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Design, Synthesis and Antifungal Activity of Novel Triazole Derivatives

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Abstract: Twenty-one 1-(1H-1,2,4-triazolyl)-2-(2,4-diflurophenyl)-3-(4-substituted-1- piperazinyl) -2-propanol derivatives were designed and synthesized, on the basis of the active site of lanosterol 14α-demethylase. *In vitro* antifungal activities showed that some of the target compounds had higher antifungal activity and broader antifungal spectrum than fluconazole.

Keywords: Antifungal, triazole, synthesis.

Life threatening infections caused by pathogenic fungi are becoming increasingly common, especially in those individuals with suppressed immune systems such as cancer patients and patients with AIDS [1]. Antifungal triazoles(*e.g.* fluconazole), which act by inhibiting the cytochrome P-450 14α-demethylase ($P450_{14DM}$), a key enzyme in fungal ergosterol biosynthesis[2], now play a leading role in the treatment of a variety of fungal infections. But recently, drug resistance, poor activity against *Aspergillus* infection and undesirable side effects of fluconazole has been reported[3]. Therefore, there is an emergent need to develop more effective, broader-spectrum and safer drugs. In our previous paper[4], we have constructed a three dimensional model of lanosterol P450 14α-demethylase of *Candida albicans*. The pharmacophoric conformations of azole antifungal agents were searched by the active analogy approach and docked into the active site of the enzyme. Subsequently, the substrate and azole-binding residues in the active site were identified, which provide us with a template to design novel trizole antifungals specific towards the target enzyme.

In general, the active site of $P450_{14DM}$ for ligand binding can be divided into four subsites: the area in contact with the heme, the hydrophilic H-bonding region, the hydrophobic region and the narrow hydrophobic cleft formed by the residues in the helix B'-meander 1 loop and N-terminus of helix I. Based on these properties of the active site, we designed and synthesized a series of 1-(1H-1, 2, 4-triazolyl)-2-(2, 4-diflurophenyl)-3-
(4-substituted-1-piperazinyl)-2-propanol derivatives to search for more potential antifungal agents. The N3 atom of the triazole was designed to be coordinated to iron

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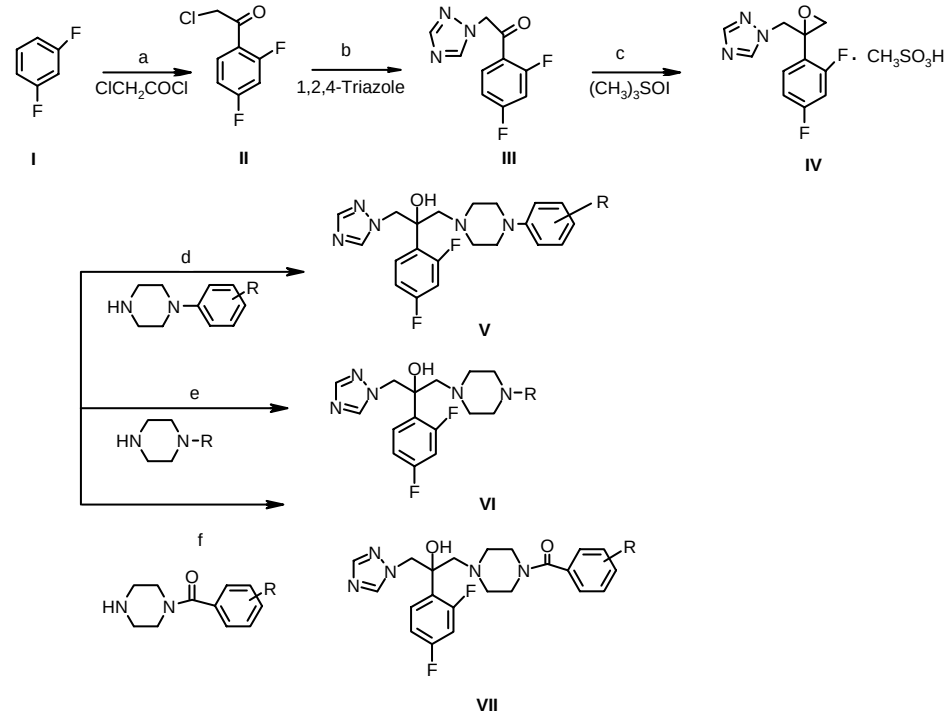
atom of the heme, the 2,4-difluorophenyl group could be located into the hydrophobic pocket and the substituted piperazinyl side chain was designed to interact with the residues of the narrow hydrophobic cleft and adjust the physico-chemical properties of the whole molecule. The substituted phenyl, pyridinyl and phenylacyl was chosen to be linked to the piperazinyl in order to increase the hydrophobic interaction with the hydrophobic cleft.

Scheme 1



Conditions: a. ClCH2COCl, AlCl3, 50℃, 5h, 87%; b. CH2Cl2, K2CO3, TEBA 0℃, 24h, 85%; c. toluene, cetyltrimethylammonium bromide, NaOH, 60℃, 5h, 62.3%; d. CH3CH2OH, Et3N, 75~80 ℃, 81~91%. e. CH3OH, NaOH, 80~85℃, 77~79%. f. CH3CH2OH, Et3N, 80~90℃, 73~94%.

