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Organocatalytic synthesis and sterol 14α-demethylase binding interactions of enantioriched 3-(1*H*-1,2,4-triazol-1-yl)butyl benzoates

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ABSTRACT

1*H*-1,2,4-Triazole reacted with 2-butenal in the presence of diaryl prolinol silyl ether **3** and benzonic acid to give 3-(1*H*-1,2,4-triazol-1-yl)butanal **4**, which was subsequently reduced and then treated with various acyl chloride to generate enantioriched 3-(1*H*-1,2,4-triazol-1-yl)butyl benzoates **6**. Some of triazoles **6** exhibited strong binding interactions with the cytochrome P450-dependent sterol 14α-demethylase (CYP51). For example, compound (*R*)-**6f** showed the best binding activity with K_d 0.3381 μM.

Because of their lower application doses, high selectivity, reduced undesired environmental impact and high therapeutic index, azoles are the most widely used and studied class of antifungal agents in both agricultural and medicinal usage.[1] The biochemical site of action of the fungicidal azoles is the cytochrome P450-dependent lanosterol 14α-demethylase (CYP51), encoded by the ERG11 gene in fungal cells.[2] This haem protein binds tetracyclic steroid molecules, inserting one oxygen atom into a C–H bond of the C-14 methyl group.[3] Azole antifungal agents inhibit CYP51 by the mechanism in which the heterocyclic nitrogen atom (N-3 of imidazole and N-4 of triazole) binds to the heme iron atom in the binding site of the enzyme. Azoles are fungistatic against yeasts, but unfortunately, the broad usage of these compounds leads to the development of resistance. Therefore, the invention of new and effective antifungal agents is of great importance.

Triazole derivatives such as diniconazole, tebuconazole, hexaconazole, triadimefon, triadimenol, and so on represent the most important category of fungicides to date. These compounds have excellent protective, curative, and eradicant power toward a wide spectrum of crop diseases. Structurally, they inherently have a chiral centre which results in two enantiomers. It is well known that both stereoisomers of above fungicides would display different fungicidal activities.[4] The questions concerning the synthetic method of active enantiomers and the influence of stereochemistry upon binding constants (K_d) of the cytochrome P450-dependent lanosterol 14α-demethylase (CYP51) for chiral triazole pesticides are therefore of particular interest in this study.

Since the rediscovery of the proline-catalyzed aldol reaction,[5] organocatalysis has expanded widely within the last few years.[6] Many chiral secondary amines are effective catalysts for enantioselective β addition to α,β-unsaturated carbonyl compounds.[7] In the case of these α,β-unsaturated systems, the catalyst activates the substrate through the iminium-ion mechanism, thereby facilitating the addition of the nucleophile to the β-carbon atom. Recently, several organocatalyzed nucleophilic nitrogen addition reactions of N-heterocyclic compounds to α,β-unsaturated carbonyl compounds have been impressively presented with high enantioselectivities using chiral organocatalysts.[8] We extend this organocatalytic strategy to the design and synthesis of potential triazole-based antifungal agents.

In previous study, we have carried out cloning, expression, and inhibition of CYP51 from *Magnaporthe grisea* and *Penicillium digitatum*. The structural characteristics of the interaction between heterologous CYP51 and commercial azoles were also analysed by binding assay.[9] In this Letter, we describe the synthesis of some new triazole compounds and their binding interactions with the cytochrome P450-dependent lanosterol 14α-demethylase.

The reaction of 1*H*-1,2,4-triazole (**1**) with 2-butenal (**2**) has been performed in the presence of racemic, (*R*)-, and (*S*)-**3**[10] and benzoic acid, respectively, to prepare racemic and both enantiomeric **4**. The result showed that full conversion to adduct **4** was achieved in toluene at room temperature for 2 h. The product 3-(1*H*-1,2,4-triazol-1-yl)butanal (**4**) was reduced with $NaBH_4$ subsequently to give 3-

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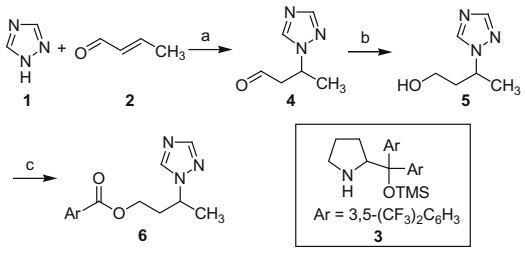

Scheme 1. Preparation of 3-(1*H*-1,2,4-triazol-1-yl)butyl benzoates **6**. Reagents and conditions: (a) 10 mol % **3**, 10 mol % $PhCO_2H$, toluene, rt; (b) $NaBH_4$, methanol, 70% in two steps; (c) Acyl chloride, NEt_3 (1.5 equiv.), 10 mol % DMAP, dichloromethane.

