Synthesis and *In Vitro* Evaluation of Novel 1, 2, 4-Triazole Derivatives as Antifungal Agents

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Received July 22, 2009: Revised August 15, 2009: Accepted August 22, 2009

Abstract: Despite the advances in medicine and the emergence of new antifungal agents, fungal infections remain a significant cause of morbidity and mortality. Azoles are widely used as antifungal agents. Azoles interfere with the conversion of lanosterol to ergosterol by inhibiting a fungal cytochrome P450enzyme, lanosterol 14α-demethylase. Resistance to azoles, particularly fluconazole, is emerging to *Candida albicans*, after long-term suppressive therapy. Thus, there is an urgent need for newer potent antifungals to combat resistance developed against widely used azoles. In present work, we report synthesis of novel triazole derivatives of 7-hydroxy-4-methylcoumarin using various substituted aromatic aldehydes and evaluated for their *in vitro* fungicidal activity against *Candida albicans* at various concentrations to obtain minimum inhibitory concentration (MIC).

Keyword: Azole, Antifungal, Minimum inhibitory concentration, Coumarin, Triazole.

INTRODUCTION

Fungal infections remain a significant cause of morbidity and mortality despite advances in medicinal chemistry. Antifungal drug discovery has identified three classes of natural products (griseofulvin [1], polyenes [2] and echinocandins [3]) and four classes of synthetic chemicals (azoles [4], allylamines [5], flucytosine [6] and phenylmorpholines [7]) with clinical value against fungal infections. The azoles class of antifungal agent is chemically either an imidazole or a triazole group joined to an asymmetric carbon atom as their functional pharmacophore. They all work by blocking the active site of an enzyme variously known as lanosterol 14α-demethylase or cytochrome $P450_{DM}$ [8]. The affinity for P450 and the activity of the azole antifungals is not only determined by the affinity of the nitrogen for the heme iron, but also by that of the N-l substituent for the apoprotein moiety of P450 [9]. This affinity for the apoprotein not only determines the activity of the azole antifungal, but also its selectivity. The remaining part of the azole antifungal fits in the similar way like lanosterol in hydrophobic groove by interacting with Met-313and the P-methyl group of Thr-318 [10]. More than any other antifungal class, the azoles have been steadily refined and improved upon over the course of almost 50 years. The earliest azole for clinical use, chlormidazole, was really not a very good pharmaceutical due to its toxicity, but the ease with which variants on the chlormidazole chemical structure could be synthesized and tested led to steady progress with azole antifungal agents [11].

For the treatment of opportunistic fungal infections, the development of new potent and broad-spectrum antifungal agents is an important challenge for modern medicine, with a potent, broad spectrum of antifungal activity, good pharmacokinetics, and excellent bioavailability. It has been reported that serious infections caused by fungi are an increasing problem because of factors such as intensive care practices, human immunodeficiency virus infections, organ transplantation, and other immunosuppressive conditions [12]. Treatments for these infections are still limited to a few agents. Various azole antifungal agents ketoconazole, fluconazole, voriconazole and itraconazole (Fig. **1**), have been developed so far for clinical use. However, the clinical values of these agents have been limited by their relatively high risks of toxicity, the emergence of drug resistance, pharmacokinetic deficiencies, and/or insufficiencies in their antifungal activities. Resistance to azoles, particularly fluconazole, is emerging to *Candida albicans*, after long-term suppressive therapy was observed [7]. There is an urgent need for newer potent antifungals to combat resistance developed against widely used azoles. Thus, much effort to develop novel antifungal agents which are more safe and efficacious is still being made.

Coumarins are also known to show the antifungal activity. Naturally occurring angelicin (furanocoumarin) (Fig. **2**) and its various synthetic derivatives were already reported to have good antifungal activity [13].

