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Identification of 1-[4-Benzyloxyphenyl)-but-3-enyl]-1*H*-azoles as New Class of Antitubercular and Antimicrobial Agents

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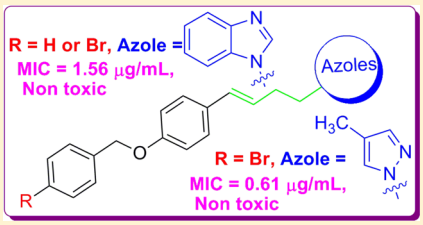
Supporting Information

ABSTRACT: A series of 1-[(4-benzyloxyphenyl)-but-3-enyl]-1*H*-azoles has been identified as potent antitubercular agents against *Mycobacterium tuberculosis*. Synthesis of compounds involved acid catalyzed ring-opening of cyclopropyl ring of phenyl cyclopropyl methanols followed by nucleophilic attack of the azoles on the carbocation intermediates. Several of the compounds **26**, **34**, and **36** exhibited significant antitubercular activities with MIC value as low as 1.56, 1.56, and 0.61 μg/mL, respectively, comparable to many standard drugs. These compounds were also screened against other strains of bacteria and fungi, and few of them showed good antifungal activity against *A. fumigatus*, responsible for lung infection.



KEYWORDS: *Antitubercular agents, azoles, cyclopropyl methanols, Mycobacterium tuberculosis*

Mycobacterium tuberculosis (*Mtb*) causing tuberculosis is one of humanity's oldest and most resilient plagues, despite the availability of a four drug (INH, ethambutol, pyrazinamide, and rifampicin) regimen to treat the disease.[1] The long duration of therapy generally results in noncompliance of the treatment and results in multidrug resistance tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB), which are highly lethal, extremely expensive and complicated to treat, posing new challenges for the prevention, treatment, and control of TB.[2−4] As per WHO, reported in 2011, about 8.7 million new cases of TB (13% coinfected with HIV) were reported with 1.4 million mortality. The latter include almost one million deaths among HIV-negative individuals and 430 000 among HIV-positive people.[5] Because of the drug−drug interactions, difficulty of the coadministration of anti-TB and anti-HIV drugs posed a new challenge before scientific community.[6] Despite the unraveling of mycobacterial genome sequence[7] and sound knowledge of mycobacterial biochemistry, none of the antitubercular drugs entered in the market for the last 50 years except a diraylquinoline (TMC 207) from Johnson & Johnson only very recently.[8,9] Unavailability of suitable vaccine and limitation of existing anti-TB drugs warrants the introduction of novel compounds or drug regimen effective against both the actively growing and latent stage mycobacterium avoiding drug−drug interactions.

Azole and triazole based compounds are good as antifungal and antimycobacterial agents.[10] Such compounds generally inhibit cytochrome P450 (CYP121), an important enzyme with several of its isoforms of great significance in *M. tuberculosis* viability and pathogenicity.[11,12] Azoles also inhibit the biosynthesis of glycopeptidolipids (GPLs), an integral part of the mycobacterial cell envelope.[13] In an ongoing program for design and development of new antitubercular agents[14−17] we have identified phenyl cyclopropyl methanones and methanols as potent leads.[16,17] One of the phenyl cyclopropyl methanols (**A**) showed very good in vitro activity (MIC 3.12 μg/mL) against susceptible and drug resistant strains of *M. Tuberculosis*, and it exhibited marginal in vivo activity, too.[17] On careful examination, it was found that **A** was unstable under gastric pH and gets degraded (HPLC) to the cyclopropyl ring opened product, phenyl butenyl derivative **B** as the only product, which also showed anti-TB activity (MIC 4.46 μg/mL) (Figure 1 and Table 1).

The starting benzyloxy phenyl cyclopropyl methanones (**1a**−**1f**) and benzyloxy phenyl cyclopropyl methanols (**2a**−**2f**) were

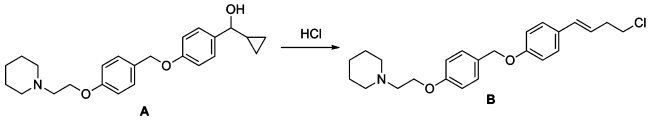


Figure 1. Effect of low pH on most active compound **A**.

