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Design and synthesis of 1*H*-1,2,3-triazoles derived from econazole as antitubercular agents

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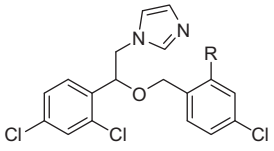
ABSTRACT

Econazole has been known to be active against *Mycobacterium tuberculosis*. We have designed and synthesized 1*H*-1,2,3-triazoles derived from econazole as antitubercular agents. The majority of triazole derivatives have been prepared by microwave-assisted click chemistry. It turned out that all of the prepared triazoles had no antifungal activities. However, most of the hydroxy-triazoles (**6a** and **10**) apparently turned out to have antitubercular activities. Overall, hydroxy-triazoles **10** were more active than their corresponding ether-triazoles **11**. While the MIC value of hydroxy-triazole **10d** was as good as econazole (16 μg/mL), the MIC value of **10a** was two-fold more active than econazole, suggesting that this 1*H*-1,2,3-triazole scaffold (**3**) could be further optimized to develop Mtb specific agents.

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), remains one of the most deadly infectious diseases, infecting 8 million and killing 2 million people annually.[1] In TB drug discovery, there are two major hurdles to overcome: lengthy regimen and drug resistance. Although there are first and second line TB drugs available, current TB chemotherapy requires lengthy treatment periods: 6–9 months for drug-susceptible patients and 18–24 months for drug-resistant patients. Since TB patients have to take TB medications for such a long time, it could have a high chance to lead to low compliance rates and severe adverse drug effects.[2] Recently, drug-resistant Mtb is getting more prevalent. For instance, 6–22% of TB patients turned out to be multidrug-resistant TB (MDR-TB) patients. MDR-TB is defined as Mtb strains resistant to both isoniazid and rifampin, which are the two most significant drugs among the first line TB agents. Moreover, extensively drug-resistant TB (XDR-TB) is becoming more ubiquitous found in over 50 countries. XDR-TB is defined as Mtb strains resistant to isoniazid, rifampin, and two series of second line drugs such as an aminoglycoside and a fluoroquinolone. For XDR-TB treatment, currently there are no evident chemotherapy methods available. However, truly novel antitubercular drugs other than repurposed drugs have not been developed since the 1960s.[3] Thus, there is an urgent need for discovery of novel drugs, which could overcome the lengthy regimen and drug resistance.

It has been shown that antifungal azoles, such as econazole (**1**) and miconazole (**2**), are active against Mtb through interactions with CYP130 in Mtb (Fig. 1).[4] In particular, econazole has encouraging activity profiles against wild-type Mtb and MDR-TB.[5,6] A recent report demonstrated that antifungal azole analogs could be modified to discover antibacterial agents.[7] Triazoles are also used very often in the medicinal applications. For instance, a variety of 1*H*-1,2,3-triazole compounds have been known to have antitubercular activity.[8–11] Castagnolo et al. reported that enantiomerically pure 1*H*-1,2,3-triazole analogs tethered with imidazole were active against Mtb.[12] As our ongoing search for novel antitubercular agents, we just reported the discovery of econazole-derived monocyclic nitroimidazoles as antitubercular agents.[13] Encouraged by these results and the background information mentioned above, we launched to explore the feasibility of 1*H*-1,2,3-triazole compounds derived from econazole as a novel scaffold for antitubercular agents. We envisioned to utilize click chemistry to



econazole (**1**, R = H)
miconazole (**2**, R = Cl)

Figure 1. Structures of econazole (**1**) and miconazole (**2**).

