

NOVEL CHROMENEIMIDAZOLE DERIVATIVES AS ANTIFUNGAL COMPOUNDS: SYNTHESIS AND *IN VITRO* EVALUATION

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Over the past two decades a significant increase in fungal infections has been observed (1). More than any other antifungal class, the azoles have been steadily refined and improved upon over the course of almost 50 years. These antifungal compounds are chemically either an imidazole or a triazole group joined to carbon atom as their functional pharmacophore and working through blocking the active site of an enzyme variously known as lanosterol 14 α -demethylase or cytochrome P450_{DM} (2). More recently, a number of chlorinated imidazole compounds have been found to have antifungal as well as antibacterial activity. Among them, clotrimazole, miconazole, sulconazole and econazole have already been used in topical and in some cases systemic therapy. Ketoconazole is a newer dioxolane imidazole which shows promise as a broad-spectrum antifungal agent effective by oral administration (3–6). Imidazole derivatives, including ketoconazole, miconazole, tioconazole, clotrimazole, and sulconazole, are recognized as potent ligands of the heme iron atom of P450s (Fig. 1) (7, 8), thereby inhibiting synthesis of normal membrane sterols in fungi. A lack of ergosterol in a fungal membrane seriously cripples the fungus and leaves it unable to grow and develop in the normal way. Resistance to azoles is emerging to *Candida albicans*, after long-term suppressive therapy (9, 10). There is an urgent need for newer potent antifungals to combat resistance developed against widely used azoles. Thus, much effort is devoted to develop novel antifungal agents, which are more safe and efficacious.

Chromenes are naturally occurring benzopyrene derivatives known to exhibit wide spectrum of

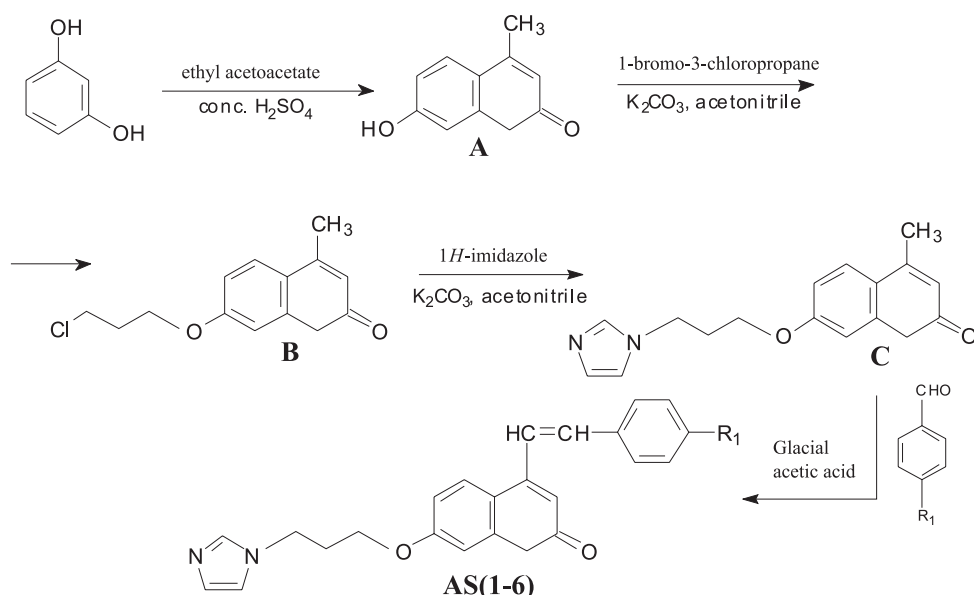
biological activities (11) like diuretic, analgesic myorelaxant (12) and antifungal (13). Naturally occurring angelicin (furanocoumarin) and its various synthetic derivatives were already reported to have good antifungal activity (14) Satyanarayana et al. reported the synthesis and antifungal screening of new Schiff base of chromenes under conventional and microwave conditions (15). It is suggested that chromene exhibit antifungal activity may be due to either killing the microbes or blocking their active sites (16).

In the present paper, we have reported synthesis of various chromeneimidazole derivatives using conventionally synthesized coumarin and further derivatized using various substituted aldehydes. Chromene and imidazole derivatives have been considered to be an ideal requirement for exhibiting wide spectrum of antifungal activity. Thus, it was considered of interest to synthesize chromene derivatives having imidazole moiety attached through a spacer bearing different acceptor as well as donor functionalities and their evaluation against resistant *Candida albicans* strain.