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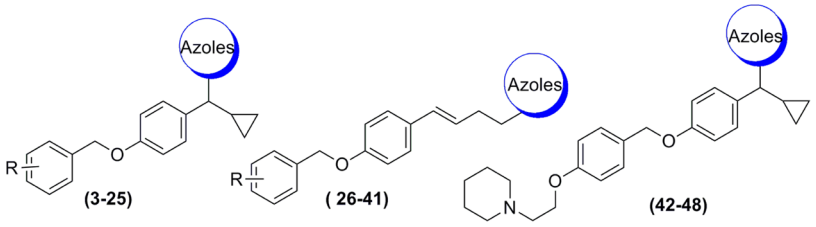
Table 1. Antitubercular Activity against *Mtb* H37Rv



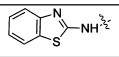
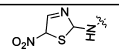
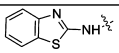
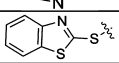
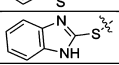
Compound	R	Azole	[a]clogP	MIC (μg/mL)	CC_{50} (μg/mL)	[b]SI (CC_{50}/MIC)
3	H		5.6	12.5	ND	ND
4	H		4.098	12.5	ND	ND
5	H		3.563	>12.5	ND	ND
6	H		4.867	6.25	65.51	10.48
7	H		3.325	>12.5	ND	ND
8	H		6.455	2.41	100.0	16
9	H		4.173	>25	ND	ND
10	2-Cl		4.728	3.12	35.83	11.48
11	2-Cl		5.497	12.5	ND	ND
12	4-Cl		6.278	6.25	73.88	11.82
13	4-Cl		4.241	12.5	ND	ND
14	4-Br		6.41	6.25	27.51	4.40
15	4-Br		4.907	3.12	2.10	0.67
16	4-Br		4.372	25	ND	ND
17	4-Br		5.676	>50	ND	ND
18	4-Br		4.132	3.12	45.48	14.57
19	4-Br		5.764	6.25	100	16.0
20	4-F		5.676	6.25	35.36	5.6
21	4-F		4.344	3.12	88.51	28.0
22	4-F		6.619	12.5	ND	ND
23	4-OCH_3		5.657	>12.5	ND	ND
24	4-OCH_3		4.154	12.5	ND	ND
25	4-OCH_3		3.62	3.12	35.12	10.25
26	H		5.77	1.56	100	64.10
27	H		3.733	>25	ND	ND
28	H		7.365	3.12	100.0	16.0
29	H		6.621	>25	ND	ND

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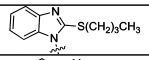
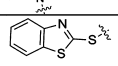
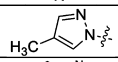
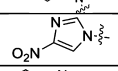
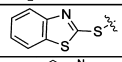
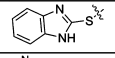
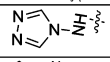
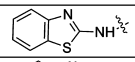
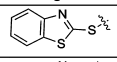
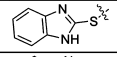
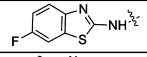
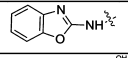
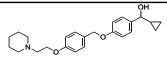
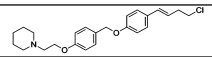
Table 1. continued

Compound	R	Azole	[a]clogP	MIC (μg/mL)	CC_{50} (μg/mL)	[b]SI (CC_{50}/MIC)
30	H		8.489	>12.5	ND	ND
31	2-Cl		7.826	6.25	28.49	4.5
32	4-Cl		6.448	6.25	ND	ND
33	4-Cl		6.307	>12.5	ND	ND
34	4-Br		6.579	1.56	100	11.25
35	4-Br		4.542	4.78	ND	ND
36	4-Br		5.298	0.61	88.94	57.0
37	4-F		5.934	3.12	100	32.0
38	4-F		4.514	50	ND	ND
39	4-F		7.422	3.12	100	32.0
40	4-OCH_3		5.827	6.25	75.17	12.0
41	4-OCH_3		6.677	>12.5	ND	ND
42			6.595	12.5	ND	ND
43			4.317	50	ND	ND
44			6.705	12.5	ND	ND
45			8.072	3.12	100	32.2
46			7.182	>50	ND	ND
47			7.208	6.25	4.93	0.78
48			6.693	6.25	11.69	1.87
A			4.06	3.12	>50	>10
B			4.948	4.46	-	-
	Fluconazole		-0.142	**2.825**	-	-
	Isoniazide		-0.969	**0.025**	-	-
	Ethambutol		0.35	**2**	-	-

[a]cLogP was determined by mol inspiration; available at hptt/www.molinspiration.com/docu/mipc. [b]SI index ≥10 can be considered as potent compound in an in vitro assay.