(1*H*-1,2,4-triazol-1-yl)butanol (**5**).[11] Further reaction of **5** with acyl chloride in the presence of NEt_3/DMAP produced 3-(1*H*-1,2,4-triazol-1-yl)butyl benzoates **6** in 53–91% total yields (Scheme 1).[12] By the use of this three-step reaction procedure, the reaction underwent with good enantioselectivities (72–82% ee) and in high yields (Table 1).

The binding interactions of triazoles **6** with CYP51 were investigated with the commercial fungicides triadimefon and Diniconazole as standards.[13] Similar to our previous results, the purified CYP51 revealed a typical absolute absorbance spectrum of oxidized P450 with Soret maximum at 417 nm and the reduced CO spectrum had a maximum at 449 nm. UV–visible absorption spectroscopy provided a simple and accurate method for the determination of the level of binding of substrates and inhibitors to P450s. Compound (*S*)-**6c** binding with the purified CYP51 was initially examined at 25 °C and induced a typical type II spectrum with a spectral peak located at 425 nm and a trough located at 398 nm, respectively (Fig. 1). Higher concentrations of (*S*)-**6c** produced higher absorbance of different values, D425-398, accordingly. These spectra resulted from an interaction of the triazole N-4 of (*S*)-**6c** with the heme of the P450 causing a shift towards the high-spin state.[14] This exhibited the displacement of the native sixth ligand of the heme iron by the nitrogen atom in the triazole ring of (*S*)-**6c**. The spectral results showed that the affinity of (*S*)-**6c** with the purified CYP51 was tight.

Table 1
Preparation of 3-(1*H*-1,2,4-triazol-1-yl)butyl benzoates **6**

Compd	Ar	Yield (%)	ee[a] (%)
(*RS*)-**6a**	Ph	72	
(*S*)-**6a**	Ph	69	76
(*R*)-**6a**	Ph	75	82
(*RS*)-**6b**	2-Cl–C_6H_4	61	
(*S*)-**6b**	2-Cl–C_6H_4	53	81
(*R*)-**6b**	2-Cl–C_6H_4	64	78
(*RS*)-**6c**	3-Cl–C_6H_4	84	
(*S*)-**6c**	3-Cl–C_6H_4	81	77
(*R*)-**6c**	3-Cl–C_6H_4	71	79
(*RS*)-**6d**	4-Cl–C_6H_4	82	
(*S*)-**6d**	4-Cl–C_6H_4	87	79
(*R*)-**6d**	4-Cl–C_6H_4	68	77
(*RS*)-**6e**	2,4-Cl_2–C_6H_3	86	
(*S*)-**6e**	2,4-Cl_2–C_6H_3	76	81
(*R*)-**6e**	2,4-Cl_2–C_6H_3	77	77
(*RS*)-6f	4-Me–C_6H_4	91	
(*S*)-**6f**	4-Me–C_6H_4	66	76
(*R*)-**6f**	4-Me–C_6H_4	78	79
(*RS*)-**6g**	4-F–C_6H_4	80	
(*S*)-**6g**	4-F–C_6H_4	60	82
(*R*)-**6g**	4-F–C_6H_4	88	81
(*RS*)-**6h**	4-MeO–C_6H_4	74	
(*S*)-**6h**	4-MeO–C_6H_4	74	78
(*R*)-**6h**	4-MeO–C_6H_4	85	72

[a] Determined by chiral HPLC.