EXPERIMENTAL MATERIALS AND METHODS

All chemicals and solvents were obtained from commercial sources. Melting points were determined in an open capillary melting point apparatus and are uncorrected. The structures of the compounds were confirmed by the IR and proton NMR spectra. The IR spectra were recorded on a JASCO V500 spectrometer using KBr pellets. Proton NMR

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Scheme 1. Synthesis of various chromeneimidazole derivatives

spectra were recorded on a Varian Mercury YH-300 MHz and using tetramethylsilane (TMS) as the 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.

Chemistry

Commercially available resorcinol was stirred with ethylacetoacetate in the presence of conc. H_2SO_4 to form 7-hydroxy-4-methylcoumarin (**A**) (17). In the next step, 7-hydroxy-4-methylcoumarin was refluxed with 1-bromo-3-chloropropane in the presence of anhydrous potassium carbonate for 12 h to afford 7-(3-chloropropoxy)-4-methyl-2H-chromen-2-one (**B**) which was condensed with 1H-imidazole to give 7-[3-(1H-imidazol-1-yl)propoxy]-4-methyl-2H-chromen-2-one (**C**). Condensation of **C** with various substituted aldehydes in the presence of glacial acetic acid (18) yielded final products **AS-1** to **AS-6**. The synthetic pathways have been illustrated in Scheme 1.

7-Hydroxy-4-methylcoumarin (7-hydroxy-4-methyl-2H-chromen-2-one) (**A**)

The method of Pechmann and Duisberg was followed for the preparation of 7-hydroxy-4-methylchromen-2-one. Hundred mL of conc. H_2SO_4

was kept in an ice-bath. When temperature fell below 10°C , a solution of resorcinol (10 g, 0.091 mol) and ethyl acetoacetate (13 mL, 0.103 mol) was added with continuous stirring during 2 h. The temperature was maintained below 10°C throughout the addition. The reaction mixture was kept at room temperature for 18 h after which it was poured with vigorous stirring into the mixture of 200 g of crushed ice and 300 mL of distilled water. The precipitate was collected by vacuum filtration and washed with cold water (45 mL). The solid was dissolved in 150 mL of 5% NaOH, filtered, and 2 M H_2SO_4 (55 mL) was added to it with vigorous stirring until the solution was acidic. The crude coumarin was collected by filtration at the pump, washed with cold water and dried. The product was recrystallized from ethanol. Yield: 89%; m.p. $183\text{--}185^\circ\text{C}$; TLC [benzene: ethyl acetate 4 : 1, v/v] R_f : 0.45. IR (KBr, cm^{-1}): 3499 (Ar-OH), 3104 (aromatic C-H), 2818 (aliphatic C-H), 1670 (C=O), 1605 (aromatic C=C), 1275 (C-O). ^1H NMR (CDCl_3 , δ , ppm.): 3.92 (bs, 1H, Ar-OH), 7.43 (d, 1H, Ar-H), 6.82 (d, 1H, Ar-H), 6.75 (s, 1H, Ar-H), 6.03 (s, 1H, HC-C=O), 2.38 (s, 3H, CH_3). Analysis: for $\text{C}_{10}\text{H}_8\text{O}_3$ calcd.: C, 68.18; H, 4.58; O, 27.25%; found: C, 68.92; H, 4.71; O, 27.04%.

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

7-Hydroxy-4-methylchromen-2-one (1.76 g, 0.01 mol) was dissolved in 45 mL of acetonitrile.

To this solution, 1-bromo-3-chloropropane (0.99 mL, 0.01 mol) and anhydrous potassium carbonate (2.76 g, 0.02 mol) were added. The resulting reaction mixture was refluxed for 12 h. Acetonitrile was distilled off at the end of the reflux period; the reaction mixture was cooled and poured in 60 mL of iced water, and immediately the final compound was precipitated as solid crystals. They were filtered, washed with water, dried to afford crude product and recrystallized using methanol. Yield: 82%; m. p.: 145–147°C; TLC [benzene : ethyl acetate 4 : 1, v/v] R_f : 0.58. IR (KBr, cm^{-1}): 3093 (aromatic C-H), 2959, 2887 (aliphatic C-H), 1678 (C=O), 1611 (aromatic C=C), 1293 (C-O stretching), 706 (C-Cl). ^1H NMR (DMSO- d_6 , δ , ppm): 7.53 (d, 1H, Ar-H), 6.88 (d, 1H, Ar-H), 6.80 (s, 1H, Ar-H), 6.09 (s, 1H, HC-C=O), 4.17 (t, 2H, $\text{CH}_2\text{-O}$), 3.62 (t, 2H, $\text{CH}_2\text{-Cl}$), 2.36 (s, 3H, CH_3), 2.32 (m, 2H, CH_2). Analysis: for $\text{C}_{13}\text{H}_{13}\text{ClO}_3$ calcd.: C, 61.79; H, 5.19; O, 18.99%; found: C, 61.95; H, 5.57; O, 18.72%.