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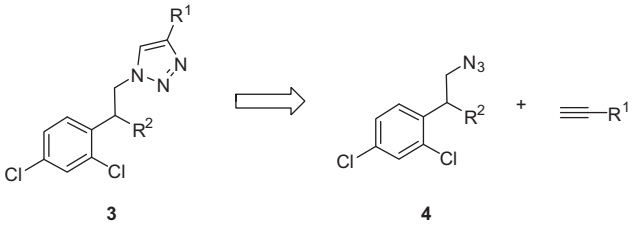
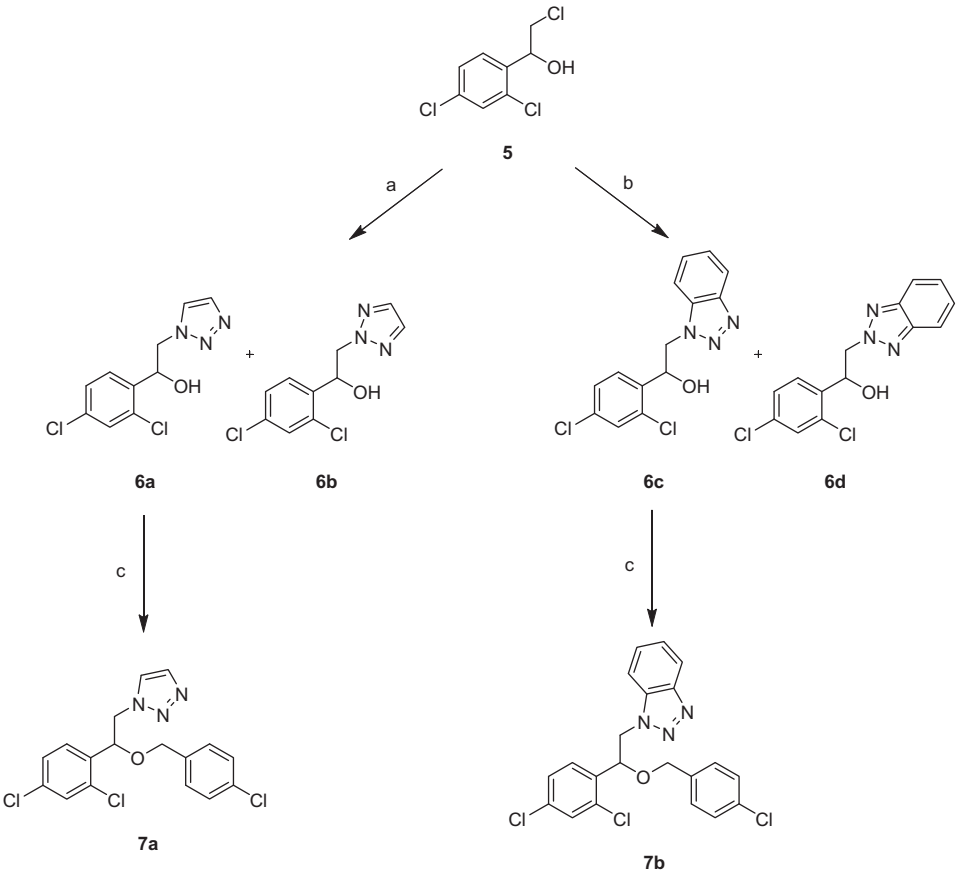


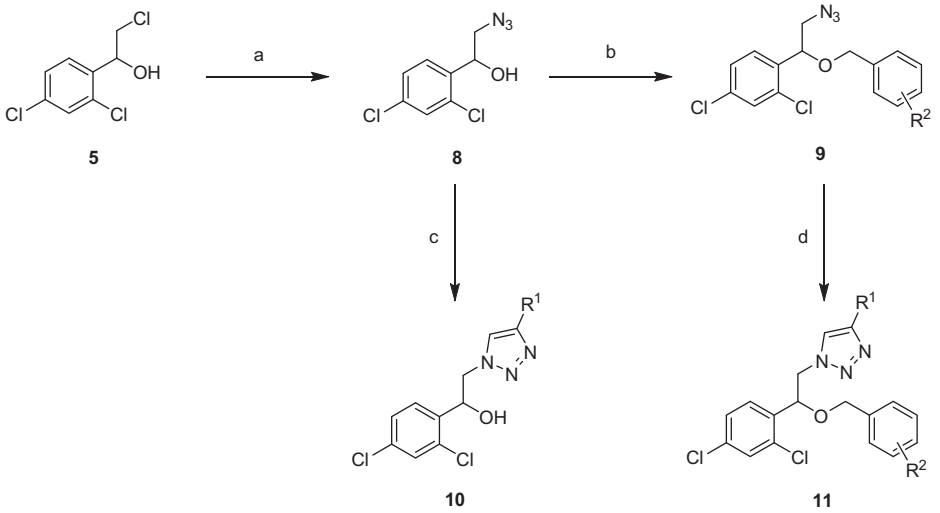
Figure 2. Design of triazoles (**3**) derived from econazole.

