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Synthesis and antifungal activities of new fluconazole analogues with azaheterocycle moiety

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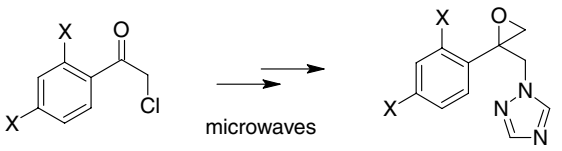
Abstract—A series of fluconazole analogues **5**–**20** incorporating azaindole and indole moieties were prepared using oxirane intermediates synthesized under microwave irradiation. All of the compounds were evaluated in vitro against two clinically important fungi, *Candida albicans* and *Aspergillus fumigatus*. Four derivatives **6**, **13**, **14** and **18** exerted high antifungal activity against *C. albicans* with MIC_{80} values 3- to 28-fold lower than that of fluconazole.

Invasive fungal infections have increased in frequency and severity over the last two decades as a result of an increasing number of immunocompromised hosts.[1] Widespread use of antifungal therapies for curative, pre-emptive or prophylactic purposes has been developed to overcome the threat of *Candida* colonisation and infection, but has also led to the development of resistance to the currently available antifungals.[2] Intrinsic (*C. krusei*, *C. glabatra*) or acquired (*C. albicans*) resistances to azole compounds have been observed with an increasing occurrence, particularly among HIV infected patients suffering from oropharyngeal candidiasis. This situation highlights the need for advent of new and effective antimycotic agents.

In our on-going interest in the preparation of new antifungals,[3] our attention has been focused on fluconazole because of its broad antifungal spectrum, its low toxicity and its excellent pharmacokinetic properties.[4] Furthermore, the indole and its azaheterocycle bioisosters such as indazole and 7-azaindole, which present considerable biological importance, are embedded in a wide range of natural products and medicinal synthetic compounds exerting antifungal activity.[5] We describe in the present study the synthesis and the antifungal activities of new fluconazole analogues incorporating indole and other azaheterocycle moieties.

Recently, our group has developed a new synthetic method of the Corey–Chaykovsky reaction under microwave heating to obtain oxiranes, precursors widely used in the synthesis of conazoles.[3b] These key intermediates were prepared in two steps from 2,2′,4′-trihalogenoacetophenones **1**, **2** by N-alkylation of 1*H*-1,2,4-triazole and then Corey–Chaykovsky reaction of phenacyltriazoles under microwave irradiation (Scheme 1).



1 2,4-diF
2 2,4-diCl
3 2,4-diF
4 2,4-diCl

Scheme 1. Two-step synthesis under microwave irradiation of intermediate oxiranes **3**–**4**.

Keywords: Oxirane; Azaheterocycle; Triazole; Indole; Microwave irradiation; Antifungal; *Candida albicans*.
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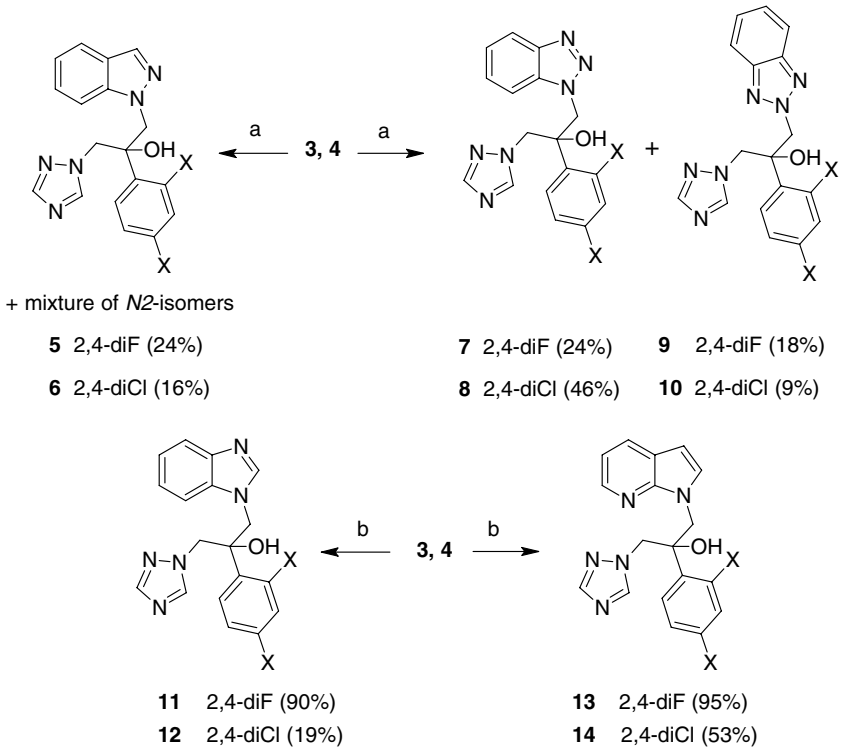
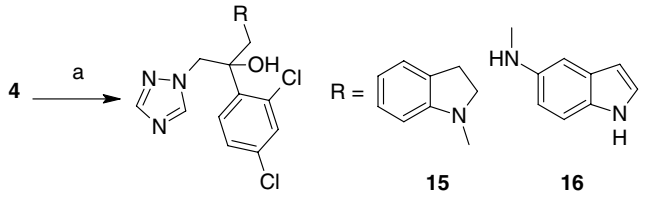