7-(3-[1H-imidazol-1-yl]propoxy)-4-methyl-2H-chromen-2-one (C)

7-(3-Chloropropoxy)-4-methyl-2H-chromen-2-one (1.87 g, 0.01 mol) (**B**) was dissolved in 45 mL of acetonitrile. To this solution, imidazole (0.68 g, 0.01 mol) and anhydrous potassium carbonate (2.76 g, 0.02 mol) were added. The resulting reaction mixture was refluxed for 32 h. Acetonitrile was distilled off at the end of the reflux period; the reaction mixture was cooled and poured in 60 mL of iced water. 7-[3-(1H-imidazol-1-yl)propoxy]-4-methyl-2H-chromen-2-one precipitated as solid crystals. The compound was filtered, washed with water, dried and recrystallized from methanol.

Yield: 80%; m. p.: 142–144°C; TLC [chloroform: methanol 4 : 1, v/v] R_f : 0.42. IR (KBr, cm^{-1}): 3061 (aromatic C-H), 2811 (aliphatic C-H), 1678 (C=O), 1605 (aromatic C=C), 1326 (C-N), 1274 (C-O). ^1H NMR (DMSO- d_6 , δ , ppm.): 7.58 (s, H, Ar-H), 7.51 (d, 1H, Ar-H), 6.98 (d, 2H, Ar-H), 6.91 (d, 1H, Ar-H), 6.85 (s, 1H, Ar-H), 6.10 (s, 1H, HC-C=O), 4.27 (t, 2H, $\text{CH}_2\text{-O}$), 4.17 (t, 2H, $\text{CH}_2\text{-N}$), 2.36 (s, 3H, CH_3), 2.32 (m, 2H, CH_2). Analysis: for $\text{C}_{16}\text{H}_{16}\text{N}_2\text{O}_3$ calcd.: C, 67.59; H, 5.67; N, 9.85%; found: C, 67.92; H, 5.77; N, 9.98%.

General procedure for synthesis of chromeneimidazole derivatives (AS 1-6)

7-[3-(1H-imidazol)propoxy]-4-methyl-2H-chromen-2-one (2.84 g, 0.01 mol) (**C**) was dissolved in 40 mL of glacial acetic acid. To this solution, various substituted aldehydes (0.01 mol) were added.

The resulting reaction mixture was refluxed for 48 h. At the end of reflux period; the reaction mixture was cooled and poured in 60 mL of iced water, when the final compounds (**AS 1-6**) precipitated as solid crystals. The compounds were filtered, washed with water, dried and recrystallized from methanol.

(E)-7-(3-(1H-imidazol-1-yl)propoxy)-4-styryl-2H-chromen-2-one (AS-1)

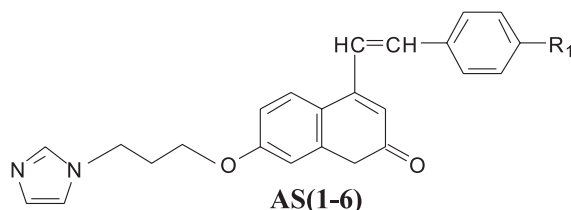
Yield: 76%; m. p.: 110–112°C; TLC [chloroform: methanol 4 : 1, v/v] R_f : 0.52. IR (KBr, cm^{-1}): 3084 (aromatic C-H), 2872 (aliphatic C-H), 1682 (C=O), 1605 (aromatic C=C), 1324 (C-N), 1278 (C-O). ^1H NMR (DMSO- d_6 , δ , ppm): 8.13 (d, 1H, $\text{CH}=\text{CHAr}$), 7.58 (s, 1H, Ar-H), 7.48 (d, 1H, Ar-H), 7.27 (d, 2H Ar-H), 7.21 (m, 3H Ar-H), 7.16 (d, 1H, $\text{RCH}=\text{CHAr}$), 6.98 (d, 2H, Ar-H), 6.87 (d, 1H, Ar-H), 6.82 (s, 1H, Ar-H), 6.10 (s, 1H, HC-C=O), 4.25 (t, 2H, $\text{CH}_2\text{-O}$), 4.21 (t, 2H, $\text{CH}_2\text{-N}$), 2.31 (m, 2H, CH_2). Analysis: for $\text{C}_{23}\text{H}_{20}\text{N}_2\text{O}_3$ calcd.: C, 74.18; H, 5.41; N, 7.52%; found: C, 74.56; H, 5.87; N, 7.77%.