prepared as reported earlier by us.[17] Reaction of the cyclopropyl methanols with different azoles was investigated under different conditions. To optimize the reaction conditions, **2a** was reacted with benzimidazole in anhydrous toluene under the influence of different catalysts, *p*-TSA, $ZnCl_2$, and Montmorillonite (K-10) at different temperatures, and the results are shown in Supporting Information S2. Reaction with *p*-TSA as catalyst at 120 °C using a Dean−Stark Column proved to be the most optimum condition giving two products (TLC). The latter were isolated and characterized to be 1-(4-benzyloxy phenyl cyclopropyl methyl)-1*H*-benzimidazole (**3**, 60%) and 1-{(4-benzyloxy phenyl)-but-3-enyl}-1*H*-benzimidazole (**26**, 35%). However, at 80 °C, the yield of the ring opened product is further reduced to 10%, and at ambient temperature none of such product was formed.

Next, the scope of the above reaction with several phenyl cylopropyl methanols (**2a−2f**) and different azoles was extended to get the desired products, phenyl cyclopropyl methyl azoles (**3−25**) and phenyl butenyl azoles (**26−41**). The formation of these products may be explained in terms of acid catalyzed nonclassical carbocation (Supporting Information S3). Temperature, nucleophilicity, and size of the azoles are important during the reaction. Bulkier nucleophiles (**A4**, **A9**, **A10**, and **A12**) easily approach the less hindered side of the intermediate (**Ib** or **Ic** in Supporting Information S3) to give the phenyl butenyl azoles as the major one and only trace amount of other isomer in some cases (TLC, unisolated) at 80 °C or at 120 °C temperature (Supporting Information S3). The structures of the compounds were confirmed on the basis of their spectroscopic and HRMS data. Both the structural

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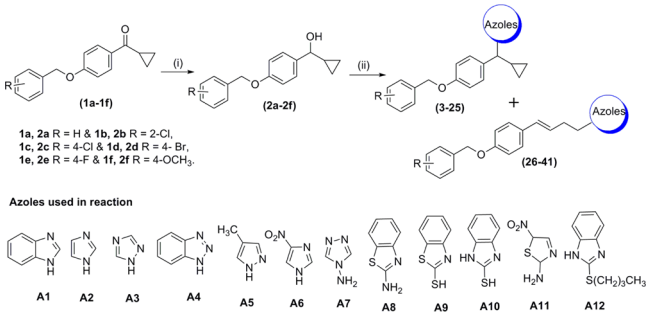
isomers, phenyl cyclopropyl methyl azoles and phenyl butenyl azoles, were prepared under different reaction conditions (Scheme 1 and Supporting Information S3,S4].

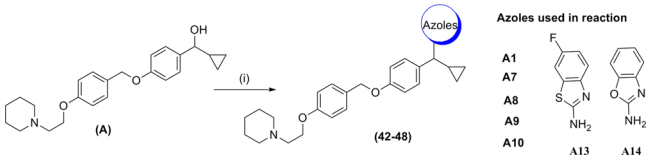
Scheme 1. Synthesis of Phenyl Cyclopropyl Methyl Azoles and Phenyl Butenyl Azoles[a]



[a]Reagents and conditions: (i) $NaBH_4$, MeOH, 0−30 °C, 1−1.5 h. (ii) Azoles, *p*-TSA, anhyd. toluene, 80−120 °C, 4−5 h.

As stated above since compound **A** was the most potent molecule,[17] it was thought to synthesize azole hybrids of it and see their antitubercular activities (Scheme 2 and Table 1).

Scheme 2. Synthesis of Cyclopropyl[4-{4-(2-(piperidin-1-yl)ethoxy)benzyloxy}phenyl]methyl Azoles[a]



[a]Reagents and conditions: (i) azoles, montmorillonite (K10), anhyd. toluene, 80 °C, 4−5 h.

Thus, reaction of compound **A** with different azoles in the presence of montmorillonite (K10) in toluene gave respective azolyl cylopropyl methane derivatives (**42**−**48**) in good yields (84−88%).