Scheme 2. Reagents and conditions: (a) K_2CO_3, indazole or benzotriazole, CH_3CN, reflux, 12 h; (b) NaH, benzimidazole or 7-azaindole, rt, 12 h.

The preparation of azaheterocycle derivatives is reported in Scheme 2. Compounds with indazole and benzotriazole moieties have been obtained by ring opening of oxiranes **3**–**4** in the presence of potassium carbonate in acetonitrile.[6] Although N1 and N2-alkylation was observed for the two heterocycles, the two regioisomers could be isolated by silica gel column chromatography only in the benzotriazole sub-series in a ratio of about 1/0.4, as evaluated in the crude extracts. In the case of the indazole sub-series, compounds from N1-alkylation have been isolated after purification, whereas N2-alkylation compounds were in mixture with N1-alkylation compounds. Benzimidazole and 7-azaindole, which are less acidic than indazole and benzotriazole rings, need a stronger base for the deprotonation of NH group. The compounds **11**–**14** have been obtained by condensation of benzimidazole or 7-azaindole on oxiranes **3**–**4** in the presence of sodium hydride in DMSO.[7]

To investigate the importance of the pyrrole ring and the N1-fixation of the propan-2-ol chain, we decided to prepare indoline and 5-aminoindole analogues, respectively (Scheme 3).

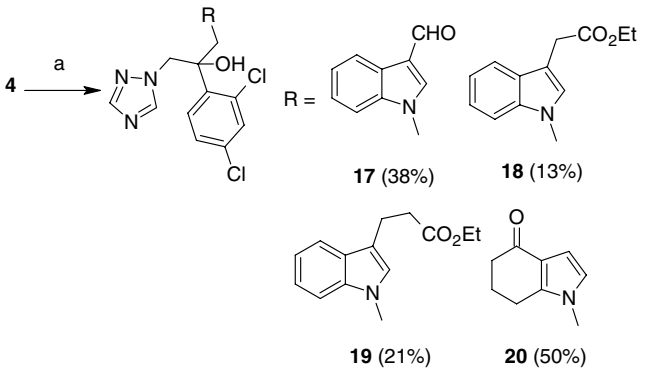


Scheme 3. Reagents and condition: (a) K_2CO_3, indoline or 5-aminoindole, DMF, 90 °C, 72 h, 8–10%.

Pharmacomodulations of the azaheterocycle moiety have been accomplished by the ring opening of oxirane **4** with appropriate azaheterocycles using potassium carbonate in DMF at 90 °C for 72 h.[8] Due to tedious purification workup, yields of the desired compounds **15** and **16** remained very poor: 8% and 10%. In the case of compound **16**, only regioisomer in position 5 has been observed without by-product resulting from the N1-alkylation of the indole ring. The NH group of 5-aminoindole is less acidic than those of indazole or benzotriazole and cannot be deprotonated by potassium carbonate. Then the most potent nucleophilic position is the amino group in position 5 of the indole nucleus.

Additionally, opening of oxirane **4** was carried out using diverse C-3 substituted indoles (3-formylindole, 3-ethoxycarbonylmethylindole, and 3-ethoxycarbonyl-ethylindole) leading, after metallation by NaH in DMSO, to compounds **17**–**19**.[9] Furthermore, condensation with 4-oxo-4,5,6,7-tetrahydro-1*H*-indole afforded selectively the N1-substituted compound **20**; this enaminone seems to be a valuable moiety.[10] The synthetic pathway to achieve these fluconazole analogues **17**–**20** is presented in Scheme 4.