(E)-7-(3-(1H-imidazol-1-yl)propoxy)-4-(4-hydroxystyryl)-2H-chromen-2-one(AS-2)

Yield: 72%; m. p.: 103–105°C; TLC [chloroform : methanol 4 : 1, v/v] R_f : 0.48. IR (KBr, cm^{-1}): 3420 (Ar-OH), 3084 (Ar-CH), 2892 (aliphatic C-H), 1682 (C=O), 1605 (aromatic C=C), 1326 (C-N), 1272 (C-O). ^1H NMR (DMSO- d_6 , δ , ppm.): 8.19 (d, 1H, $\text{CH}=\text{CHAr}$), 7.58 (s, 1H, Ar-H), 7.49 (d, 1H, Ar-H), 7.15 (d, 1H, $\text{RCH}=\text{CHAr}$), 7.10 (d, 2H Ar-H), 6.98 (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, HC-C=O), 4.62 (bs, 1H, Ar-OH), 4.19 (t, 2H, $\text{CH}_2\text{-O}$), 4.05 (t, 2H, $\text{CH}_2\text{-N}$), 2.22 (m, 2H, CH_2). Analysis: for $\text{C}_{23}\text{H}_{20}\text{N}_2\text{O}_4$ calcd.: C, 71.12; H, 5.19; N, 7.21%; found: C, 71.53; H, 5.49; N, 7.47%.

(E)-7-(3-(1H-imidazol-1-yl)propoxy)-4-(4-nitrostyryl)-2H-chromen-2-one(AS-3)

Yield: 73%; m. p.: 123–125°C; TLC [chloroform: methanol 4 : 1, v/v] R_f : 0.47. IR (KBr, cm^{-1}): 3086 (Ar-CH), 2878 (aliphatic C-H), 1684 (C=O), 1610 (aromatic C=C), 1552 (C-NO $_2$), 1326 (C-N), 1272 (C-O). ^1H NMR (DMSO- d_6 , δ , ppm): 8.24 (d, 1H, $\text{CH}=\text{CHAr}$), 8.10 (d, 2H Ar-H), 7.58 (s, 1H, Ar-H), 7.51 (d, 1H, Ar-H), 7.45 (d, 2H Ar-H), 7.17 (d, 1H, $\text{RCH}=\text{CHAr}$), 6.98 (d, 2H, Ar-H), 6.88 (d, 1H, Ar-H), 6.76 (s, 1H, Ar-H), 6.05 (s, 1H, HC-C=O), 4.24 (t, 2H, $\text{CH}_2\text{-O}$), 4.08 (t, 2H, $\text{CH}_2\text{-N}$), 2.23 (m, 2H, CH_2). Analysis: for $\text{C}_{23}\text{H}_{19}\text{N}_3\text{O}_5$ calcd.: C, 66.18; H, 4.59; N, 10.07%; found: C, 66.49; H, 4.93; N, 10.39%.

Table 1. *In-vitro* antifungal activity

Molecule Code	R ₁	MIC (μg/mL)
AS-1	4-H	100
AS-2	4-OH	125
AS-3	4-NO ₂	12.5
AS-4	4-Cl	25
AS-5	4-OCH ₃	>200
AS-6	4-F	12.5
Standard	Ketoconazole	12.5

(E)-7-(3-(1H-imidazol-1-yl)propoxy)-4-(4-chlorostyryl)-2H-chromen-2-one (AS-4)

Yield: 69%; m. p.: 117–120°C; TLC [chloroform: methanol 4 : 1, v/v] R_f: 0.42. IR (KBr, cm⁻¹): 3092 (Ar-C-H), 2884 (aliphatic C-H), 1684 (C=O), 1605 (aromatic C=C), 1326 (C-N), 1284 (C-O), 724 (C-Cl). ¹H NMR (DMSO-d₆, δ, ppm): 8.22 (d, 1H, CH=CHAr), 7.58 (s, 1H, Ar-H), 7.49 (d, 1H, Ar-H), 7.32 (d, 2H Ar-H), 7.28 (d, 2H Ar-H), 7.21 (d, 1H, RCH=CHAr), 6.98 (d, 2H, Ar-H), 6.92 (d, 1H, Ar-H), 6.79(s, 1H, Ar-H), 6.09 (s, 1H, HC-C=O), 4.26 (t, 2H, CH₂-O), 4.13 (t, 2H, CH₂-N), 2.27 (m, 2H, CH₂). Analysis: for C₂₃H₁₉ClN₂O₃ calcd.: C, 67.90; H, 4.71; N, 6.89%; found: C, 68.29; H, 4.97; N, 7.17%.

