J Chem Biol (2017) 10:35–44
DOI 10.1007/s12154-016-0162-8

ORIGINAL ARTICLE

2-(chromon-3-yl)imidazole derivatives as potential antimicrobial agents: synthesis, biological evaluation and molecular docking studies

Shweta Sharma[1] · Vishal Sharma[1] · Gurpreet Singh[1] · Harpreet Kaur[2] · Shubham Srivastava[3] · Mohan Paul S. Ishar[1]

Received: 28 May 2016 / Accepted: 26 October 2016 / Published online: 16 November 2016

Abstract A series of novel 2-(chromon-3-yl)-4,5-diphenyl-1*H*-imidazoles (**4a**-**h**) were synthesized by one pot condensation of substituted 3-formylchromones (**1a**-**h**), benzil (2) and ammonium acetate (3) in refluxing acetic acid at 110 °C under N2 atmosphere. Allylation of compounds **4a**-**h** with allyl bromide in the presence of fused K_2CO_3 furnished *N*-allyl-2-(chromon-3-yl)-4,5-diphenyl-1*H*-imidazoles (**6a**-**h**). The synthesized compounds were characterized spectroscopically and evaluated for in vitro antimicrobial activity against various pathogenic bacterial and fungal strains by disc diffusion method. Compounds bearing electron withdrawing substituents such as *bromo* (**4f**) showed significant inhibitory activity against *S. cerevisiae* (MIC 1.4 μg/ml) and **4g** containing *chloro* substituent, displayed more inhibitory potential against *C. albicans* (MIC 1.5), as compared to the standard drugs. Compounds **6a** and **4c** exhibit remarkable inhibitory potential against *B. subtilis* with MIC 0.98 and 1.23, respectively. The time kill assay for active compound **6a** was performed by viable cell count (VCC) method to elucidate the microbicidal nature of 2-(chromon-3-yl)imidazoles. A molecular docking study of most active compounds with target 'lanosterol 14α-demethylase' (CYP51) was performed to unravel the mode of antifungal action.

Keywords 3-Formylchromones · 2-Chromonylimidazoles · Antibacterial activity · Antifungal activity · Molecular docking

Electronic supplementary material The online version of this article (doi:10.1007/s12154-016-0162-8) contains supplementary material, which is available to authorized users.

✉ Mohan Paul S. Ishar
mpsishar@yahoo.com

[1] Bio-organic and Photochemistry Laboratory, Department of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar, Punjab -143005, India

[2] Department of Microbiology, Guru Nanak Dev University, Amritsar, Punjab -143005, India

[3] Department of Pharmacy, School of Chemical Sciences and Pharmacy, Central University of Rajasthan, Ajmer, Rajasthan 305817, India

Introduction

Heterocyclics containing nitrogen and sulphur are found to possess a variety of biological activities [1–5]. Amongst them, imidazoles, pyrazoles, -1,2,3 and -1,2,4, dithiazoles are endowed with interesting biological activities, in particular, antimicrobial activity [6–11]. According to literature reports, clinically used azoles such as clotrimazole, miconazole and econazole containing imidazole nucleus exhibit significant antimicrobial spectrum though inhibition of enzyme lanosterol 14α-demethylase [12]. Various imidazole-based lead compounds such as 3-aryl-4-(4,5-diphenyl-1H-imidazol-2-yl)-1H-pyrazole [13], *N*-(4-aryl-*1H*-imidazol-2-yl)cinnamamide [14] and substituted 1-[2-(1*H*-imidazol-1-yl)acetyl]-2,6-diarylpiperidin-4-one [15] have also been screened for in vitro antibacterial and antifungal activities against various pathogenic microbial strains and displayed promising inhibitory potential.

At the same time, chromones of both synthetic and natural origin are also known to display valuable antimicrobial activity. For instance, 8-hydroxy-6-methyl-2,3-dihydrocyclopenta[b]chromen-1,9-dione, isolated from a *carpophilus* fungus and synthetic 2-phenylchromone derivative 2-benzo[1,3]dioxol-5-yl-7-benzyloxy-chromen-4-one have displayed valuable antifungal activity [16]. Another important synthetic chromone-based antifungal agent is *N*-isobutyl-2-(4-oxo-2-phenyl-4H-chromen-6-yl)-1H-benzoimidazole-5-