The target azaheterocycle analogues of fluconazole **5**–**20** were screened for antifungal activities against *C. albicans* and *Aspergillus fumigatus*. The growth inhibition test for drug evaluation against *C. albicans* and *A. fumigatus* was carried out by the method based on the fluorometric properties of alamar blue.[11] Amphotericin B, itraconazole and fluconazole were used as positive controls. The minimum inhibitory concentration (MIC_{80}) values (in $\mu g\,mL^{-1}$) are gathered in Table 1.



Scheme 4. Reagents and condition: (a) NaH, azaheterocycle, DMSO, rt, 12 h.

The MIC_{80} values of the azaindole derivatives indicate that the most active compounds were the 2,4-dichlorophenyl derivatives **6**, **8**, **10**, **12**, **14** which showed generally remarkable activities against *C. albicans*, in particularly **6** (indazole) and **14** (7-azaindole) (IC_{80} = 0.0007 and 0.0031 μg mL^{-1}, respectively) which were more potent than fluconazole (IC_{80} = 0.020 μg mL^{-1}). The difluoro analogues **5**, **7**, **9**, **11**, **13** were consistently less active against *C. albicans* but still some of them (**5**, **7**, **13**) are among the most potent compounds.

The MIC_{80} values of the indole derivatives **17**–**19** denote that compound **17** showed comparable antifungal activity to that of fluconazole and that compound **18** (IC_{80} = 0.006 μg mL^{-1}) was the most potent.

Only three compounds **5**, **13** and **14** exerted moderate inhibitory activity against *A. fumigatus*; they remain 50 times less active than itraconazole: ~25 and 0.50 μg mL^{-1}. Surprisingly, comparison between **5**, **13** and **6**, **14**, respectively, brings to the fore, in that case, the favourable influence of a 2,4-difluorophenyl grouping.

These data demonstrate that the presence of a 2,4-dichlorophenyl ring increases the in vitro activity against *C. albicans* by comparison with the 2,4-difluorophenyl moiety. The fact that indazole and 7-azaindole derivatives are the most potent compounds could highlight the influence of number and position of nitrogen atoms in the heterocycle; these atoms may contribute to the formation of hydrogen bonds within the target enzyme active site and thereby stabilize the drug–enzyme complex.

Replacement of the azaindole ring by other azaheterocycles such as indoline (compound **15**), 5-aminoindole (compound **16**) or 4-oxo-4,5,6,7-tetrahydro-1*H*-indole (compound **20**) decreases the activity against *C. albicans*. Thus, the activity seems to be related to the aromaticity of pyrrole and benzene rings in the indole moiety, and the N1-fixation of the propanol chain on the heterocycle. Incorporation of a ethoxycarbonylalkyl chain at C-3 position of the indole core gives disparate results; the highest activity in this sub-series has been obtained with a 3-ethoxycarbonylmethyl chain.

Analysis of the activity of this series of compounds against *C. albicans* and *A. fumigatus* shows a high selectivity of action against *C. albicans*. As fluconazole, these analogues seem to be selective of the cytochrome P450-dependent 14α-lanosterol demethylase ($P450_{14DM}$) of *C. albicans*. We detected high levels of 14α-méthylsterols after treatment of a *C. albicans* strain with NL114, a 1*H*-1,2,4-triazole derivative. No significative inhibitions of aromatase and 17α-hydroxylase/17,20-lyase were also found.[12]

Encouraging in vitro results obtained with both 2,4-dichlorophenyl and difluorophenyl derivatives, and especially **6**, **13**, **14** and **18**, prompt us to carry out in vivo evaluation so as to confirm their surprisingly higher antifungal potency. In parallel, we are developing a chiral HPLC method to separate the racemic compounds presenting highest anti-*Candida* activities.

