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Synthesis and biological evaluation of new enantiomerically pure azole derivatives as inhibitors of *Mycobacterium tuberculosis*

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ABSTRACT

A series of novel enantiomerically pure azole derivatives was synthesized. The new compounds, bearing both an imidazole as well as a triazole moiety, were evaluated as antimycobacterial agents. One of them proved to have activity against *Mycobaterium tuberculosis* comparable to those of the classical antibacterial/antifungal drugs Econazole and Clotrimazole.

Tuberculosis (TB) is a common and deadly infectious disease caused by mycobacteria, mainly *Mycobacterium tuberculosis*. Over one-third of the world's population has been exposed to the TB bacterium, and new infections occur at a rate of one per second.[1] In 2005, mortality and morbidity statistics included 14.6 million chronic active TB cases, 8.9 million new cases, and 1.6 million deaths, mostly in developing countries.[2] In addition, the rising number of people who are contracting TB because of their immune systems compromised by immunosuppressive drugs or substance abuse or HIV/AIDS, is a serious threat to TB control and prevention. Moreover the emergence of multi-drug resistance TB (MDR-TB), defined as resistance to at least isoniazid and rifampin, and the extensively drug resistant (XDR-TB) strains make the discovery and the development of new drugs a priority.[3] In the last few years, the determination of the genome sequence of *Mycobacterium tuberculosis*[4,5] (MTB) provided a much needed boost for research into new drug targets against this pathogen. In particular, recent promising data suggest that targeting the lipid metabolism pathways of MTB may provide an excellent route to attenuating or killing the bacterium. The genome of MTB encodes for a relatively large number of cytochrome P450 enzymes. These data indicate important physiological roles for these enzymes which, given that the substrate preference of the majority of P450s is for hydrophobic molecules, most are likely to be involved in lipid metabolism. McLean and co-workers cloned and expressed MTB CYP51 and CYP121, two types of P450 from MTB.[6] They demonstrated that CYP51 and CYP121 bind azole antifungal drugs tightly and that azole compounds are potent inhibitor of cell growth of *Mycobacterium bovis* and *Mycobacterium smegmatis*, two mycobacterial species which closely resemble MTB. Azole drugs proved to have also antitubercular activities in mice.[7] Finally, recent studies furnished the crystal structure of MTB cytochrome P450 CYP121 in complex with the fluconazole.[8] It has been found that MTB-CYP121 binds commercially available azole drugs as clotrimazole or econazole and that it may be the true target for the antimycobacterial activity of the azoles in vivo as suggested by gene-knockout studies.[9,10]

In this context, as a continuation of our previous works on the discovery of new antimicrobial agents,[11,12] we decided to focus our attention on the synthesis and preliminary biological evaluation against MTB of novel azole analogues with polycyclic structure **A** which resemble the classical antifungal/antibacterial azole drugs (Fig. 1). Being the target compounds chiral, we were also attracted by the possibility of synthesizing these derivatives in enantiomerically pure form in order to evaluate the biological profile of the single enantiomers.

Desired compounds were synthesized starting from different arylpropargylamides **1a–e** (Scheme 1) which were obtained in

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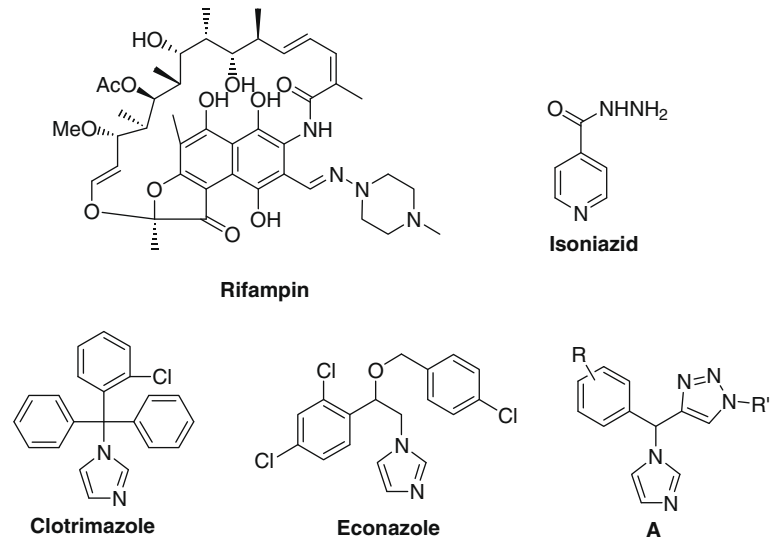
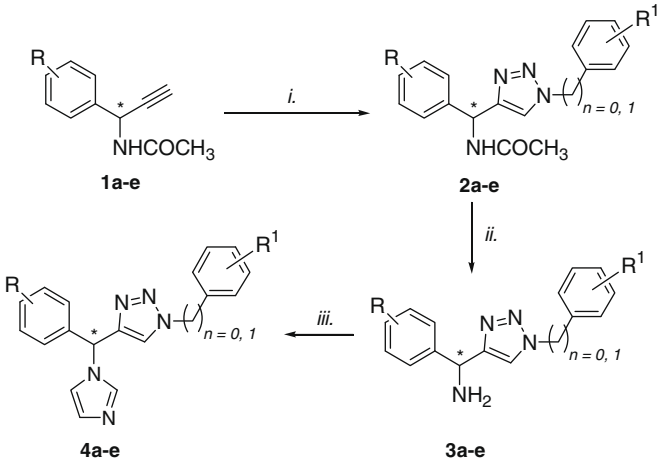


Figure 1. Common drugs used in anti-TB therapy and target compounds A.