carboxamidine, which displays valuable antimycotic properties against *Candida albicans* and *Candida krusei* [17]. Chromone-based pyrazoles such as 3-hydroxy-2-(1-phenyl-3-aryl-4-pyrazolyl)chromones have also been reported as potential antifungal agents [18]. Earlier, we have reported chromone-based 1,2,3-dithiazoles as potent antimicrobial agents, which were also evaluated against number of cancer cell lines [11, 19]. In continuation to our earlier work and taking cognizance of the literature reports on bioactive imidazole- and chromone-based compounds, it was decided to synthesize chromone-based imidazoles and evaluate their in vitro antifungal and antibacterial activity. With the aim of rationalizing the antimicrobial data obtained from synthesized compounds, a molecular docking study is performed to understand the possible interaction of these compounds with fungus enzyme 'lanosterol 14α-demethylase' (CYP51).

Materials and methods

General information

Starting materials and reagents were purchased from commercial suppliers and used after further purification (crystallization/distillation). JEOL (300 MHz) NMR spectrometer was used to record the ^{1}H NMR and ^{13}C NMR (**75** MHz) spectra. Tetramethylsilane was used as the internal standard; chemical shifts are reported in ppm and *J* values are in Hertz. IR spectra of compounds were recorded on Shimadzu 8400S FT-IR spectrophotometer as thin film between KBr discs. Mass spectra were recorded on Bruker Dalonics Esquire 300 (ESI) mass spectrometer. Elemental analysis was carried out on a Thermoelectron EA-112 elemental analyser and are reported in percent abundance. In docking studies, the ligands were initially sketched in Maestro by using 2D-sketcher and prepared by using LigPrep. The prepared ligands were subjected to docking into respective protein grid using XP docking mode. After XP docking, the protein-ligand complexes were subjected to free energy analysis of the binding (ΔG) which was calculated using Prime MMGB/SA function.

Chemistry

*Synthesis of substituted 2-(chromon-3-yl) -4,5-diphenyl-1H-imidazoles (**4a**-**h**)*

A mixture consisting of benzil (2, 1.05 g, 5 mmol), substituted 3-formylchromones (**1a**-**h**, 5 mmol), and ammonium acetate (3, 0.385 g, 5 mmol) in 30 ml of glacial acetic acid was stirred at 110 °C under N_2 atmosphere.[20] The reaction was monitored by the TLC. After completion of reaction, the reaction mixture was allowed to stand at room temperature, followed by treatment with ice cold water and further extracted with chloroform, which was dried by adding anhydrous sodium sulphate, the residue obtained on removal of solvent by filtration under vacuum was purified by column chromatography, using neutral silica gel (60–120 mesh) and eluted with 12–14% ethyl acetate in hexane. The purified products (**4a**-**h**) were characterized spectroscopically (IR, ^{1}H and ^{13}C NMR, mass) and elemental analysis.

*Synthesis of N-allyl-2-(chromon-3-yl) -4,5-diphenyl-1H-imidazoles (**6a**-**h**)*

Substituted imidazoles (**4a**-**h**, 5 mmol) were dissolved in dry *N*,*N*-dimethylformamide (DMF, 10 ml); then, allylbromide was added along with fused potassium carbonate (K_2CO_3) in dry RBF fitted with moisture guard tube and reaction mixture were stirred overnight at room temperature for 24 h. The reaction mixture was filtered and collected filtrate further extracted with chloroform. The solid allylated products (6a-h) were obtained on concentrate the chloroform extract by vacuum distillation. The crude products were purified by column chromatography over silica gel (60–120 mesh) with 10–12% ethylacetate in hexane. The purified products were characterized spectroscopically (IR, ^{1}H and ^{13}C NMR, mass) and elemental analysis.