Table 1. In vitro antifungal activity of azaheterocyclic derivatives **5**–**20**

Compound	Azaheterocycle	X	MIC_{80} (μg mL^{-1})	
			Candida albicans (CA980001)	*Aspergillus fumigatus* (AF980003)
5	*N*1-Indazole	2,4-diF	0.019 ± 0.003	24 ± 0.4
6	*N*1-Indazole	2,4-diCl	0.0007 ± 0.00001	>100
7	*N*1-Benzotriazole	2,4-diF	0.025 ± 0.001	>100
8	*N*1-Benzotriazole	2,4-diCl	0.020 ± 0.004	>100
9	*N*2-Benzotriazole	2,4-diF	>0.036	>100
10	*N*2-Benzotriazole	2,4-diCl	0.010 ± 0.002	>100
11	Benzimidazole	2,4-diF	0.233 ± 0.001	>100
12	Benzimidazole	2,4-diCl	0.026 ± 0.001	>100
13	7-Azaindole	2,4-diF	0.007 ± 0.002	21 ± 1.0
14	7-Azaindole	2,4-diCl	0.0031 ± 0.0004	29 ± 3.0
15	Indoline	2,4-diCl	2.720 ± 0.80	>100
16	5-Aminoindole	2,4-diCl	0.026 ± 0.001	>100
17	3-Formyl-1*H*-indole	2,4-diCl	0.045 ± 0.003	>100
18	3-Ethoxycarbonylmethyl-1*H*-indole	2,4-diCl	0.006 ± 0.003	>100
19	3-Ethoxycarbonylethyl-1*H*-indole	2,4-diCl	0.330 ± 0.005	>100
20	4-Oxo-4,5,6,7-tetrahydro-1*H*-indole	2,4-diCl	0.070 ± 0.030	>100
Amphotericin B			0.120 ± 0.01	0.14 ± 0.04
Fluconazole			0.020 ± 0.001	—
Itraconazole			—	0.50 ± 0.10