prepare 1*H*-1,2,3-triazoles (**3**) from azides (**4**) and alkynes (Fig. 2). Lately, Ankati et al. demonstrated that this click chemistry would be feasible by successfully performing a couple of related reactions to our synthetic design.[14]

At first, we began our studies to elucidate the replacement effect of the imidazole on econazole with 1*H*-1,2,3-triazole or benzotriazole by constructing triazoles **7a** or **7b**, respectively (Scheme 1). Chlorohydrin **5** was treated with 1*H*-1,2,3-triazole under microwave conditions to give hydroxy-triazoles **6a** (43%) as a major product in addition to **6b** (14%) as a minor product.

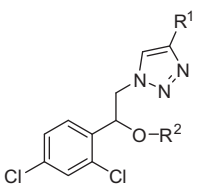


Scheme 1. Reagents and conditions: (a) 1*H*-1,2,3-triazole, CH_3CN, μW, 80 °C, 50 min, **6a** (43%), **6b** (14%); (b) benzotriazole, CH_3CN, μW, 80 °C, 50 min, **6c** (25%), **6d** (8%); (c) 4-chlorobenzyl bromide, NaH, TBAI, DMF, −78 °C to rt, 2 h, **7a** (77%), **7b** (73%).

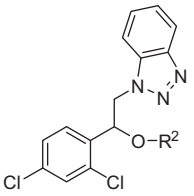


Scheme 2. Reagents and conditions: (a) NaN_3, DMF, reflux, 12 h, 69%; (b) benzyl bromides, NaH, TBAI, DMF, −78 °C to rt, 2–5 h, 90–98%; (c) alkynes, CuI, DIPEA, CH_2Cl_2, μW, 150 °C, 10 min, 74–94%; (d) alkynes, CuI, DIPEA, CH_2Cl_2, μW, 150 °C, 10 min, 72–94%.

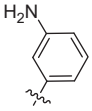
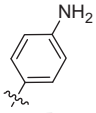
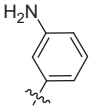
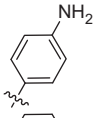
Table 1
Antitubercular and antifungal activities and cytotoxicity of 1*H*-1,2,3-triazoles



6a, **7a**,**1 0a-h, 11a-l**



6c, **7b**

Compound number	R^1	R^2	MIC[a] *Mtb*	MIC[b] *C. albicans*	IC_{50}[c] vero cell
6a	H	H	256	>256	>200
6c	—	4-Cl-Bn[d]	>256	>256	>100
7a	H	4-Cl-Bn	64	>256	>12.5
7b	—	4-Cl-Bn	128	>256	>12.5
10a	*n*-Bu	H	8	>256	23.28
10b	CH_2CH_2OH	H	256	>256	274.1
10c	CH_2OH	H	>256	ND[e]	>100
10d	Cyclohexyl	H	16	>256	31.94
10e	*t*-Bu	H	32	256	33.94
10f		H	32	>256	26.8
10g		H	32	>256	33.94
10h		H	32	ND	ND
11a	*n*-Bu	4-Cl-Bn	128	>256	10.61
11b	*n*-Bu	4-OCF_3-Bn	>256	>256	63.38
11c	*n*-Bu	2,4-diF-Bn	>256	>256	21.02
11d	CH_2CH_2OH	4-Cl-Bn	>256	>256	24.34
11e	CH_2CH_2OH	4-OCF_3-Bn	>64	>64	ND
11f	CH_2CH_2OH	2,4-diF-Bn	128	>256	33.68
11g	CH_2OH	4-Cl-Bn	>256	>256	19.79
11h	Cyclohexyl	4-Cl-Bn	>256	>256	>12.5
11i	*t*-Bu	4-Cl-Bn	>256	>256	>25
11j		4-Cl-Bn	>256	>256	>50
11k		4-Cl-Bn	256	>256	>50
11l		4-Cl-Bn	256	>256	>50
1 (econazole)			16	16	
rifampicin			0.0625	ND	

[a] MIC against *M. tuberculosis* (H37Rv) (μg/mL).
[b] MIC against *C. albicans* (μg/mL).
[c] IC_{50} against VERO cells (μg/mL).
[d] Bn: benzyl.
[e] ND: not determined.

