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Synthesis and antitubercular activity of monocyclic nitroimidazoles: Insights from econazole

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ABSTRACT

We have designed and synthesized econazole-derived nitroimidazoles to investigate the antitubercular activity of the nitroimidazole compounds. The introduction of a nitro group at the 4-position of the imidazole on econazole abolished the antitubercular activity. However, alcoholic nitroimidazoles **4** and **6** compounds were active against *Mycobacterium tuberculosis* (Mtb). While the MIC value of econazole was 16 μg/mL, the MIC of **6a** and **6f** turned out to be 0.5 μg/mL. In particular, the activity of **6f** against non-replicating Mtb was as good as PA-824, which is currently in clinical phase II studies as an antitubercular agent. Overall, alcohol compounds **4** and **6** tend to be more active than ether compounds **5** and **7**.

Tuberculosis (TB) still kills about 2 million people annually worldwide.[1] There have been no novel TB drugs developed for the past four decades. Recently, nitroimidazole series compounds such as PA-824[2] and OPC-67683[3] (Fig. 1) entered into clinical studies and they are now in clinical phase II. Since then nitroimidazoles have been explored as a promising scaffold for the TB drug development.[4–8] Since nitroimidazole compounds are active against both replicating and non-replicating *Mycobacterium tuberculosis* (Mtb), in the field of TB drug discovery this scaffold has been thought to contribute to shortening the current lengthy TB drug regimen.[4] While PA-824 and OPC-67683 are bicyclic compounds it has been reported that monocyclic nitroimidazoles also have antitubercular activities such as metronidazole.[4]

Antifungal azole agents are known to have antimycobacterial activity.[9,10] Econazole, one of the antifungal agents, has been known to be active against both replicating and non-replicating Mtb,[11] multi-drug resistant TB (MDR-TB),[12] and even in mouse TB model.[11] The potential use of econazole as TB chemotherapeutics was also investigated by using drug delivery systems.[13,14]

In an effort to discover novel antitubercular compounds, we explored the antitubercular activity of nitroimidazoles derived from econazole. In terms of structure, econazole has an imidazole ring moiety. In particular, we investigated the antitubercular activity effect of the nitro functionality introduction on the imidazole of econazole. Since both PA-824 and OPC-67683 have 4-nitroimidazole structural motif, our interest was to investigate the antitubercular activity of 4-nitroimidazoles derived from econazole (**5** in Fig. 2). It has been reported that it is advantageous to have an oxy-

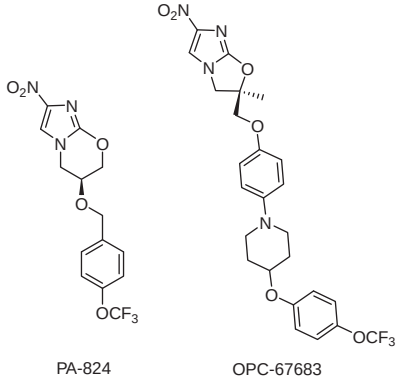

Figure 1. PA-824 and OPC-67683.

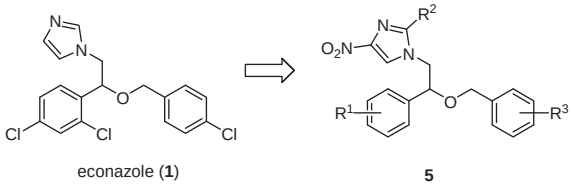

Figure 2. Design of econazole-derived nitroimidazoles.