All the synthesized compounds were tested for their antitubercular activity against *M. tuberculosis* H37Rv.[18] Subsequently cytotoxicities of compounds showing antitubercular activity (MIC ≤ 6.25 μg/mL) against mammalian VERO cell line were determined.[17,19] As evident from Table 1, most of phenyl butenyl azoles showed better antitubercular activity as compared to phenyl cyclopropyl methyl azoles. The phenyl butenyl azoles **26**, **34**, **36** and **37** showed potent anti-TB activities with MIC values as low as 1.56 μg/mL, 1.56 μg/mL, 0.61 μg/mL, and 3.12 μg/mL respectively; while the corresponding phenyl cyclopropyl methyl derivatives **3**, **14**, **17** and **20** exhibited MIC values of 12.5 μg/mL, 6.25 μg/mL, 50 μg/mL, and 6.25 μg/mL respectively. Results of CC50 and SI were also very interesting because phenyl butenyl azoles (**26**, **34**, **36** and **37**) showed CC50 ≥ 88.94 μg/mL and SI of these compounds were 64.10, 11.25, 57, and 32 respectively which were better than their cyclic isomer phenyl cyclopropyl methyl azoles (Table 1). Substituent on the 4-benzyloxy ring did not display any significant change on the antitubercular activity profile of the compounds. It is interesting to note that compounds with imidazole and benzimidazole moieties in both the series (phenyl cyclopropyl methane and phenyl butenyl derivatives) compounds **4**, **11**, **15**, **24**, **3**, **12**, **14**, **20**, **26**, **32**, **34**, **37** and **40** showed promising antitubercular activity with MIC values from 1.56 μg/mL to 12.5 μg/mL. The triazole bearing compounds **25** and **35** exhibited MIC 3.12 μg/mL and 4.78 μg/mL respectively while compounds **5**, **13**, and **16** showed MIC ≥12.5 μg/mL. The phenyl butenyl benzotriazole **33** did not show any significant inhibition of bacterial growth. Cyclopropyl phenyl methyl aminothiazole derivatives **8** and **19** showed anti-TB activity with MIC 2.41 μg/mL and 6.25 μg/mL respectively. The antitubercular activities of phenyl butenyl mercaptothiazoles **28**, **31** and **39** were also of the same order with MIC in 3.12−6.25 μg/mL range. Compound **28** and **39** are non toxic with SI ≥16. On the other hand 4-nitro imidazole and 2- mercapto benzimidazole derivatives resulted in loss of activity as compared to imidazole derivatives. Among the azole

Table 2. Antifungal Activity of Compounds[a]

	minimum inhibitory concentration (MIC) in μg/mL									
	bacteria				fungi					
compd	Ec	Psu	Sa	Kpn	Ca	Cn	Ss	Tm	Af	Cp
10	>50	>50	0.19	3.12	12.5	25	0.39	0.39	0.39	50
15	>50	>50	>50	>50	1.56	1.56	3.12	0.78	1.56	12.5
18	>50	>50	>50	>50	>50	>50	>50	12.5	50	50
24	>50	50	25	25	25	50	3.12	3.12	6.25	50
26	>50	>50	12.5	>50	>50	>50	>50	>50	25	>50
28	>50	>50	25	>50	>50	>50	>50	50	>50	>50
34	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
36	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
39	>50	>50	>50	>50	50	50	>50	>50	>50	>50
45	>50	>50	12.5	>50	6.25	50	12.5	6.25	25	>50
48	>50	>50	25	>50	12.5	50	25	12.5	>50	50
fluconazole					1	2	4	16	>32	0.5
clotrimazole					0.25	0.25	4	2	8	1
ampicillin	3.12	>50	0.04	50						

[a]*E. coli* (Ec, ATCC 9637), *P. aeruginosa* (Psu, ATCC BAA427), *S. aureus* (Sa, ATCC 25923), *K. pneumoniae* (Kpn, ATCC 27736), *C. albicans* (Ca, patient isolate), *C. neoformans* (Cn,CDRI isolate), *S. schenckii* (Ss, patient isolate), *T. mentagrophytes* (Tm, patient isolate), *A. fumigatus* (Af, patient isolate), and *C. parapsilosis* (Cp, ATCC 22019).

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derivatives of earlier identified HIT compound **A** the 2-mercapto benzothiazolyl derivative (**45**) proved to be equipotent (MIC 3.12 μg/mL) to that of compound **A** and it was non toxic with SI 32.2 against *M. tuberculosis*. However, oxazole (**47**) and thiazole (**48**) derivatives exhibited (MIC 6.25 μg/mL) against *M. tuberculosis*.