Likewise, compound **5** was reacted with benzotriazole to obtain **6c** (25%) and **6d** (8%). Benzylation of **6a** and **6C** facilitated ether-triazoles **7a** (77%) and **7b** (73%) in the presence of NaH and 4-chlorobenzyl bromide.

In order to construct a variety of 1*H*-1,2,3-triazoles derived from econazole, we proposed to use click chemistry.[15] Azidation of commercially available chlorohydrin **5** was carried out under refluxing NaN_3 in DMF to afford azido-alcohol **8** (Scheme 2). Treatment of **8** with various benzyl bromides and NaH furnished azido-ethers **9** in over 90% yields. With the azides **8** and **9** in hand, we have screened many different reaction conditions to optimize the click chemistry conditions. Among the click chemistry conditions used, a microwave condition with CuI and DIPEA in CH_2Cl_2 gave the best yield. The click chemistry of **8** or **9** under the microwave conditions gave hydroxy-triazoles **10** or ether-triazoles **11**, respectively.[14,16,17]

The antitubercular activity of each compound against *Mycobacterium tuberculosis* H37Rv was measured by the green

fluorescent protein reporter assay.[18] Briefly, the compound was initially dissolved in dimethylsulfoxide (DMSO), and two-fold dilutions were made in 7H9 broth in microplates. The initial inoculum of 2×10^5 CFU/mL of Mtb H37Rv-GFP, which was grown in Middlebrook 7H9 media, was exposed to the compounds for 7 days. The fluorescence was measured in a Fluostar Optima microplate fluorometer (BMG Labtech, Germany), and the MIC was defined as the lowest concentration of compounds that inhibited fluorescence by 90%, compared with the fluorescence of bacteria only wells.

We investigated the antitubercular activity of triazole **7a** and **7b** as controls. Triazole **7a** gave the MIC value of 64 μg/mL. Although **7a** is 4 times less active than econazole, it still maintains the antitubercular activity, indicating that replacing the imidazole on econazole with 1*H*-1,2,3-triazole could be tolerated in terms of the activity (Table 1). However, in case of **7b**, the activity was abolished, compared with econazole. While we have tried several different alkynes to prepare ether-triazoles **11**, most of them were not active against Mtb. Moreover, when the benzyl group was replaced with 4-trifluoromethoxybenzyl or 2,4-difluorobenzyl substituents, the activity was not improved. This led us to investigate the activity of hydroxy-triazoles **10**. It turned out that hydroxy-triazoles **10** appeared to be far more active, compared with ether-triazoles **11**. Some of the hydroxy-triazoles such as **10e**, **10f**, **10g**, and **10h** were two-fold less active than econazole. While **10d** (R^1 = cyclohexyl) was as active as econazole, **10a** (R^1 = *n*-Bu) was two-fold more active than econazole. However, their corresponding ether-triazoles **11h** and **11a**, respectively, were not active, confirming that hydroxy-triazoles **10** are more active than ether-triazoles **11**. This trend appears to be applicable in the case of amine containing triazoles. While the MIC values of hydroxy-triazoles such as **10f**, **10g**, and **10h** were 32 μg/mL, their corresponding ether-triazoles (**11j**, **11k**, and **11l**, respectively) were inactive.

The antifungal activity of the compounds prepared against *Candida albicans* ATCC90027 was measured by broth microdilution method. The initial inoculum of *C. albicans* that was grown in tryptic soy broth (Difco, USA) was exposed to the two-fold dilutions of the compound for 48 h, which were made in the same media. The growth of *C. albicans* was visualized by Alamar Blue dye (AbD Serotec, UK), and the MIC was defined as the lowest concentration of compounds that inhibited the color change of the Alamar Blue from blue to pink. While econazole gave the MIC value of 16 μg/mL against *C. albicans*, all of the triazoles in Table 1 were not active at all. Surprisingly, substituting the imidazole on econazole with 1*H*-1,2,3-triazole led to the complete loss of antifungal activities. These data suggest that the triazole series compounds prepared could have quite different interaction mode with CYP51 from *C. albicans*, compared with that of econazole.[19]