The synthetic route was outlined in the Scheme 1. Compound II was easily prepared by Friedel-Crafts reaction. According to the literature5, the ketone III was prepared by reacting II with 1,2,4-triazole under reflux in the presence of a base such as K2CO3 and Et3N in 40% yield. In the present paper, we chose TEBA as phase transfer catalyst and CH2Cl2 as solvent, and as a result, the yield was increased to 85%. The oxirane copound IV could be obtained by reacting III with dimethyloxosulfonium methylide, which is prepared from trimethylsulfoxonium iodide and cetyltrimethylammonium bromide in the presence of aqueous sodium hydroxide. Then, compound IV can be isolated by forming the methanesulfonate. The yield of IV was poor at first and was increased from 21.8% to 62.3% by optimizing the reaction condition with orthogonal experimental design. Reacting

excess piperazine with electrowithdrawing group substituted chlorobenzene, substituted pyridine and substituted benzoyl chloride gave the piperazinyl side chain in good yield. The title copmpound V, VI and VII were prepared by ring-open reaction of epoxide VI with excess piperazinyl side chain on heating at 70-95℃. This reaction proceeded more smoothly in the protic solvent, such as alcohol, than in nonprotic solvents.

Table 1. Structure and in vitro antifungal activity of the target compounds

Compound.	R	*in vitro* antifungal activity(MIC_{80})[5]						
		C. alb.	C. par.	C. tro.	C. neo.	A.fum.	F. ped.	T. rub.
V_1	4-$COCH_3$	0.25	<0.125	0.25	2	<0.125	1	
V_2	4-CHO	<0.125	0.5	0.25	4	16	2	>64
V_3	4-CN	<0.125	16	4	<0.125	16	0.25	4
V_4	3-CF_3	<0.125	<0.125	<0.125	0.5	0.5	0.25	2
V_5	4-$C(CH_3)_3$	<0.125	<0.125	<0.125	<0.125	0.25	<0.125	<0.125
VI_1	4-pyridine	<0.125	32	4	>64	>64	16	>64
VI_2	2-pyridine	1	<0.125	0.25	1	0.25	0.25	0.5
VI_3	a	<0.125	<0.125	<0.125	<0.125	16	<0.125	0.5
VII_1	4-Cl	<0.125	0.25	0.25	4	8	2	4
VII_2	4-F	0.25	1	1	8	8	4	8
VII_3	2-Cl	1	8	2	32	8	16	>64
VII_4	4-NO_2	1	1	0.5	16	4	4	8
VII_5	3-NO_2	0.5	8	2	>64	>64	>64	64
VII_6	3-Me	8	2	0.5	16	16	16	16
VII_7	4-Me	1	0.5	0.5	4	16	4	4
VII_8	4-OMe	8	1	0.5	8	4	4	8
VII_9	4-Me	0.125	4	>64	64	>64	32	>64
VII_{10}	4-NH_2	16	>64	>64	>64	32	64	64
VII_{11}	H	16	2	1	8	16	16	16
VII_{12}	2-OMe	1	16	8	32	32	64	64
VII_{13}	4--$C(CH_3)_3$	8	1	0.125	4	16	8	4
Flu		0.5	0.5	0.5	16	32	64	8
		0.25						

a. 2-Cl-4-CF_3-2-pyridine; C. alb. Candida albicans; C. par. Candida paropsilosis; C. tro. Candida tropicalis; C. neo. Cryptococcus neoformans; A. fum. Aspergillus fumigatus; F. ped. Fonsecaea pedrosoi; T. rub. Trichophyton rubrum; Flu: Fluconazole.

All the target compounds have not been reported in literature before and their structures were confirmed by ^{1}HNMR, MS, IR and elemental analysis. The structure and *in vitro* antifungal activities were listed in **Table 1**. All of the designed compounds showed antifungal activites against seven pathogenic fungi. In general, phenyl(**VI**) and pyridinyl(**VII**) analogues showed higher antifungal activity than the phenylacyl(**VII**) analogues. Some of the target compounds(**V_1, V_4, V_5, VI_2, VI_3**) showed good MIC values less than 0.125μg/mL and proved to be more potent than fluconazole. **V_5** and **VI_3** had excellent potency against a broad range of fungal

pathogen including *Aspergillus fumigatus*. Further biological evaluation of the two compounds is in progress. The mode of action of this class of compounds will be explored by molecular modeling, which will lead us to design and synthesize more potent antifungal agents.

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6. *In vitro* antifungal activity was measured by means of the minimal inhibitory concentrations (MIC) using the serial dilution method with 96-well microtest plates. Test fungal strains were obtained from ATCC. Minimal inhibitory concentrations (MIC) were determined in RPM 1640 medium. The MIC was defined as the lowest concentration which resulted in a culture with turbidity less than or equal to the 80% inhibition, when compared with the growth of the control. Test compounds were dissolved in DMSO, serially diluted in growth medium, inoculated and incubated at 35°C. Growth MIC was determined at 24 hr for Candida species, 72 hr for Cryptococcus neoformans and 7 days for filamentous fungi.

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