds have relatively low solubility and have *C*log *P* values greater than 5, which is likely one reason why they have limited in vivo efficacy.[23] Based on the observed relationship between lipophilicity and



Scheme 2. Reagents and conditions: (a) K_2CO_3, DMAc, Δ, Y; (b) $(CuOTf)_2{\cdot}PhH$, Cs_2CO_3, EtOAc, toluene, 120 °C, Y; (c) hexyl ZnCl, $Pd(P(t\text{-}Bu)_3)_2$, THF/NMP, 130 °C; (d) BBr_3, DCM, 0 °C to rt.



Scheme 3. Reagents and conditions: (a) K_2CO_3, DMAc, Δ; (b) hexyl ZnCl, $Pd(P(t\text{-}Bu)_3)_2$, THF/NMP, 130 °C; (c) Zn, HCl, EtOH, rt; (d) acyl chloride, NEt_3, DCM; (e) BBr_3, DCM, 0 °C to rt.



Scheme 4. Reagents and conditions: (a) K_2CO_3, DMAc, Δ; (b) *N*-methyl piperazine, $NaBH(OAc)_3$, DCE; (c) hexyl ZnCl, $Pd(P(t\text{-}Bu)_3)_2$, THF/NMP, 130 °C; (d) BBr_3, DCM, 0 °C to rt.

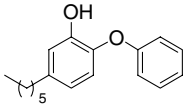
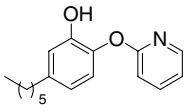
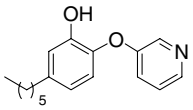
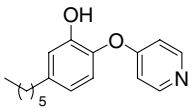
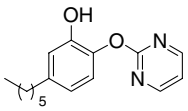
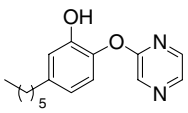
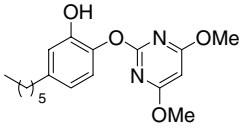
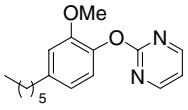
in vivo efficacy, especially as it pertains to antibacterial compounds,[24,25] we synthesized a series of analogues that incorporated functionalities designed to increase the polarity of the parent diphenyl ether InhA inhibitors. The effect on compound polarity was estimated by calculating the log *P* value (*C*log *P*) for each compound synthesized.

In this study we describe two classes of molecules in which alterations have been made to the diphenyl ether 'B' ring. In one series of compounds we have replaced the B ring with isosteric heterocycles that incorporate nitrogen atoms within the ring, thereby causing little steric perturbation to the overall structure of the molecule (Scheme 2). The second series of compounds have nitro, amino, amide, and piperazino functionalities incorporated at the *ortho*, *meta*, or *para* positions of the B ring (Schemes 3 and 4). This second series of compounds was synthesized not only to improve solubility but also to systematically identify positions on the B ring which could be substituted without diminishing biological activity.

The synthesis of the heterocyclic diaryl ether compounds was initiated either by nucleophilic aromatic substitution or by Buchwald–Hartwig cross-coupling of the appropriate nitrogen heterocycle with 4-bromo or chloro-2-methoxy phenol producing **1a–f** (Scheme 2).[26,27] This was followed by palladium catalyzed Negishi coupling of the diaryl ethers with hexyl zinc chloride to give **2a–f**.[28] Boron tribromide cleavage of the methyl ether was used subsequently to generate the respective phenols, **3a–f**.[29] Structural characterization of all compounds was performed using ^{1}H NMR and ESI/MS.

The synthesis of the nitro, amino, and amide-substituted compounds was performed using the series of reactions shown in Scheme 3. Nucleophilic aromatic substitution reactions with fluoronitrobenzenes were first used to generate compounds **4a–c**.[27] This was followed by Negishi coupling giving **5a–c** followed by boron tribromide cleavage to give compounds **13a–c** or zinc-mediated reduction giving anilines **6a–c**.[28–30] Cleavage of the methyl ether gave **14a–c** while acylation of the anilines

Table 1. Inhibition and solubility data for B-ring heterocycles

Compound	Structure	IC_{50} [a] (nM)	MIC_{90} (μg mL $^{-1}$)	*C*log *P* [b]	log *P*
19		11 ± 1[c]	2.1 ± 0.9	6.47	5.76
3a		11,500 ± 1160	50	4.97	5.06
3b		236 ± 31	3.13	4.97	
3c		160 ± 16	3.13	4.97	4.93
3d		8200 ± 980	100.0 ± 0	4.01	4.46
3e		650 ± 60	6.25 ± 0	4.01	4.764
3f		NI[d]	100	5.50	
2d		>100,000	75 ± 0	4.50	

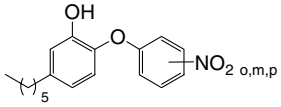
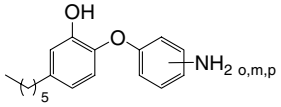
[a] Enzyme concentration is 100 nM.
[b] *C*log *P* values determined using ChemDraw 8.0.
[c] Enzyme concentration is 1 nM.
[d] No inhibition.

with acyl chlorides afforded compounds **7**, **8**, and **9a–c**.[29,31] Boron tribromide cleavage then gave the final compounds **10**, **11**, and **12a–c**.[29]