The cytotoxicity of the compounds was tested in Vero cells by MTT assay (Promega, USA) in accordance to the manufacturer's instruction. The cell suspension, which was in early log phase, was exposed to serially diluted solution of the compounds for 3 days. Both the cell suspension and the compound dilutions were made in RPMI 1640 medium. The cytotoxic effect was normalized by comparing with the effect of DMSO only wells, and the IC_{50} value was calculated using Prism software (Graphpad, CA, USA). We selected the active triazoles with the MIC values lower than 32 μg/mL, and investigated the selective indexes (SI). The SI values were ranged from 1 to 3, indicating that cytotoxicity profile should be improved further to develop this triazole series as antitubercular agents.

In summary, replacing the imidazole on econazole with 1*H*-1,2,3-triazole could be tolerable to maintain antitubercular activities, based on the activity profiles of the hydroxy-triazoles (**10**) prepared. Overall, hydroxy-triazoles (**10**) tend to be more active than ether-triazoles (**11**). While **10d** was as active as econazole, **10a** was two-fold more active than econazole. Moreover, the triazole series compounds prepared were not active at all against *C. albicans*, suggesting that the mode of action of the triazoles prepared might be quite different from that of econazole.[4,19] Thus, this 1*H*-1,2,3-triazole scaffold could be further optimized to develop Mtb specific agents.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.09.041.

References and notes

1. http://www.who.int/topics/tuberculosis/en/ (accessed on July 17, 2012).
2. http://www.cdc.gov/tb/ (accessed on July 17, 2012).
3. Ma, Z.; Lienhardt, C.; McIlleron, H.; Nunn, A. J.; Wang, X. *Lancet* **2010**, *375*, 2100.
4. Ouellet, H.; Podust, L. M.; de Montellano, P. R. *J. Biol. Chem.* **2008**, *283*, 5069.
5. Ahmad, Z.; Sharma, S.; Khuller, G. K. *FEMS Microbiol. Lett.* **2006**, *261*, 181.
6. Ahmad, Z.; Sharma, S.; Khuller, G. K.; Singh, P.; Faujdar, J.; Katoch, V. M. *Int. J. Antimicrob. Agents* **2006**, *28*, 543.
7. Güven, Ö. Ö.; Erdoğan, T.; Göker, H.; Yildiz, S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2233.
8. Shanmugavelan, P.; Nagarajan, S.; Sathishkumar, M.; Ponnuswamy, A.; Yogeeswari, P.; Sriram, D. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 7273.
9. Labadie, G. R.; de la Iglesia, A.; Morbidoni, H. R. *Mol. Divers.* **2011**, *15*, 1017.
10. Chen, L.; Wilson, D. J.; Xu, Y.; Aldrich, C. C.; Felczak, K.; Sham, Y. Y.; Pankiewicz, K. W. *J. Med. Chem.* **2010**, *53*, 4768.
11. Upadhayaya, R. S.; Kulkarni, G. M.; Vasireddy, N. R.; Vandavasi, J. K.; Dixit, S. S.; Sharma, V.; Chattopadhyaya, J. *Bioorg. Med. Chem. Lett.* **2009**, *17*, 4681.
12. Castagnolo, D.; Radi, M.; Dessi, F.; Manetti, M.; Saddi, M.; Meleddu, R.; Logu, A. D.; Botta, M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2203.
13. Lee, S.-H.; Kim, S.; Yun, M.-H.; Lee, Y. S.; Cho, S.-N.; Oh, T.; Kim, P. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1515.
14. Ankati, H.; Yang, Y.; Zhu, D.; Biehl, E. R.; Hua, L. *J. Org. Chem.* **2008**, *73*, 6433.
15. Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2004**, *2001*, 40.
16. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596.
17. Himo, F.; Lovell, T.; Hilgraf, R.; Rostovtsev, V. V.; Noodleman, L.; Sharpless, K. B.; Fokin, V. V. *J. Am. Chem. Soc.* **2005**, *127*, 210.
18. Collins, L. A.; Torrero, M. N.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1998**, *42*, 344.
19. Sheehan, D. J.; Hitchcoch, C. A.; Sibley, C. M. *Clin. Microbiol. Rev.* **1999**, *12*, 40.