Spectroscopic data of compounds 4 a-h and 6a-h

*2-(Chromon-3-yl)-4,5-diphenyl-1H-imidazole (**4a**)*

Brownish yellow solid, (0.695 g, 79%); m.p. 160–165 °C; IR (KBr), ν_{max} (cm^{-1}) 3342, 1643 (C=O), 1462, 1174; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.18 (s, 1H, –NH), 8.30 (dd, 1H, J = 8.1 and 1.5 Hz, C5′–H), 7.97 (m, 2H, C7′–H and C2′–H), 7.75-7.61 (m, 3H, C6′–H, C8′–H and Ar–H), 7.58−7.20 (m, 9H, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$) 176.2 (C=O), 155.9 (q), 154.8 (Ar–CH), 139.8 (q), 134.8 (Ar–CH), 134.7 (Ar–CH), 134.0 (Ar–CH), 132.8 (q), 129.8 (Ar–CH), 128.9 (Ar–CH), 128.7 (q), 128.5 (Ar–CH), 128.2 (q), 127.6 (Ar–CH), 125.78 (Ar–CH), 125.76 (Ar–CH), 123 (q), 118 (Ar–CH), 114.5 (q); MS (ESI) m/z 365 $(M + H)^{+}$; analysis: calculated for ($C_{24}H_{16}O_2N_2$) C 79.11, H 4.43, 8.78, N 7.69%, found C 79.16, H 4.46, N 7.73%.

*2-(6-Methylchromon-3-yl)-4,5-diphenyl-1H-imidazole (**4b**)*

Brownish yellow solid, (0.744 g, 78%); m.p. 180–185 °C; IR (KBr), ν_{max} (cm^{-1}) 3364, 1640 (C=O), 1482, 1170; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.01 (s, 1H, –NH), 7.96 (dist d, 2H, J = 2.1 Hz, C5′–H & C2′–H), 7.49–7.32 (m, 6H, C3′–H & Ar–H), 7.30-7.14 (m, 6H, Ar–H), 1.21 (s, 3H, CH_3); MS (ESI) m/z

378 (M^+); analysis: calculated for ($C_{25}H_{18}O_2N_2$) C 79.35, H 4.79, N 7.40% found C 79.59, H 4.85, N 7.42%.

*2-(6-Chlorochromon-3-yl)-4,5-diphenyl-1H-imidazole (**4c**)*

Brownish yellow solid (0.840 g, 80%); m.p. 162–168 °C; IR (KBr), ν_{max} (cm^{-1}) 3364, 1645 (C=O), 1465, 1185; ^1H NMR δ 9.18 (s, 1H, –NH), 8.44 (d, 1H, *J* = 2.4 Hz, C5′–H), 7.99 −7.91 (m, 2H, C2′–H and Ar–H), 7.82 (dd, 1H, *J* = 9 and 2.4 Hz, C7′–H), 7.66-7.26 (m, 10H, C8′–H and Ar–H); MS (ESI) *m*/*z* 399.5 $(M + H)^+$; analysis: calculated for ($C_{24}H_{15}O_2N_2Cl$) C 72.27, H 3.79, N 7.02%, found C 72.36, H 3.84, N 7.05%.

*2-(6-Bromochromon-3-yl)-4,5-diphenyl-1H-imidazole (**4d**)*

Brownish yellow solid, (1.04 g, 82%); m.p. 135–140 °C; IR (KBr), ν_{max} (cm^{-1}) 3365, 1645 (C=O), 1462, 1174; ^1H NMR (300 MHz, $CDCl_3$) δ 9.19 (s, 1H, –NH), 8.44 (d, 1H, *J* = 2.4 Hz, C5′–H), 7.98 (m, 2H, C2′–H and Ar–H), 7.82 (dd, 1H, *J* = 2.4 and 9, C7′–H), 7.73-6.82 (m, 10H, C8′–H and Ar–H); MS (ESI) *m*/*z* 444 $(M + H)^+$; analysis: calculated for ($C_{24}H_{15}O_2N_2Br$) C 65.03, H 3.41, N 6.32%, found C 65.06, H 3.43, N 6.34%.

*2-(6-Fluorochromon-3-yl)-4,5-diphenyl-1H-imidazole (**4e**)*

Brownish solid, (0.809 g, 84%); m.p. 218–220 °C; IR (KBr), ν_{max} (cm^{-1}) 3365, 1647 (C=O), 1470, 1190; ^1H NMR (300 MHz, $CDCl_3$), δ 9.21 (s, 1H, -NH), 7.96 (dd, 1H, *J* = 8.4 and 3.3 Hz, C5′–H), 7.66–7.58 (m, 3H, C2′–H and Ar–H), 7.51–7.46 (m, 3H, C7′–H & Ar–H), 7.41–7.16 (m, 7H, C8′–H and Ar–H); MS (ESI) *m*/*z* 382 $(M + H)^+$; analysis: calculated for ($C_{24}H_{15}O_2N_2F$) C 75.38, H 3.95, N 7.83%, found C 75.35, H 3.91, N 7.79%.