Scheme 1. Synthesis of enantiomerically pure azoles. Reagents and conditions: (i) (For $n = 1$): NaN_3, benzyl chloride, sodium ascorbate/$CuSO_4$, H_2O/*t*-BuOH, MW, 125 °C, 10 min; (For $n = 0$): PhN_3, sodium ascorbate/$CuSO_4$, H_2O/*t*-BuOH, MW, 120 °C, 10 min; (ii) HCl 3 N, 100 °C; (iii) H_3PO_4, paraformaldehyde, NH_4Cl, H_2O/dioxane, MW, 80 °C, 10 min.

enantiopure form via kinetic enzymatic resolution as described in our previous works.[13] Propargylamides **1a**–**e** were converted into 1,2,3-triazoles **2a**–**e** via a microwave assisted Click reaction in the presence of Cu/Na Ascorbate to generate in situ the Cu(I) catalyst. Compounds **2a**–**e** were obtained in pure form after simple aqueous work-up and were used in the next step without any further chromatographic purification. Hydrolysis of triazoles **2a**–**e** in HCl 3 N at reflux afforded the corresponding unstable amines **3a**–**e** which were immediately used in the next step without further purification. The synthesis of the imidazole ring from a primary amine requires generally a multistep procedure and long reaction times. On the basis of our experience in the microwave-assisted organic synthesis,[13] we decided to circumvent the problem performing a one-pot synthesis of the imidazole ring under microwave irradiation.

Amines **3a**–**e** were dissolved in a 9:1 mixture of water and dioxane, and polyphosphoric acid, solid paraformaldehyde, glyoxal aqueous solution (40%) and NH_4Cl saturated solution were added and the mixture was finally irradiated with microwaves for 6 min at 120 °C. Imidazole derivatives **4a**–**e** were isolated from the reaction mixtures in good yields. HPLC–MS analysis revealed that compounds **4a**–**e** are enantiomerically pure and no racemization occured during the reaction. The enantiomeric excesses (ee) and reaction yields are reported in Table 1.

Compounds (*R*)-**4a**–**e** and (*S*)-**4a**–**e** were assayed for their inhibitory activity toward *M. tuberculosis* H37Rv (ATCC27294). The min-

Table 1
Yields and ee values of azoles **4**

Entry	Compounds						
		R	R_1	*n*	ee[a] (%)	Yield[b] (%)	$[\alpha]_D^{20}$
1	(*S*)-**4a**	4-F	H	0	95	43	−9.9 (*c* 1.0 $CHCl_2$)
2	(*R*)-**4a**	4-F	H	0	95	40	+8.3 (*c* 1.0 $CHCl_2$)
3	(*S*)-**4b**	4-Br	H	0	89	45	−10.9 (*c* 1.0 $CHCl_2$)
4	(*R*)-**4b**	4-Br	H	0	89	43	+9.2 (*c* 1.2CH_2Cl_2)
5	(*S*)-**4c**	4-F	H	1	92	52	−8.7 (*c* 1.1 $CHCl_2$)
6	(*R*)-**4c**	4-F	H	1	93	53	+7.6 (*c* 1.3CH_2Cl_2)
7	(*S*)-**4d**	4-Cl	4-Cl	1	95	62	−9.4 (*c* 1.1 $CHCl_2$)
8	(*R*)-**4d**	4-Cl	4-Cl	1	95	60	+9.9 (*c* 1.0CH_2Cl_2)
9	(*S*)-**4e**	4-Br	H	1	94	55	−9.6 (*c* 0.9 $CHCl_2$)
10	(*R*)-**4e**	4-Br	H	1	95	49	+10.1 (*c*1.0CH_2Cl)

[a] Determined by chiral HPLC–MS using an (*S*,*S*)-Whelk-O1 column (methanol/water 95:5, flow rate 0.8 mL/min, UV-254 nm).
[b] Isolated yields were reported.