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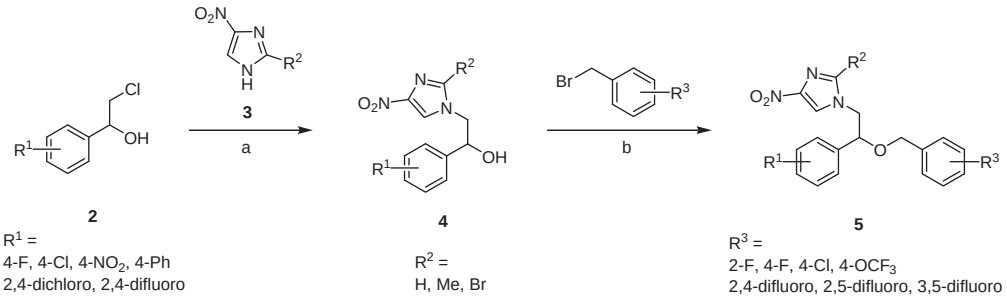


Scheme 1. Reagents and conditions: (a) **3**, TBAI, K_2CO_3, MeOH or EtOH, reflux, 12 h, 38–50%; (b) benzyl bromides, NaH, TBAI, DMF, −78 °C to rt, 2.5 h, 90–94%.

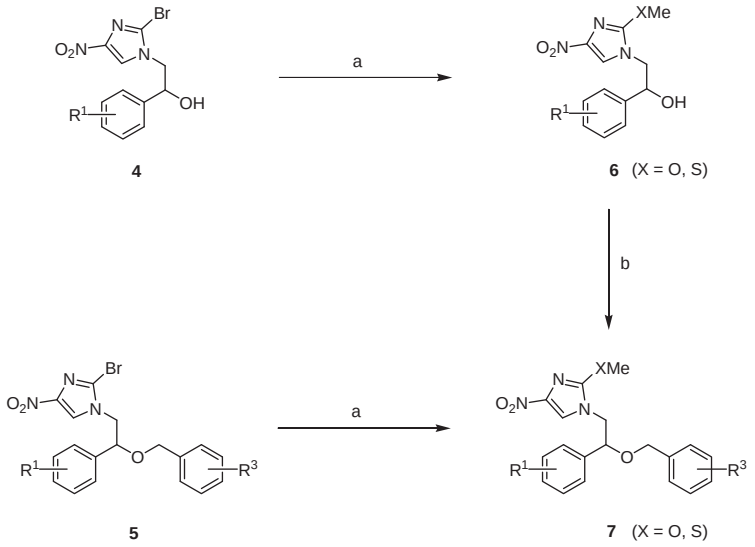
gen or electron donating groups at the 2-position of nitroimidazoles in order to have antitubercular activity.[7,8] Thus, we also studied the effect of the 2-position substituents of the nitroimidazoles for the activity.

All of the compounds were prepared as racemic. At first, we prepared 4-nitro-econazole (**5a**) as a control (Scheme 1). The synthesis of **5a** started from 2-chloro-1-(2,4-dichlorophenyl)ethanol (**2a**; R^1 = 2,4-dichloro**)** via introduction of 4-nitroimidazole (**3a**; R^2 = H) and subsequent benzylation. Compound **2a** was refluxed with 4-nitroimidazole (**3a**), TBAI, and K_2CO_3 in EtOH to obtain 4-nitroimidazole **4a** as a major product and corresponding 5-nitroimidazole compound (not shown) as a minor product.[7] Benzylation of **4a** was accomplished with 4-chlorobenzyl bromide under NaH and TBAI in DMF at −78 °C to rt to facilitate **5a** (R^3 = Cl**)** in good yield.[7] With this chemistry in hand, the two benzene rings of **5a** were derivatized with various substituents by using several different chlorohydrins (**2**) and benzyl bromides. In case of benzyl bromides, 4-trifluoromethoxy group was chosen because of the presence of this substituent in both PA-824 and OPC-67683. Other benzyl bromides were selected based on the structure of known antifungal azole compounds. The 2-position of the nitroimidazole was also varied with either 2-methyl-4-nitroimidazole (**3b**) or 2-bromo-4-nitroimidazole[15] (**3c**).