*2-(7-Bromochromon-3-yl)-4,5-diphenyl-1H-imidazole (**4f**)*

Light brownish yellow solid, (1.02 g, 80%); m.p. 142–147 °C; IR (KBr), ν_{max} (cm^{-1}) 3364, 1645 (C=O), 1462, 1174; ^1H NMR (300 MHz, $CDCl_3$) δ 9.12 (s, 1H, –NH), 8.17 (d, 1H, *J* = 8.4 Hz, C5′–H), 7.97 (d, 1H, *J* = 8.4 Hz, C6′–H), 7.90 (m, 2H, C2′–H and Ar–H), 7.78 (s, 1H, C8′–H), 7.73-7.26 (m, 9H, Ar–H); MS (ESI) *m*/*z* 444 $(M + H)^+$; analysis: calculated for ($C_{24}H_{15}O_2N_2Br$) C 65.03, H 3.41, N 6.32%, found C 65.07, H 3.45, N 6.30%.

*2-(8-Chlorochromon-3′-yl)-4,5-diphenyl-1H-imidazole (**4g**)*

Brownish solid, (0.850 g, 81%); m.p. 157–163 °C; IR (KBr), ν_{max} (cm^{-1}) 3361, 1648 (C=O), 1460, 1175; ^1H NMR (300 MHz, $CDCl_3$) δ 9.24 (s, 1H, –NH), 8.22 (dist.dd, 1H, *J* = 8.1 and 1.2 Hz, C5′–H), 7.96 (dist.dd, 1H, *J* = 7.5 and 1.2 Hz, C7′–H), 7.79 (m, 2H, C2′–H and C6′–H), 7.66–7.11 (m,10H, Ar–H); MS (ESI) *m*/*z* 399 $(M + H)^+$; analysis: calculated for ($C_{24}H_{15}O_2N_2Cl$) C 72.27, H 3.79, N 7.02%, found C 72.33, H 3.85, N 7.04%.

*2-(6,8-Dichlorochromon-3-yl)-4,5-diphenyl-1H-imidazoles (**4h**)*

Brownish solid, (1.025 g, 83%); m.p. 173–177 °C; IR (KBr), ν_{max} (cm^{-1}) 3362, 1650 (C=O), 1470, 1180; ^1H NMR (300 MHz, $CDCl_3$) δ 9.22 (s, 1H, –NH), 8.11 (d, 1H, *J* = 2.4 Hz, C5′–H), 7.96 (m, 2H, C2′–H), 7.77 (d, 1H, *J* = 2.4 Hz, C7′–H), 7.66-6.90 (m, 9H, Ar–H); MS (ESI) *m*/*z* 433 $(M + H)^+$; analysis: calculated for ($C_{24}H_{14}O_2N_2Cl_2$) C 66.53, H 3.26, N 6.47%, found C 66.48 H 3.20, N 6.49%.

*N-Allyl-2-(chromon-3-yl)-4,5-diphenyl-1H-imidazole (**6a**)*

Brownish yellow solid (0.900 g, 60%); m.p. 135–140 °C; IR (KBr), ν_{max} (cm^{-1}) 2924, 1650 (C=O), 1468, 1165; ^1H NMR (300 MHz, $CDCl_3$) δ 7.83 (m, 1H, C5′–H), 7.58-7.53 (m, 2H, Ar–H and C2′–H), 7.49-7.11 (m, 12H, Ar–H), 5.73–5.63 (m, 1H, C2″–H), 4.95 (d, $1H_a$, *J* = 10.5, C3″–H), 4.67 (d, $1H_b$, *J* = 17.1, C3″–H), 4.54 (d, 2H, *J* = 5.1, C1″–H); ^{13}C NMR (75 MHz, $CDCl_3$), 174.7 (C = O), 157.7 (Ar–CH), 152.4 (q), 139.6 (q), 134.3 (Ar–CH), 133.7 (Ar–CH), 131.3 (Ar–CH), 131.0 (q), 130.8 (Ar–CH), 129.2 (Ar–CH), 128.9 (Ar–CH), 128.8(q), 128.7 (Ar–CH), 128.1 (Ar–CH), 126.7 (Ar–CH), 126.4 (q), 125.4 (Ar–CH), 122.9 (q), 120.5 (q),111.5 (CH_2), 47.2 (CH_2); MS (ESI) *m*/*z* 405.1 $(M + H)^+$; analysis: calculated for ($C_{27}H_{20}O_2N_2$) C 80.18 H 4.98, N 6.93%, found C 80.25, H 5.01, N 6.98%.