Mouri *et al.* reported fifty-three new 3-(2-diethylaminoethyl)-4-methyl-7-substituted coumarins [14] which were synthesized by four different routes utilizing commercially available 3-(2-diethylaminoethyl)-7-hydroxy-4-methylcoumarin hydrochloride. Their antifungal activities were measured against phytopathologic fungi, *Botrytis cinerea*. Satyanarayana *et al.* reported the synthesis and antifungal screening of new schiff base 2-[(4-methyl-2-oxo-*2H*-chromen-7-yl)oxy]-N'-(substitutedmethylene) aceto hydrazides were under conventional and microwave conditions [15].

In the present work, we have used chemical hybridization approach and combined triazole moiety with coumarin. Coumarin and triazole derivatives have been considered to be an ideal requirement for exhibiting wide spectrum of antifungal activity. Thus it was considered of interest to synthesize novel coumarin derivatives having triazole moiety attached through a spacer.

EXPERIMENTAL

Materials and Method

All chemicals and solvents were obtained from commercial sources and purified using standard procedures whenever required. All melting points were recorded on Veego-540 melting point apparatus and are uncorrected. The structures of the compounds were

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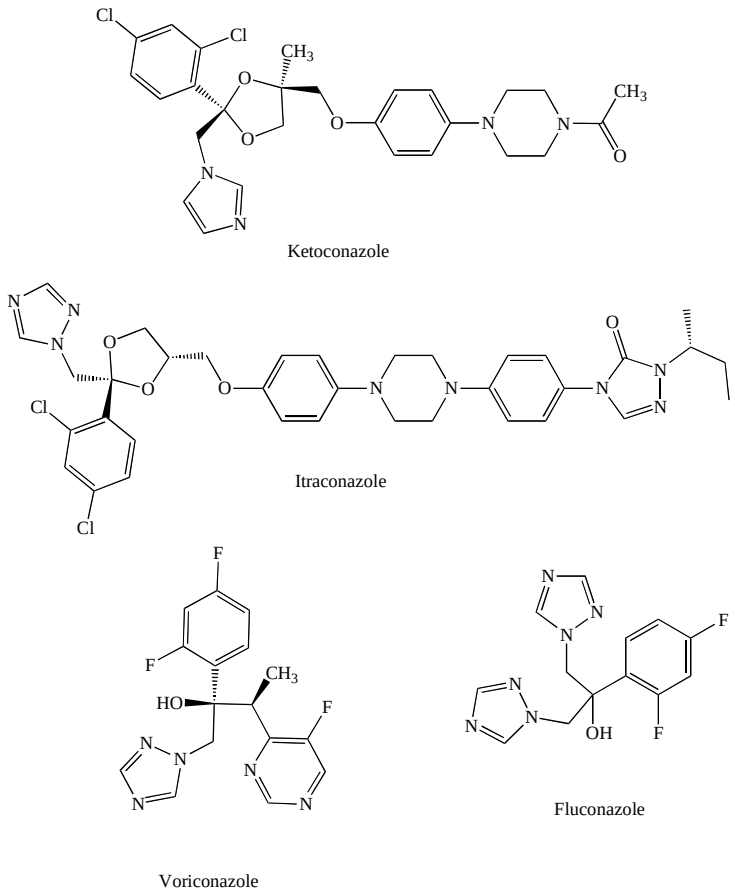


Fig. (1). Various azoles clinically used for treatment of fungal infection.

confirmed by IR and ^{1}HNMR spectra. IR spectra were recorded on JASCO V500 spectrometer using KBr pellets. ^{1}HNMR spectra were recorded on a Varian Mercury YH-300 MHz and using tetramethylsilane (TMS) as internal standard. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; b, broad. Elemental analysis has been carried out using Perkin–Elmer 2400 elemental analyzer. Thin layer chromatography (TLC) was performed on pre-coated aluminium sheets coated with Silica Gel 60 F254, 0.2 mm thickness.

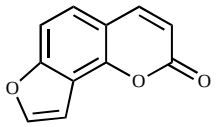


Fig. (2). Furanocoumarin.