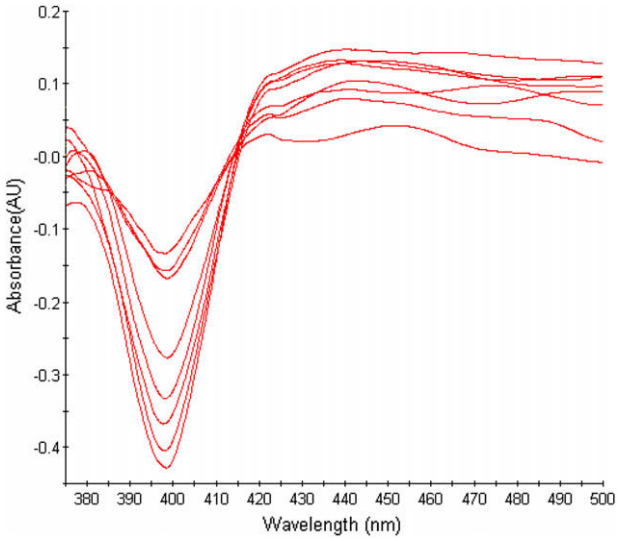

Figure 1. CYP51 type II binding spectrum in the presence of compound (*S*)-**6c**. Type II spectral response to (*S*)-**6c** and the concentrations of the (*S*)-**6c** added to the reaction mixture were 0.1, 0.2, 0.4, 0.8, 1, 2, 3 and 8 μM.

The K_d values of some of the compounds **6** are highlighted in Table 2. The results showed that some of them exhibited strong binding interactions with CYP51. As indicated in Table 2, most of the compounds showed strong associative interactions with CYP51. Compounds (*R*)-**6f** showed the best binding activities with K_d value of 0.3381 μM. Although these compounds displayed less binding activities than Diniconazole did (K_d 0.2473 μM), some samples (e.g., (*S*)-**6b**, (*R*)-**6b**, (*RS*)-**6c**, (*S*)-**6c**, (*R*)-**6d**, (*S*)-**6e**, and (*R*)-**6f**) did indeed exhibit stronger binding activities than triadimefon (K_d 0.9355 μM). The presence of the groups like 3-Cl ((*S*)-**6c**), 2,4-Cl_2 ((*S*)-**6e**) and 4-Me ((*R*)-**6f**) on the benzene ring plays a significant role in imparting binding activity. The absolute configuration of triazoles **6** also shows influence on the binding activity. For example, (*S*)-**6c** (K_d 0.4724 μM) is much more active than (*R*)-**6c** (K_d 2.3568 μM), whereas (*R*)-**6f** (K_d 0.3381 μM) displays much stronger binding activity to CYP51 than (*S*)-**6f** (K_d 10.4903 μM) does.

In conclusion, we have synthesized some enantioriched 3-(1*H*-1,2,4-triazol-1-yl)butyl benzoates **6** via organocatalytic Michael addition and subsequent reduction and functionalization. The pre-

Table 2
In vitro binding constants (K_d, μM) of compounds **6**

Compd	K_d
(*R*,*S*)-**6a**	1.1824
(*S*)-**6a**	1.4173
(*R*)-**6a**	1.6183
(*R*,*S*)-**6b**	1.1163
(*S*)-**6b**	0.5600
(*R*)-**6b**	0.5801
(*R*,*S*)-**6c**	0.7429
(*S*)-**6c**	0.4724
(*R*)-**6c**	2.3568
(*R*,*S*)-**6d**	1.3759
(*S*)-**6d**	0.9881
(*R*)-**6d**	0.6824
(*R*,*S*)-**6e**	0.9734
(*S*)-**6e**	0.4524
(*R*)-**6e**	/
(*R*,*S*)-**6f**	1.7392
(*S*)-**6f**	10.4903
(*R*)-**6f**	0.3381
Triadimefon	0.9355
Diniconazole	0.2473

liminary investigation on the biological activities of **6** show that some of them exhibited strong binding interactions with the cytochrome P450-dependent lanosterol 14α-demethylase.

Acknowledgements

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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.2009.03.028.