A recent report suggested that electron donating groups at the 2-position of the nitroimidazole play a significant role in antitubercular activity.[7] Thus, the 2-position bromine of nitroimidazole **4** was transformed to give rise to **6** in good yields with either methoxy or thiomethoxy group (Scheme 2). Benzylation of **6** facilitated ether **7** with various benzyl bromides. Ether **7** was also prepared from bromoether **5** under the same reaction conditions as above.

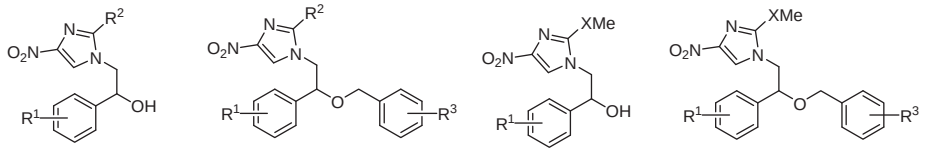
The antitubercular activity of the synthesized compounds against Mtb H37Rv was determined by the microdilution Alamar blue assay.[16] The MIC against non-replicating Mtb was determined by using the green fluorescent protein (GFP) expressing Mtb strain[17] in the Wayne hypoxia model (anaerobic conditions).[18]

We found out that 4-nitro-econazole (**5a**) was not active, while the MIC of econazole was 16 μg/mL, suggesting that the nitro group introduction on econazole was ineffective to maintain the antitubercular activity of econazole. In addition, all of the benzyl ether series compounds **5** turned out to be inactive (Table 1). These discouraging results led us to investigate the antitubercular activity of alcohol compounds **4**, which are the precursors of **5**. In the case of alcohol series compounds **4**, most of them were not active except **4c** (MIC 2 μg/mL), while compounds **4a** and **4j** were moderately active (MIC 32 μg/mL). Under these circumstances, our interest was to introduce electron donating groups at the 2-position of the imidazoles to enhance the activity. Overall, when the 2-position bromine of the nitroimidazole was substituted with a methoxy group, the activity was increased. In case of **6a**, **6d**, **6e**, and **6h**, the activity was increased 4-fold, compared with **4c**, **4g**, **4h**, and **4j**, respectively. While **6c** and **6l** were 8-fold more active than **4f** and **4k**, respectively, **6b** was 16-fold more active than **4e**. In particular, the MIC values of both **6a** and **6f** were 0.5 μg/mL. However, when the 2-position of the nitroimidazole was substituted with a thiomethoxy group, the activity was decreased by 8-fold (**4C→6g**). This data suggest that the oxygen atom at the 2-position



Scheme 2. Reagents and conditions: (a) NaOMe or NaSMe, MeOH, rt, 12 h, 60–93%; (b) benzyl bromides, NaH, TBAI, DMF, −78 °C to rt, 2.5 h, 90–94%.

Table 1
Antitubercular activity and cytotoxicity of nitroimidazoles



4a-k **5a-q** **6a-i** (X = O, S) **7a-k** (X = O, S)