Chemistry

In the first step, commercially available resorcinol was stirred with ethylacetoacetate in presence of conc. H_2SO_4 to form 7-hydroxy-4-methylcoumarin **(A)** [16,17]. 7-hydroxy-4-methylcoumarin **(A)** obtained from first step was refluxed with 1-bromo-3-chloropropane in presence of anhydrous potassium carbonate for twelve hours to afford 7-(3-chloropropoxy)-4-methyl-2H-chromen-2-one **(B)** which was condensed with 1,2,4-triazole to give **7**-(3-(1H-1,2,4-triazol-1-yl)propoxy)-4-methyl-2H-chromen-2-one **(C)**. Condensation of **7**-(3-(1H-1,2,4-triazol-1-yl)propoxy)-4-methyl-2H-chromen-2-one with various substituted aldehyde in presence of glacial acetic acid to afford final products **D-1** to **D-5**. The synthetic pathways have been illustrated in Scheme **1**.

Characterization Data

A) 7-hydroxy-4-methyl-coumarin [7-hydroxy-4-methyl-2H-chromen-2-one] (A)

Yield: 89%; m. p.: 183-185˚C; TLC [benzene: ethyl acetate:: 4 : 1] R_f: 0.45

IR (KBr; cm^{-1}): 3499, 3104, 2818, 1670, 1605, 1275. **^{1}H NMR peaks (δ, ppm, $CDCl_3$):** 3.92 (bs, 1H, Ar-O*H*), 7.43(d, 1H, Ar- *H*), 6.82(d, 1H, Ar- *H*) , 6.75(s, 1H, Ar- *H*), 6.03 (s, 1H, *H*C-C=O), 2.38 (s, 3H, C*H*$_3$). **Anal**.: Calcd. For $C_{10}H_8O_3$. C(68.18), H (4.58), O (27.25). Found: C (68.92), H (4.71), O (27.04).

B) 7-(3-chloropropoxy)-4-methyl-2H-chromen-2-one (B)

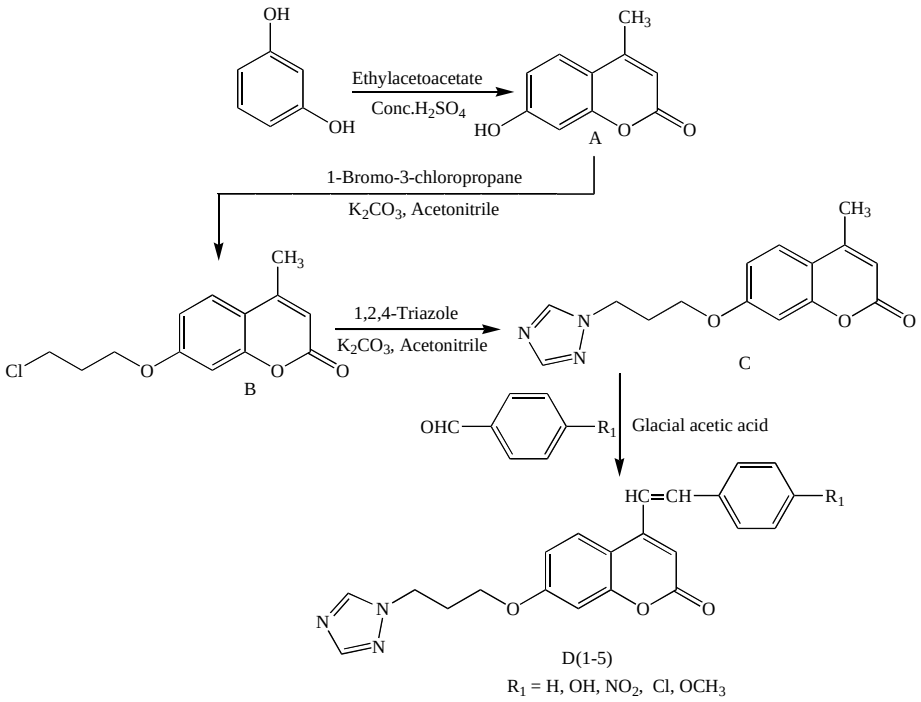
Yield: 82%; m. p.: 145-147˚C; TLC [benzene: ethyl acetate:: 4 : 1] R_f: 0.58