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11. *Preparation of 3-(1H-1,2,4-triazol-1-yl)butanol* **5**: A mixture of 2-butenal (**2**) (0.6 mL, 7.5 mmol), catalyst **3** (10 mol %, 0.5 mmol, 298.8 mg), toluene (50 mL), and benzoic acid (10 mol %, 0.5 mmol, 61.1 mg) were introduced into a sample vial equipped with a magnetic stirring bar. The mixture was stirred for 30 min at ambient temperature, 1*H*-1,2,4-triazole (**1**) (345.4 mg, 5 mmol) was then added. After 2 h (monitored by TLC), the reaction was diluted with pre-cooled MeOH (10 mL) and $NaBH_4$ (0.3 g, 8 mmol) was added. After 1 h, the reaction was quenched with NH_4Cl (satd). The mixture was extracted with CH_2Cl_2 several times and dried over $MgSO_4$. The solvent was evaporated and the product was purified by flash chromatography (first Et_2O and then EtOAc). Evaporate the solvent to provide the alcohol which was used in next step direclty.
12. *Preparation of 3-(1H-1,2,4-triazol-1-yl)butyl benzoates* **6**: To 3-(1*H*-1,2,4-triazol-1-yl)butanol **5** (3 mmol) in CH_2Cl_2 (24 mL) was added acyl chloride (4.5 mmol), Et_3N (4.5 mmol) and DMAP (10 mol %, 0.04 g, 0.3 mmol) at 0 °C. The reaction mixture was stirred overnight and was quenched with satd $NaHCO_3$. The mixture was extracted with CH_2Cl_2 several times and dried over $K_2CO_3/MgSO_4$. The solvent was evaporated and the pure product was then obtained by flash chromatography (eluent:PE–Et_2O = 3:1 first, and then neat Et_2O).*3-(1H-1,2,4-Triazol-1-yl)butyl benzoate* **6a**: ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.13 (s, 1H, triazolyl-5-H), 7.98 (s, 1H, triazolyl-3-H), 8.06–7.46 (m, 5H, Ar–H), 4.68–4.63 (m, 1H, NCH), 4.36–4.02 (m, 2H, OCH_2), 2.44–2.28 (m, 2H, CH_2), 1.62 (d, *J* = 6.8 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 165.9, 151.7, 141.9, 132.9, 129.5, 129.2, 128.1, 60.9, 53.1, 35.1, 20.7 MS *m/z* (%): 246 (M^++1, 49), 177 (8), 123 (26), 105 (100), 77 (67). Anal. Calcd for $C_{13}H_{15}N_3O_2$: C, 63.66; H, 6.16; N, 17.13. Found: C, 63.41; H, 6.28; N, 17.21.*3-(1H-1,2,4-Triazol-1-yl)butyl 2-chlorobenzoate* **6b:** ^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm) 8.14 (s, 1H, triazolyl-5-H), 7.98 (s, 1H, triazolyl-3-H), 7.78–7.34 (m, 4H, Ar–H), 4.72–4.68 (m, 1H, NCH), 4.45–4.03 (m, 2H, OCH_2), 2.43–2.27 (m, 2H, CH_2), 1.61 (d, *J* = 6.6 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 150 MHz): δ (ppm) 165.2, 151.7, 142.1, 133.1, 132.5, 131.2, 130.8, 129.6, 126.5, 61.5, 53.0, 35.0, 20.7.MS *m/z* (%): 244 (M^+-Cl, 6), 231 (5), 139 (100), 111 (88). Anal. Calcd for $C_{13}H_{14}ClN_3O_2$: C, 55.82; H, 5.04; N, 15.02. Found: C, 55.97; H, 4.97; N, 15.27.*3-(1H-1,2,4-Triazol-1-yl)butyl 3-chlorobenzoate* **6c**: ^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm) 8.12 (s, 1H, triazolyl-5-H), 7.96 (s, 1H, Ar–H), 7.96 (s, 1H, triazolyl-3-H), 7.87–7.40 (m, 3H, Ar–H), 4.69–4.60 (m, 1H, NCH), 4.34–4.09 (m, 2H, OCH_2), 2.48–2.25 (m, 2H, CH_2), 1.62 (d, *J* = 6.6 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 164.5, 151.6, 141.8, 134.0, 132.7, 131.1, 129.4, 129.0, 127.2, 61.3, 53.0, 34.9, 20.7 MS *m/z* (%): 156 (3), 139 (24), 111 (77), 69 (84), 42 (100). Anal. Calcd for $C_{13}H_{14}ClN_3O_2$: C, 55.82; H, 5.04; N, 15.02. Found: C, 55.61; H, 4.90; N, 15.16.