Compound number	R^1	R^2	R^3	X	MIC[a] (μg/mL)	IC_{50}[b] (μg/mL)
4a	2,4-Dichloro	H			32	>250
4b	2,4-Dichloro	CH_3			>256	>250
4c	2,4-Dichloro	Br			2 (8)[c]	100
4d	2,4-Difluoro	H			256	292
4e	2,4-Difluoro	Br			64	>200
4f	4-F	Br			128	>100
4g	4-Cl	Br			64	>100
4h	4-NO_2	Br			64	>100
4i	4-Phenyl	Br			>256	>100
4j	H	Br			32	>100
4k	2,4-Dimethyl	Br			128	22
5a	2,4-Dichloro	H	4-Cl		>256	>16
5b	2,4-Dichloro	CH_3	4-Cl		>256	>31
5c	2,4-Dichloro	Br	4-Cl		>256	>62.5
5d	2,4-Difluoro	H	4-Cl		>256	>25
5e	2,4-Difluoro	Br	4-Cl		>256	>25
5f	2,4-Dichloro	H	2-F		>256	>63
5g	2,4-Dichloro	H	4-F		>256	>16
5h	2,4-Dichloro	Br	2-F		>256	>63
5i	2,4-Dichloro	Br	4-F		>256	>16
5j	2,4-Dichloro	H	2,4-Difluoro		>256	>25
5k	2,4-Dichloro	H	2,5-Difluoro		>256	>25
5l	2,4-Dichloro	H	4-OCF_3		>256	>25
5m	2,4-Dichloro	H	3,5-Difluoro		>256	>25
5n	2,4-Dichloro	Br	3,5-Difluoro		>256	>6
5o	2,4-Difluoro	H	4-F		>256	>50
5p	2,4-Difluoro	H	4-OCF_3		>256	>50
5q	2,4-Difluoro	H	3,5-Difluoro		>256	>50
6a	2,4-Dichloro			O	0.5 (4)[c]	100
6b	2,4-Difluoro			O	4 (4)[c]	>100
6c	4-F			O	16	>100
6d	4-Cl			O	16	>100
6e	4-NO_2			O	16	>100
6f	4-Phenyl			O	0.5 (1)[c]	>100
6g	2,4-Dichloro			S	16	59
6h	H			O	8	>100
6i	2,4-Dimethyl			O	16	84
7a	2,4-Dichloro		4-Cl	O	>256	>8
7b	2,4-Dichloro		4-F	S	>256	>13
7c	2,4-Dichloro		4-OCF_3	O	>256	>13
7d	2,4-Difluoro		4-Cl	O	>16	>13
7e	2,4-Difluoro		4-F	O	>32	>25
7f	2,4-Difluoro		4-OCF_3	O	>256	>25
7g	2,4-Dichloro		3,5-Difluoro	O	>256	>50
7h	2,4-Dichloro		4-F	O	>256	>13
7i	2,4-Dichloro		4-Cl	S	>256	>13
7j	2,4-Dichloro		3,5-Difluoro	S	>256	>135
7k	2,4-Difluoro		4-Cl	S	>256	>13
Econazole					16	
PA-824					0.13 (2)[c]	

[a] MIC against H37Rv.
[b] IC_{50} against VERO cells.
[c] Values in parenthesis represent MIC under anaerobic conditions.

of the nitroimidazole plays a significant role in the antitubercular activity. In the case of ether series compounds **7**, all of them were inactive, suggesting that introducing lipophilic side chains on the benzylic alcohol group in **6** abolishes the activity. The compounds of which MICs are lower than 8 μg/mL were tested against non-replicating Mtb (**4c**, **6a**, **6b**, and **6f**). All four of them were active against non-replicating Mtb. In particular, the activity of **6f** against non-replicating Mtb was as good as PA-824. It is noteworthy that in case of **6f** the MIC value difference between aerobic and anaerobic assays was just 2-fold, while PA-824 has more than 15-fold difference, suggesting that this monocyclic nitroimidazole could be optimized further to enhance anaerobic activity.

Since all of the nitroimidazole compounds have been derived from the econazole scaffold, the synthesized compounds were tested against *Candida albicans* ATCC90027 for their antifungal activity. The broth microdilution method was used to determine MIC of the compounds against *Candida albicans*.[19] The yeast was exposed to the compounds of which concentrations ranged from

256 to 0.5 μg/mL for 48 h, and then its growth was visualized using Alamar blue dye.[16] All of the nitroimidazole compounds were inactive in the antifungal assays performed. While the MIC of econazole was 16 μg/mL against *Candida albicans*, the MICs of all of the nitroimidazoles in Table 1 were higher than 256 μg/mL. Based on this dramatic difference on the antifungal activity between econazole and the nitroimidazoles synthesized, we could speculate that introduction of nitro functionality on econazole scaffold led to a different mode of action, compared with that of econazole.