IR (KBr; cm^{-1}): 3093, 2959, 2887, 1678, 1611, 1293, 706. **^{1}H NMR peaks (δ, ppm, DMSO-d_6):** 7.53(d, 1H, Ar- *H*), 6.88(d, 1H, Ar- *H*) , 6.80(s, 1H, Ar- *H*), 6.09 (s, 1H, *H*C-C=O), 4.17 (t, 2H, C*H*$_2$-O) ,3.62 (t, 2H, C*H*$_2$-Cl), 2.36 (s, 3H, C*H*$_3$). 2.32 (m, 2H, C*H*$_2$). **Anal**.: Calcd. for $C_{13}H_{13}ClO_3$. C (61.79), H (5.19), O (18.99). Found: C (61.95), H (5.57), O (18.72).

C) 7-(3-(1H-1, 2, 4-triazol-1-yl) propoxy)-4-methyl-2H-chromen-2-one(C)

Yield: 79%; m. p.: 122-124˚C; TLC [benzene: ethyl acetate:: 4 : 1] R_f: 0.41

IR (KBr; cm^{-1}): 3064, 2849, 1678, 1601, 1326, 1274. **^{1}H NMR peaks (δ, ppm, DMSO-d_6):** 7.88(s, 2H, *H*C=N), 7.53(d, 1H, Ar- *H*), 6.91(d, 1H, Ar- *H*) , 6.85(s, 1H, Ar- *H*), 6.10 (s, 1H, *H*C-C=O), 4.27 (t, 2H, C*H*$_2$-O), 4.17 (t, 2H, C*H*$_2$-N) , 2.36 (s, 3H, C*H*$_3$). 2.32



Scheme 1. Synthesis of various 1, 2, 4-triazole derivatives.

(m, 2H, CH_2). **Anal**.: Calcd. for $C_{15}H_{15}N_3O_3$. C (63.15), H (5.30), O (16.82), N (14.73). Found: C (63.85), H (5.77), O (16.12), N (14.52).

D) (E)-7-(2-(1H-1, 2, 3-triazol-1-yl) propoxy)-4-styryl-2H-chromen-2-one (D-1)

Yield: 76%; m. p.: 110-112˚C; TLC [chloroform: methanol:: 4 : 1] R_f : 0.54

IR (KBr; cm^{-1}): 3082, 2857, 1684, 1609, 1326, 1282. **^{1}H NMR peaks (δ, ppm, DMSO-d_6):** 8.13(d,1H,C*H*=CHAr,J=15.9Hz), 7.92 (s, 2H, *H*C=N), 7.48(d, 1H, Ar- *H*), 7.27 (d, 2H Ar-*H*), 7.21 (m, 3H Ar-*H*)7.16 (d,1H, RCH=C*H*Ar,J=15.9Hz), 6.87(d, 1H, Ar- *H*) , 6.82(s, 1H, Ar- *H*), 6.10 (s, 1H, *H*C-C=O), 4.25 (t, 2H, CH_2-O), 4.21 (t, 2H, CH_2-N) , 2.31 (m, 2H, CH_2). **Anal**.: Calcd. for $C_{22}H_{19}N_3O_3$. C (70.76), H (5.13), O (12.85), N (11.25). Found: C (71.25), H (5.51), O (12.72), N (10.89).