*3-(1H-1,2,4-Triazol-1-yl)butyl 4-chlorobenzoate* **6d**: ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.12 (s, 1H, triazolyl-5-H), 7.97 (s, 1H, triazolyl-3-H), 7.93–7.42 (m, 4H, Ar–H), 4.67–4.61 (m, 1H, NCH), 4.36–4.12 (m, 2H, OCH_2), 2.45–2.26 (m, 2H, CH_2), 1.62 (d, *J* = 6.8 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 164.7, 151.4, 141.7, 138.9, 130.4, 128.2, 127.7, 61.0, 52.9, 34.8, 20.5. MS *m/z* (%): 280 (M^++1, 2), 156 (4), 139 (82), 111 (100), 75 (75). Anal. Calcd for $C_{13}H_{14}ClN_3O_2$: C, 55.82; H, 5.04; N, 15.02. Found: C, 55.94; H, 5.17; N, 14.93.*3-(1H-1,2,4-Triazol-1-yl)butyl 2,4-dichlorobenzoate* **6e**: ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.12 (s, 1H, triazolyl-5-H), 7.97 (s, 1H, triazolyl-3-H), 7.97–7.33 (m, 3H, Ar–H), 4.67–4.62 (m, 1H, NCH), 4.43 - 4.04 (m, 2H, OCH_2), 2.43–2.23 (m, 2H, CH_2), 1.61 (d, *J* = 6.8 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 164.0, 151.4, 141.8, 138.0, 134.2, 132.2, 130.5, 127.5, 126.7, 61.6, 52.9, 34.8, 20.6.MS *m/z* (%): 173 (5), 145 (6), 109 (19), 69 (62), 42 (100). Anal. Calcd for $C_{13}H_{13}ClN_3O_2$: C, 49.70; H, 4.17; N, 13.38. Found: C, 49.51; H, 4.25; N, 13.52.*3-(1H-1,2,4-Triazol-1-yl)butyl 4-methylbenzoate* **6f**: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.14 (s, 1H, triazolyl-5-H), 7.97 (s, 1H, triazolyl-3-H), 7.88–7.25 (m, 4H, Ar–H), 4.65–4.62 (m, 1H, NCH), 4.34–4.07 (m, 2H, OCH_2), 2.47–2.27 (m, 5H, CH_2 and CH_3), 1.62 (d, *J* = 6.6 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 166.0, 151.7, 143.7, 142.0, 129.3, 128.9, 126.7, 60.7, 53.1, 35.1, 21.4, 20.8.MS *m/z* (%): 260 (M^++1, 59), 191 (8), 119 (100), 91 (52). Anal. Calcd for $C_{14}H_{17}N_3O_2$: C, 64.85; H, 6.61; N, 16.20. Found: C, 64.91; H, 6.57; N, 16.43.*3-(1H-1,2,4-Triazol-1-yl)butyl 4-fluorobenzoate* **6g**: ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.14 (s, 1H, triazolyl-5-H), 7.97 (s, 1H, triazolyl-3-H), 8.04–7.12 (m, 4H, Ar–H), 4.64–4.62 (m, 1H, NCH), 4.34–4.08 (m, 2H, OCH_2), 2.44 -2.24 (m, 2H, CH_2), 1.62 (d, *J* = 6.6 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 166.1, 164.5, 151.4, 141.8, 131.6, 125.6, 115.1, 61.0, 53.1, 34.9, 20.5.MS *m/z* (%): 264 (M^++1, 71), 195 (8), 123 (100), 95 (78). Anal. Calcd for $C_{13}H_{14}FN_3O_2$: C, 59.31; H, 5.36; N, 15.96. Found: C, 59.57; H, 6.25; N, 15.89.*3-(1H-1,2,4-Triazol-1-yl)butyl 4-methoxybenzoate* **6h**: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.12 (s, 1H, triazolyl-5-H), 7.97 (s, 1H, triazolyl-3-H), 8.00–6.93 (m, 4H, Ar–H), 4.72–4.54 (m, 1H, NCH), 4.33–4.03 (m, 2H, OCH_2), 3.87 (s, 3H, OCH_3), 2.42–2.26 (m, 2H, CH_2), 1.62 (d, *J* = 6.8 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 165.3, 162.9, 151.4, 141.7, 131.0, 121.6, 113.1, 60.4, 54.8, 52.9, 34.9, 20.5.MS *m/z* (%): 275 (M^+, 5), 152 (17), 135 (100), 92 (19). Anal. Calcd for $C_{13}H_{17}N_3O_3$: C, 61.08; H, 6.22; N, 15.26. Found: C, 61.00; H, 6.10; N, 15.32.
13. The *E. coli* membrane fragments containing expressed fungal *P. digitatum* CYP51 were used for binding spectra analysis. Binding assays to *P. digitatum* CYP51 were repeated three times. Azole binding spectra were recorded on an S3100 UV–visible scanning spectrophotometer (Sinco, Korea) as described by the following reference: Venkateswarlu, K.; Denning, D. W.; Manning, N. J.; Kelly, S. L. *Antimicrob. Agents Chemother* **1996**, *40*, 1382.
14. Yoshida, Y.; Aoyama, Y. *Biochem. Pharmacol.* **1987**, *36*, 229.