The *in vitro* antibacterial and antifungal activities of all the compounds showing potent antitubercular activity (≤3.25 μg/mL) were determined against different strains of bacteria and fungi by microbroth dilution technique as per guidelines of NCCLS (Table 2 and Supporting Information S31,S32).[20] The results are shown in Table 2. Imidazolyl phenyl cyclopropyl methanes **10**, **15**, and **24** showed better antibacterial and antifungal activities as compared to other azolyl derivatives of the series. Compounds **10**, **15**, and **24** with MIC 0.39, 1.56, and 6.25 μg/mL, respectively, against *A. fumigatus* proved to be most potent. Compounds **45** and **48** bearing thiazole and oxazole ring showed moderate inhibitory activity against different strain of fungi with MIC values in the range of 6.25−12.5 μg/mL. Other compounds did not exhibit significant activities.

The most potent compounds of the series, **8**, **26**, **28** and **36**, were studied for their pharmacokinetic parameters, also (Figure 2 and Supporting Information S5−S8). These compounds were

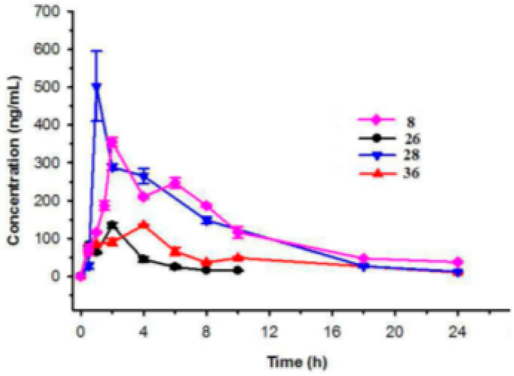

Figure 2. Concentration−time profile of compounds (**8**, **26**, **28**, and **36**) after a single 10 mg/kg oral dose in male Sprague−Dawley rats.

quickly absorbed, distributed, and eliminated from the serum with an MRT of 3.22 to 7.90 h after 10 mg/kg oral dose.[21] The results indicated that the compounds have negligible extra-hepatic elimination as the clearance was smaller than the hepatic blood flow of the rat, and no peculiarities in the animal's behavior were observed indicating that these compounds are promising drug candidates. HPLC chromatograms of compounds **26**, **28**, and **36** have been given in Supporting Information S4,S5.

Since azoles are known to target CYP450 enzyme and CYP121 of *M. tuberculosis* has been shown to be crucial for viability of *M. tuberculosis*, we were curious to see whether these compounds could bind and inhibit CYP121 of *M. tuberculosis* using molecular docking approach with fluconazole as standard cytochrome P450 inhibitor Supporting Information S9).[11,12] The spatial disposition of compounds **26** and **36** was observed to be similar to that of fluconazole (Figure 3A). Compound **26** exhibits maximum interactions with important active site residues (Figure 3B). Polar interactions and the binding distance between the protein and inhibitors (**26** and **36**) were visualized using PyMOL.[22]

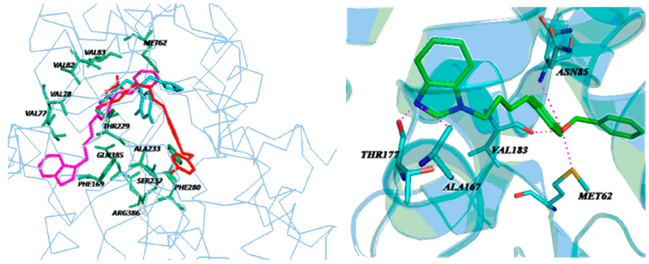

Figure 3. (left) Ribbon diagram of CYP121 showing active site key residues, bound with fluconazole, and compounds **26** and **36** showing the same pattern of conformation. Active site residues are depicted in ball and stick mode in pale green color. The fluconazole ligand is shown in cyan; the compound **26** and **36** are shown in magenta and red color, respectively. (right) CYP121-compound **26** docked structure. Fluconazole and compound **26** are shown in atom colored sticks with green carbons, and key amino acid interacting residues are shown with cyan carbons. Residues are labeled in black.

In conclusion, we have discovered the benzyloxy phenyl butenyl azoles by chemical modification of phenyl cyclopropyl methanols with promising attributes of synthetic accessibility and a good structure−activity relationship. The compounds possess potent antitubercular activities with MIC comparable to the standard drugs. This series also possesses antifungal activity against *A. fumigatus* causing lung infection. These compounds have good in vitro activity, and ongoing studies are focused on improving the efficacy through further analogue generation.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Details for synthesis and characterization of all compounds together with protocols for biological assays, pharmacokinetic parameters, and computational method for molecular docking. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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