Table 2
MIC values for compounds **4a**–**e**

Entry	Compound	Log P[a,b]	MIC (μg mL^{-1}) *M. tuberculosis*
1	(*S*)-**4a**	4.184	32
2	(*R*)-**4a**	4.184	32
3	(*S*)-**4b**	4.525	32
4	(*R*)-**4b**	4.525	32
5	(*S*)-**4c**	4.329	64
6	(*R*)-**4c**	4.329	64
7	(*S*)-**4d**	5.103	32
8	(*R*)-**4d**	5.103	32
9	(*S*)-**4e**	4.670	32
10	(*R*)-**4e**	4.670	16
11	Clotrimazole[c]	5.399	20.4
12	Econazole[c]	5.605	12.5
13	Isoniazid	0.318	0.2[11]

[a] Physicochemical properties were predicted using QikProp v2.5.[16]
[b] Predicted octanol/water partition coefficient; range of recommended values(−2.0)–(+6.5).
[c] According to the literature.[14]

imum inhibitory concentration ($\mu g\ mL^{-1}$) was determined for each compound. Clotrimazole and econazole were used a reference compounds.[14] Resulting data are reported in Table 2. Compounds **4a** and **b** showed medium activity toward MTB as well as compounds **4d** (*entries* 1–4 and 7 and 8). In all these cases, both enantiomers presented the same biological profile. On the contrary, componds **4c** resulted to be inactive (*entries* 5 and 6). Finally the bromo derivatives **4e** showed good MIC values. In particular one of the two enantiomers, namely (*R*)-**4e** (*entry* 10), proved to have very promising antimycobacterial activity, showing a biological profile similar to econazole (*entry* 12) and better than clotrimazole (*entry* 11).[15]

In conclusion, a new class of azole derivatives has been synthesised. All the compounds were obtained in good yield and in enantiomerically pure form and were tested as new potential antitubercular agents. One of those compounds, derivative (*R*)-**4e**[17,18] showed good activity against MTB. Interestingly, its enantiomer, derivative (*S*)-**4e** presented higher MIC values, confirming the importance of synthesizing enantiomerically pure compounds due to the different interactions of single enantiomers with chiral biological systems. Attempts to design and synthesize novel azole derivatives of **4** to improve the activity against MTB are currently in progress.

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15. *M. tuberculosis* H37Rv ATCC 27294 was used in this study. It was maintained on Löwenstein-Jensen (bioMérieux, Marcy l'Étoile, France) agar slants until needed. MICs were determined by a standard twofold agar dilution method. Briefly, 1 mL of Middlebrook 7H11 agar (Becton Dickinson BBL, Sparks, MD) supplemented with 10% oleic acid-albumin-dextrose-catalase enrichment containing the testing compounds in 24-multiwell plates at concentrations ranging between 0.0312 and 64 μg/mL was inoculated with 10 μL of a suspension containing *M. tuberculosis* H37Rv 1.5×10^5 cfu/mL grown on Middlebrook 7H9 broth (Difco Laboratories, Detroit, MI) supplemented with 10% albumin–dextrose–catalase enrichment. Final inoculum was 1.5×10^3 per well and was obtained as described previously.[11,12] Plates were incubated for 21–28 days and MICs were read as minimal concentrations of compounds completely inhibiting visible growth of mycobacteria.
16. QikProp, version 2.5, Schrödinger, LLC, New York, NY, 2005.
17. *Synthesis of azoles* **4**. *General procedure*.Amine **3** (0.38 mmol) was dissolved into a solution of H_2O/dioxane (3/1 mL) and H_3PO_4 was added until pH 2. Then solid paraformaldehyde (15 mg) and glyoxal sol. 40% in water (0.1 mL) were added and the mixture was stirred at 80 °C for 10 min. NH_4Cl saturated solution (0.5 mL) was added at this temperature and the resulting solution, in a 10-mL sealed glass vial, was irradiated by microwave for 6 min at 120 °C. The mixture was then cooled at 0 °C and NaOH was added until pH 12. The alkaline solution was extracted with AcOEt two times. The combined organic layers were washed with brine, dried (Na_2SO_4), filtered and evaporated to give the crude **4**. The crude product was purified by flash chromatography on silica gel, using AcOEt/MeOH 9:1 as eluant, and obtained as a tan oil.
18. *Characterization of azole (R)*-**4e.** Yield: 49%. ^1H NMR ($CDCl_3$): δ 7.48–7.46 (3H, m, NC*H*N and Ph), 7.39–7.38 (2H, m, Ph), 7.27–7.24 (3H,m, CC*H*NBn and Ph), 7.08 (1H, s, NC*HCH*N), 7.03–7.07 (2H, d, *J* = 8.21, Ph), 6.91(1H, s, NC*HCH*N), 6.59 (1H, s, C*H*N), 5.25 (2H, s, CH_2Ph) ppm. ^{13}C NMR ($CDCl_3$): δ 146.06, 136.76, 133.70,131.86, 129.48, 128.94, 128.94, 128.75, 128.53, 127.71, 122.51, 122.29, 56.62, 54.09 ppm. MS: 393.04 (M^+), 395.04 $(M+H)^+$ 416.04 $(M+Na)^+$. $[\alpha]_D^{20}$ +10.1 (c 1.0 CH_2Cl_2). Anal. Calcd for $C_{19}H_{16}BrN_5$: C, 57.88; H, 4.09; N, 17.76. Found: C, 57.97; H, 4.32; N, 17.87.