The cytotoxic effect of the compounds was tested on Vero cells by MTT assay (Promega, USA) in accordance to the manufacturer's instruction. Initial compound solution was prepared in DMSO, and 2-fold serial dilutions were made in RPMI 1640 medium. Due to limited solubility, the highest concentration at which each compound was tested varied. The cell suspension which was in early log phase was exposed to serially diluted compounds solution. The cytotoxic effect was quantified by measuring the amount of formazan that was generated in each well. Most of the nitroimidazole compounds have encouraging cytotoxicity profiles. For example, the IC_{50} values of active compounds, such as **4c**, **6a**, **6b**, and **6f**, were either 100 μg/mL or above.

In conclusion, starting from 4-nitro-econazole (**5a**), various monocyclic nitroimidazole compounds were designed and synthesized. 4-Nitro-econazole (**5a**) turned out to be inactive against both Mtb and *Candida albicans*, suggesting that the introduction of nitro functionality on econazole led to the loss of both antitubercular and antifungal activities. However, in lieu of ether functionality in **5** or **7**, having an alcohol group such as **4** and **6** was advantageous for the antitubercular activity. In both **4** and **6** series compounds, the 2-position oxygen of the nitroimidazole turned out to be important for the activity. The alcohol compounds **4** and **6** tend to be more active than ether compounds **5** and **7**. In particular, the MIC of both **6a** and **6f** turned out to be 0.5 μg/mL and the anaerobic MIC of **6f** was as good as that of PA-824.

To our knowledge, monocyclic nitroimidazoles active against both aerobic and anaerobic Mtb have not been reported before. Metronidazole, a class of monocyclic 5-nitroimidazole compound, is known to have activities against not aerobic but anaerobic Mtb.[4] A recent study reported that some monocyclic nitroimidazoles are active against not anaerobic but aerobic Mtb.[7] Thus, in our present study we have discovered monocyclic nitroimidazoles active against both aerobic and anaerobic Mtb for the first time.

Although the mode of action of the nitroimidazole compounds prepared has not been studied, based on the trend of antitubercular activities, we could speculate that the antitubercular activities come from similar mode of action as that of PA-824. It is also clear that an oxygen atom at the 2-position of nitroimidazoles helps to increase the antitubercular activity. However, in terms of antifungal activities, regardless of the presence of an oxygen atom at the 2-position of nitroimidazoles, all of the nitroimidazoles prepared did not have antifungal activities. Thus, we could also speculate that the mode of action of the nitroimidazoles prepared is quite different from that of econazole.

Encouraging biological data including anaerobic activities suggested that the monocyclic nitroimidazole compounds prepared could be further pursued to optimize as antitubercular agents. Optimization efforts and pharmacokinetic profile studies are underway and will be reported in due course.

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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.2010.12.128.

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The detailed experimental procedure for the MIC under anaerobic conditions is as follows: Mtb H37Rv-GFP was grown in tight-sealed test tube with 0.5-head space ratio for 14 days. Initial compound solution was prepared in DMSO, and two fold serial dilutions were made in DMSO in a microplate. The serially-diluted compound solution was dispensed in a microplate, and Mtb H37Rv-GFP in non-replicating phase was dispensed in the plate in an anaerobic chamber. The plates containing compounds dilutions and Mtb H37Rv-GFP were incubated at 37 °C for 7 days in the anaerobic chamber. After exposure Mtb H37Rv-GFP was diluted 10 fold in fresh media, and incubated for 2 days. The fluorescence was measured in a Fluostar Optima microplate fluorometer (BMG Labtech., Germany) in the bottom-reading mode with excitation at 485 nm and emission at 520 nm. The MIC against non-replicating Mtb was defined as the lowest concentration of compounds that inhibited fluorescence by 80%, compared to the fluorescence of bacteria only wells.
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