E) (E)-7-(2-(1H-1, 2, 3-triazol-1-yl) propoxy)-4-(4-hydroxystyryl)-2H-chromen-2-one (D-2)

Yield: 81%; m. p: 98-100˚C; TLC [chloroform: methanol:: 4 : 1] R_f : 0.44

IR (KBr; cm^{-1}): 3420, 3084, 2892, 1684, 1610, 1326, 1274. **^{1}H NMR peaks (δ, ppm, DMSO-d_6):** 8.19(d,1H,C*H*=CHAr,J= 15.7Hz), 7.96 (s, 2H, *H*C=N), 7.49(d, 1H, Ar- *H*), 7.15(d,1H, RCH=C*H*Ar, J=15.7Hz), 7.10 (d, 2H Ar-*H*), 6.89(d, 1H, Ar- *H*) , 6.78(s, 1H, Ar- *H*), 6.62 (d, 2H Ar-*H*), 6.05 (s, 1H, *H*C-C=O), 4.62 (bs, 1H, Ar-O*H*), 4.19 (t, 2H, CH_2-O), 4.05 (t, 2H, CH_2-N) , 2.22 (m, 2H, CH_2). **Anal**.: Calcd. for $C_{22}H_{19}N_3O_4$. C (67.86), H (4.92), O (16.43), N (10.79). Found: C (67.39), H (4.78), O (16.76), N (10.54).

F) (E)-7-(2-(1H-1, 2, 3-triazol-1-yl) propoxy)-4-(4-nitrostyryl)-2H-chromen-2-one (D-3)

Yield: 84%; m. p.: 103-105˚C; TLC [chloroform: methanol:: 4 : 1] R_f : 0.46

IR (KBr; cm^{-1}): 3084, 2872, 1682, 1610, 1354, 1326, 1276. **^{1}H NMR peaks (δ, ppm, DMSO-d_6):** 8.24(d,1H,C*H*=CHAr, J=16.2Hz), 8.10 (d, 2H Ar-*H*), 7.94 (s, 2H, *H*C=N), 7.51(d, 1H, Ar- *H*), 7.45 (d, 2H Ar-*H*), 7.17(d,1H, RCH=C*H*Ar, J=16.2Hz), 6.88(d, 1H, Ar- *H*) , 6.76(s, 1H, Ar- *H*), 6.05 (s, 1H, *H*C-C=O), 4.24 (t, 2H, CH_2-O), 4.08 (t, 2H, CH_2-N) , 2.23 (m, 2H, CH_2). **Anal**.: Calcd. for $C_{22}H_{18}N_4O_5$. C (63.15), H (4.34), O (19.12), N (13.39). Found: C (63.39), H (4.64), O (19.31), N (13.30).

G) (E)-7-(2-(1H-1, 2, 4-triazol-1-yl) propoxy)-4-(4-chlorostyryl)-2H-chromen-2-one (D-4)

Yield: 77%; m. p.: 101-103˚C; TLC [chloroform: methanol:: 4 : 1] R_f : 0.48

IR (KBr; cm^{-1}): 3086, 2892, 1682, 1609, 1324, 1274, 722. **^{1}H NMR peaks (δ, ppm, DMSO-d_6):** 8.22(d,1H,C*H*=CHAr,J= 16.3Hz), 7.91 (s, 2H, *H*C=N), 7.49(d, 1H, Ar- *H*), 7.32 (d, 2H Ar-*H*), 7.28 (d, 2H Ar-*H*), 7.21(d,1H, RCH=C*H*Ar,J=16.3Hz), 6.92(d, 1H, Ar- *H*) , 6.79(s, 1H, Ar- *H*), 6.09 (s, 1H, *H*C-C=O), 4.26 (t, 2H, CH_2-O), 4.13 (t, 2H, CH_2-N) , 2.27 (m, 2H, CH_2). **Anal**.: Calcd. for $C_{22}H_{18}ClN_3O_3$. C (64.79), H (4.45), O (11.77), N (10.30). Found: C (64.98), H (4.57), O (11.72), N (10.55).