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6. Synthesis of 2-(2,4-dichlorophenyl)-3-(1*H*-indazol-1-yl)-1-(1*H*-1,2,4-triazol-1-yl)propan-2-ol **6**. Potassium carbonate (0.87 g, 6.29 mmol), indazole (0.75 g, 6.29 mmol) and 2-(2,4-dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)-1,2-epoxypropane **4** (0.85 g, 3,15 mmol) were added in 20 mL of acetonitrile and stirred for 12 h at reflux. After cooling, the mixture was filtered and evaporated under vacuo. The residue was diluted with H_2O and extracted with dichloromethane. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated in vacuo. The residue was purified by silica gel column chromatography (ethanol/dichloromethane, 1:10) and **6** was obtained (16%) as a white powder. mp: 122–123 °C; ^{1}H NMR (DMSO-d_6): δ 8.34 (s, 1H), 8.02 (s, 1H), 7.78 (s, 1H), 7.73 (d, 1H, J = 7.3 Hz), 7.68 (d, 1H, J = 7.3 Hz), 7.62 (d, 1H, J = 2.1 Hz), 7.40 (dd, 1H, J = 7.3 Hz), 7.33 (d, 1H, J = 8.9 Hz), 7.21 (dd, 1H, J = 8.9 Hz, J = 2.1 Hz), 7.14 (dd, 1H, J = 7.3 Hz), 6.25 (s, 1H, OH), 5.35 (d, 1H, J = 14.3 Hz), 5.17 (d, 1H, J = 15.0 Hz), 5.03 (d, 1H, J = 15.0 Hz), 4.69 (d, 1H, J = 14.3 Hz); MS *m*/*z*: 387 (M^+), 242, 131, 82.
7. Synthesis of 3-(1*H*-7-azaindol-1-yl)-2-(2,4-dichlorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)propan-2-ol **14**. Sodium hydride (0.08 g, 3.33 mmol) was dissolved in DMSO (20 mL) and 1*H*-7-azaindole (0.39 g, 3.33 mmol) was added portionwise. After 1 h, 2-(2,4-dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)-1,2-epoxypropane **4** (0.75 g, 2.78 mmol) in 5 mL of DMSO was added, and the mixture was further stirred for 12 h under argon. The mixture was diluted with H_2O and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated in vacuo. The residue was purified by silica gel column chromatography (ethyl acetate/hexane, 1:1) and **14** was obtained (53%) as a white powder. mp: 144–146 °C; ^{1}H NMR (DMSO-d_6): δ 8.37 (s, 1H), 8.26 (d, 1H, J = 4.9 Hz), 7.97 (d, 1H, J = 7.9 Hz), 7.78 (s, 1H), 7.56 (d, 1H, J = 2.1 Hz), 7.46 (d, 1H, J = 3.5 Hz), 7.41 (d, 1H, J = 8.9 Hz), 7.17 (dd, 1H, J = 8.9 Hz, J = 2.1 Hz), 7.11 (dd, 1H, J = 7.9 Hz, J = 4.9 Hz), 6.43 (d, 1H, J = 3.5 Hz), 5.83 (s, 1H, OH), 5.22 (d, 1H, J = 14.3 Hz), 5.07 (d, 1H, J = 15.0 Hz), 4.96 (d, 1H, J = 15.0 Hz), 4.58 (d, 1H, J = 14.3 Hz); MS *m*/*z*: 387 (M^+), 242, 131, 82.
8. Synthesis of 2-(2,4-dichlorophenyl)-3-(1*H*-indol-5-ylamino)-1-(1*H*-1,2,4-triazol-1-yl)propan-2-ol **16**. Potassium carbonate (1.71 g, 12.37 mmol), 5-amino-1*H*-indole (0.82 g, 6.18 mmol) and 2-(2,4-dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)-1,2-epoxypropane **4** (1.67 g, 6.18 mmol) were added in 20 mL of DMF and stirred for 72 h at 90 °C. After cooling, the mixture was filtered. The residue was diluted with H_2O and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated in vacuo. The residue was purified by recrystallisation (methanol/chloroform, 1:3) and **16** was obtained (10%) as a brown powder. mp: 114–116 °C; ^{1}H NMR ($CDCl_3$): δ 10.68 (s, 1H, NH), 8.37 (s, 1H), 7.78 (s, 1H), 7.62 (d, 1H, J = 8.2 Hz), 7.58 (d, 1H, J = 1.8 Hz), 7.35 (dd, 1H, J = 8.2 Hz, J = 1.8 Hz), 7.17 (d, 1H, J = 3.7 Hz), 7.15 (d, 1H, J = 8.5 Hz), 6.75 (d, 1H, J = 1.8 Hz), 6.56 (dd, 1H, J = 8.5, 1.8 Hz), 6.23 (s, 1H, OH), 6.14 (d, 1H, J = 13.7 Hz), 5.10 (d, 1H, J = 14.7 Hz), 4.77 (d, 1H, J = 14.7 Hz), 3.86 (d, 1H, J = 13.7 Hz), 3.67 (d, 1H, J = 13.7 Hz); MS *m*/*z*: 402 (M^+).
9. Synthesis of 2-(2,4-dichlorophenyl)-3-(3-ethoxycarbonylmethyl-1H-indol-1-yl)-1-(1H-1, 2,4-triazol-1-yl)propan-2-ol **18**. Sodium hydride (0.22 g, 9.26 mmol) was dissolved in DMSO (30 mL) and 3-ethoxycarbonylmethyl-1H-indole (1.71 g, 8.41 mmol) was added portionwise. After 1 h, 2-(2,4-dichlorophenyl)-3-(1H-1,2,4-triazol-1-yl)-1,2-epoxypropane 4 (2.27 g, 8.41 mmol) in 10 mL of DMSO was added, and the mixture was further stirred for 12 h under argon. The mixture was diluted with H_2O and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated in vacuo. The residue was purified by silica gel column chromatography (ethyl acetate/hexane, 1:1) and 18 was obtained (13%) as a yellow powder. mp: 78–79 °C; ^{1}H NMR (DMSO-d_6): δ 8.32 (s, 1H), 7.79 (s, 1H), 7.63 (d, 1H, J = 2.1 Hz), 7.47 (d, 2H, J = 7.6 Hz), 7.40 (d, 1H, J = 8.5 Hz), 7.23 (dd, 1H, J = 8.5, 2.1 Hz), 7.20 (s, 1H), 7.14 (dd, 1H, J = 7.6 Hz), 7.02 (dd, 1H, J = 7.6 Hz), 6.39 (s, 1H, OH), 5.30 (d, 1H, J = 14.3 Hz), 4.84 (d, 1H, J = 15.3 Hz), 4.75 (d, 1H, J = 15.3 Hz), 4.56 (d, 1H, J = 14.3 Hz); 4.08 (q, 2H, J = 7.0 Hz); 3.70 (s, 2H); 1.23 (t, 3H, J = 7.0 Hz); MS *m*/*z*: 472 (M^+), 399, 327, 216, 82.
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