The piperazine derivatives were synthesized in a similar fashion starting with nucleophilic aromatic substitution with the 2- or 4-fluorobenzaldehyde to give **13a** and **b** (Scheme 4).[27] Subsequently, reductive amination with methyl piperazine and sodium triacetoxyborohydride produced **14a** and **b**,[32] whereas Negishi coupling followed by boron tribromide cleavage gave the final compounds **16a** and **b**.[28,29]

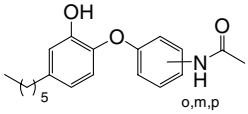
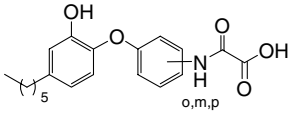
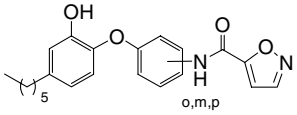
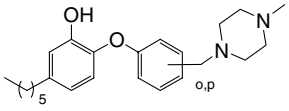
The in vitro activities of the ultimate products were evaluated using enzyme inhibition and whole cell antibacterial assays as described previously (Tables 1–3).[22,33,34] In general, addition of a bulky substituent at either the *ortho*, *meta*, or *para* position of the B ring of **19** or incorporation of the most aromatic nitrogen heterocycles resulted in a significant reduction in both enzyme inhibition and antibacterial activity (Tables 1 and 3). In contrast, introduction of either amino or nitro substituents at the *ortho* and *para* positions had only a minimal effect on activity (Table 2). The two most active compounds, **3c** and **14a**, have MIC_{90} values of 3.13 μg/mL, similar to that of **19**, and have *C*log *P* values of 4.97 and 5.24, respectively, compared to 6.47 for the parent compound (Tables 1 and 2). In addition it is also worth noting that the pyrazine derivative **3e** has a *C*log *P* value that is more than one log lower than **19**, but still only shows a 3-fold increase in MIC_{90} compared to the parent (Table 1). In general the MIC values correlated with the IC_{50} values for enzyme inhibition. Thus *ortho* and *para* amino substituents (**14a,c**) were well tolerated in addition to the *meta* nitro substituent (**13b**). In these three cases the IC_{50} values obtained using 100 nM InhA approached 50% of the enzyme concentration, indicating that these compounds are tight-binding enzyme inhibitors. Additional IC_{50} values were determined using 10 and 50 nM InhA in the enzyme assays. Subsequent linear regression analyses of the IC_{50} values as a function of enzyme concentration yielded estimates for K_i^{app} of 21 ± 3 nM (**13b**), 16 ± 12 nM (**14a**), and 40 ± 3 nM (**14c**). Thus, introduction of a *meta* nitro (**13b**) or an *ortho* amino (**14a**) group into the B ring of the parent compound **19** has only aa minor effect on the affinity of the inhibitor for the enzyme. Com-

Table 2. Inhibition and solubility data for nitro and aniline compounds

Compound	Structure	IC_{50}[a] (nM)	MIC_{90} (μg mL^{-1})	*C*log *P*[b]	log *P*
13a		180 ± 20	12.50	6.21	5.50
13b		48 ± 6[c]	12.50	6.21	
13c		90 ± 10	25.0 ± 0	6.21	5.64
14a		62 ± 5	3.13	5.24	5.27
14b		1090 ± 90	100 ± 0	5.24	
14c		55 ± 6[c]	12.50	5.24	4.93

[a] Enzyme concentration is 100 nM.
[b] *C*log *P* values determined using ChemDraw 8.0.
[c] Actual IC_{50} may be lower than this value.

Table 3. Inhibition and solubility data for amide and piperazine compounds

Compound	Structure	IC_{50}[a] (nM)	MIC_{90} (μg mL^{-1})	*C*log *P*[b]	log *P*
10a		1550 ± 460	>200 ± 0	4.90	5.28
10b		5600 ± 770	100	4.90	
10c		1300 ± 200	50.0 ± 0	4.90	
11a		2360 ± 200	100.00	4.24	
11b		580 ± 40	130 ± 58	4.24	
11c		1930 ± 90	>200 ± 0	4.24	
12a		3220 ± 550	>200 ± 0	5.76	5.60
12b		1220 ± 60	>200 ± 0	5.76	5.22
12c		130 ± 34	>200 ± 0	5.76	5.15
18a		1315 ± 256		6.66	
18b		306 ± 46	>100	6.66	

[a] Enzyme concentration is 100 nM.
[b] *C*log *P* values determined using ChemDraw 8.0.

pound **14a** is of particular interest because this derivative has an MIC_{90} value that is close to the value determined for **19**. These data provide important information on the structural flexibility of the inhibitor binding-site that will be useful in directing the design of additional compounds.

In conclusion, a series of hexyl diaryl ethers were synthesized in which the B ring of compound **19** has been substituted with a variety of groups, or replaced with nitrogen-containing aromatic heterocycles. Several of these new compounds possess MIC_{90} and K_i^{app} values similar to that of **19** while having significantly improved *C*log *P* values. Studies are currently underway to determine whether the modifications that we have introduced have resulted in an increase in compound bioavailability and an improvement in their in vivo antibacterial activity.

Acknowledgment

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.04.038.

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