(E)-7-(3-(1H-imidazol-1-yl)propoxy)-4-(4-methoxystyryl)-2H-chromen-2-one(AS-5)

Yield: 65%; m. p.: 110–112°C; TLC [chloroform: methanol 4 : 1, v/v] R_f: 0.59. IR (KBr, cm⁻¹): 3068 (Ar-C-H), 2873 (aliphatic C-H), 1684 (C=O), 1610 (aromatic C=C), 1324 (C-N), 1288 (C-O). ¹H NMR (DMSO-d₆, δ, ppm): 8.24 (d, 1H, CH=CHAr), 7.58 (s, 1H, Ar-H), 7.45 (d, 1H, Ar-H), 7.21 (d, 1H, RCH=CHAr), 7.11 (d, 2H Ar-H), 6.98 (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, HC-C=O), 4.24 (t, 2H, CH₂-O), 4.05 (t, 2H, CH₂-N), 3.79 (s, 3H, CH₃-O), 2.27 (m, 2H, CH₂). Analysis: for C₂₄H₂₂N₂O₄ calcd.: C, 71.60; H, 5.51; N, 6.96%; found: C, 71.94; H, 5.87; N, 7.29%.

(E)-7-[3-(1H-imidazol-1-yl)propoxy]-4-(4-fluorostyryl)-2H-chromen-2-one(AS-6)

Yield: 67%; m. p.: 122–123°C; TLC [chloroform: methanol 4 : 1, v/v] R_f: 0.42. IR (KBr, cm⁻¹): 3082 (Ar-C-H), 2890 (aliphatic C-H), 1682 (C=O), 1609 (aromatic C=C), 1324 (C-N), 1274 (C-O), 742 (C-F). ¹H NMR (DMSO-d₆, δ, ppm): 8.25 (d, 1H, CH=CHAr), 7.58 (s, 1H, Ar-H), 7.50 (d, 1H, Ar-H), 7.32 (d, 2H Ar-H), 7.25 (d, 2H Ar-H), 7.20 (d, 1H, RCH=CHAr), 6.97 (d, 2H, Ar-H), 6.90 (d, 1H, Ar-H), 6.78 (s, 1H, Ar-H), 6.10 (s, 1H, HC-C=O), 4.25 (t, 2H, CH₂-O), 4.15 (t, 2H, CH₂-N), 2.28 (m, 2H, CH₂). Analysis: for C₂₃H₁₉FN₂O₃ calcd.: C, 70.76; H, 4.91; N, 7.18%; found: C, 71.11; H, 5.36; N, 7.32%.

***In vitro* antifungal activity**

Screening of finally synthesized derivatives (AS 1-6) for their *in vitro* antifungal activity against strain of *Candida albicans* ATCC 24433 (NCIM 3557) was carried out using tube dilution method and the minimum inhibitory concentration (MIC) and was determined by visual comparison with the positive and negative control tubes (19). A stock solution of the compound was prepared using dimethyl sulfoxide. To 2 mL 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 $37 \pm 1^\circ\text{C}$ and observed for growth at the end of 24 and 48 h. The activity of the compounds was determined by visual observation of the presence or absence of turbidity, used as a marker indicating the growth of the organism. 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.

RESULTS AND DISCUSSION

Novel chromeneimidazoles have been designed by combining two functionalities i.e., chromene and imidazole known to exhibit antifungal potency against various fungus strains. The above designed chromenimidazoles were synthesized and further derivatized using various substituted aldehydes bearing different donor as well as acceptor functionalities (AS 1-6) and characterized using $^1\text{H-NMR}$, FT-IR and elemental analysis. Finally the above synthesized derivatives were evaluated *in vitro* against *Candida albicans* using ketoconazole as a reference standard. All compounds exhibit moderate to good activity except compound AS-5 (MIC > 200 µg/mL) having *p*-methoxy substituent. Compounds AS-3 and AS-6 antifungal activity (MIC~12.5 µg/mL) was comparable to that of the reference standard ketoconazole.

CONCLUSION

We have successfully accomplished synthesis of novel chromeneimidazole derivatives (AS 1-6) using various substituted aldehydes and determined their antifungal activity. All compounds showed moderate to good antifungal activity against *Candida albicans* strain. The results indicate that the presence of electron withdrawing functionalities at *para* position results in compounds with higher potency, while electron donating substituents yielded lower activity. These results may prove to be a guideline for the design and optimization of new agents for the treatment of resistant fungal infection.

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