H) (E)-7-(2-(1H-1, 2, 4-triazol-1-yl) propoxy)-4-(4-methoxystyryl)-2H-chromen-2-one (D-5)

Yield: 75%; m. p.: 113-115˚C; TLC [chloroform: methanol:: 4 : 1] R_f : 0.68

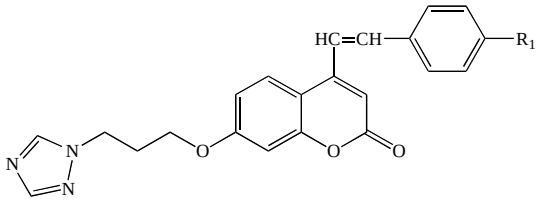
IR (KBr; cm^{-1}): 3084, 2892, 1686, 1610, 1326, 1292. **^{1}H NMR peaks (δ, ppm, DMSO-d_6):** 8.24(d,1H,C*H*=CHAr,J=16.1Hz), 7.95 (s, 2H, *H*C=N), 7.45(d, 1H, Ar- *H*), 7.21 (d,1H, RCH=C*H*Ar, J=16.1Hz), 7.11 (d, 2H Ar-*H*), 6.89(d, 1H, Ar- *H*) , 6.77(s, 1H, Ar-*H*), 6.92(d, 2H, Ar- *H*) , 6.03 (s, 1H, *H*C-C=O), 4.24 (t, 2H, CH_2-O), 4.05 (t, 2H, CH_2-N) , 3.79(s, 3H, CH_3-O) , 2.27 (m, 2H, CH_2). **Anal**.: Calcd. for $C_{23}H_{21}N_3O_4$. C (68.47), H (5.25), O (15.86), N (10.42). Found: C (69.10), H (5.82), O (15.22), N (10.73).

In-Vitro Antifungal Activity

All the compounds were screened for their *in vitro* antifungal activity against strain of *Candida albicans* ATCC 24433 (NCIM 3557) using tube dilution method [18] and the minimum inhibitory

concentration was determined by visual comparison with the positive and negative control tubes. A stock solution of the compound was prepared using dimethyl sulphoxide. To 2mL of sterile Sabourauds dextrose broth taken in a test tube, 10 to 80 µL of the stock solution was added, followed by a loopful of an authentic culture of *Candida albicans* ATCC 24433 (NCIM 3557). This corresponds to a concentration range of 12.5, 25, 37.5, 50, 75, 100, 125, 150 and 200 µg/mL of the compounds. The tests were carried out in duplicate. The tubes were incubated at 37(±1) °C and observed for growth at the end of 24 and 48 hours [19]. The activity of the compounds was determined by visual observation of the presence or absence of turbidity, used as a marker for indicating the growth of the organism. Minimum inhibitory concentration (MIC) was taken as the minimum concentration of the compound at which the clarity of the medium in the tube was the same as the negative control indicating complete inhibition of growth given in Table **1**.

Table 1. ***In-Vitro*** **Antifungal Activity**



Molecule Code	R_1	MIC (µg/mL)
D1	H	>200
D2	OH	25
D3	NO_2	12.5
D4	Cl	75
D5	OCH_3	37.5
	Ketoconazole	12.5

RESULT AND DISCUSSION

We have successfully synthesized novel compounds using combination of two functionalities active against various fungus strains. Novels triazole derivative with coumarin moiety along with different donor as well as acceptor functionalities were synthesized and characterized using ^{1}H-NMR, FT-IR and elemental analysis and evaluated *in vitro* against *Candida albicans* using ketoconazole as reference standard. All compounds except compound D-1 (MIC> 200(µg/mL) showed moderate antifungal activity while (D-3) with nitro substituent at *para*-position showed antifungal activity (MIC~12.5(µg/mL) comparable to that of ketoconazole.

CONCLUSION

Fungal infections remain a significant cause of morbidity and mortality despite advances in medicine and the emergence of new antifungal agents in the armamentarium against fungal infections. We have combined triazole as well as coumarin moiety together as both having antifungal activity in order to explore their antifungal activity. All compounds showed moderate antifungal activity against *Candida albicans* strain while compound D-3 showed activity comparable to ketoconazole. Present work may prove to be a lead for the development of new agents against resistant strain for the treatment of fungal infection.

ACKNOWLEDGEMENTS

We gratefully acknowledge Principal, Sinhgad Institute of Pharmaceutical Sciences and Director, GVM College of Pharmacy for providing necessary facilities to carry out research work

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