*N-Allyl-2-(6-methylchromon-3-yl) -4,5-diphenyl-1H-imidazole (**6b**)*

Yellowish semisolid (0.75 g, 50%); IR ($CHCl_3$), ν_{max} (cm^{-1}) 2947, 1647 (C=O), 1469, 1169; ^1H NMR (300 MHz, $CDCl_3$) δ 8.01 (d, 1H, *J* = 2.4 Hz, C5′–H), 7.86 (d, 1H, *J* = 7.2 Hz, C8′–H), 7.74 (m, 2H, C2′–H and Ar–H), 7.55-7.12 (m, 10H, Ar–H), 5.78-5.66 (m, 1H, C2″–H), 5.13 (d, $1H_a$, *J* = 11.1 Hz, C3″–H), 4.87 (d, $1H_b$, *J* = 17.4 Hz, C3″–H), 4.47 (dist.d, 2H, *J* = 2.4 Hz, C1″–H), 1.20 (s, 3H, CH_3); MS (ESI) *m*/*z* 419 $(M + H)^+$; analysis: calculated for ($C_{28}H_{22}O_2N_2$) C 80.36, H 5.30, N 6.69%, found C 80.85, H 5.31, N 6.67%.

*N-Allyl-2-(6-chlorochromon-3-yl)-4,5-diphenyl-1H-imidazole (**6c**)*

Light yellowish semisolid (0.96 g, 64%); IR ($CHCl_3$) ν_{max} (cm^{-1}) 2968, 1645 (C=O), 1466, 1178; ^1H NMR (300 MHz, $CDCl_3$) δ 8.42 (d, 1H, *J* = 2.1 Hz, C5′–H), 7.74 (dd, 1H, *J* = 2.1 and 8.1 Hz, C7'–H), 7.59-7.06 (m, 12H, C2′–H,

C8′–H and Ar–H), 5.77–5.68 (m, 1H, C2″–H), 5.13 (d, $1H_a$, J = 10.2 Hz, C3″–H), 4.85 (d, $1H_b$, J = 17.7 Hz, C3″–H), 4.45 (dist.d, 2H, J = 4.2 Hz, C1″–H); MS (ESI) m/z 439 $(M + H)^+$; analysis: calculated for ($C_{27}H_{19}O_2N_2Cl$) C 73.89, H 4.36, N 6.38%, found C 73.85, H 4.34, N 6.33%.

*N-Allyl-2-(6-bromochromon-3-yl) -4,5-diphenyl-1H-imidazole (**6d**)*

Light yellowish semisolid (0.885 g, 59 %); IR ($CHCl_3$), ν_{max} (cm^{-1}) 2957, 1645 (C=O), 1469, 1170; ^1H NMR (300 MHz, $CDCl_3$) δ 8.24 (d, 1H, J = 2.4, C5′–H), 7.74 (m, 2H, C2′-H and Ar–H), 7.64 (dd, 1H, J = 2.4 and 8.7, C7′–H), 7.57-7.01 (m, 10H, C8′–H and Ar–H), 5.96–5.09 (m, 1H, C2″–H), 5.32-4.84 (m, 2H, C3″–H), 4.47-4.41 (m, 2H, C1″–H); MS (ESI) m/z 484 $(M + H)^+$; analysis: calculated for ($C_{27}H_{19}O_2N_2Br$) C 67.09, H 3.96, N 5.80%, found C 67.11, H 3.93, N 5.78%.

*N-Allyl-2-(6-fluorochromon-3-yl)-4,5-diphenyl-1H-imidazole (**6e**)*

Brownish yellow solid, (0.855 g, 57%); m.p. 110–114 °C; IR (KBr), ν_{max} (cm^{-1}) 2978, 1649 (C=O), 1470, 1168; ^1H NMR (300 MHz, $CDCl_3$) δ 8.44-8.32 (m, 2H, C5′–H and C2′–H), 7.74-7.65 (m, 1H, C7′–H), 7.54-7.35 (m, 3H, C8′–H and Ar–H), 7.31-7.16 (m, 8H, Ar–H), 5.20 (m, 1H, C3″–H), 5.77-5.60 (m, 1H, C2″–H), 5.02-4.93 (m, 1H, C3″–H), 4.69-4.99 (m, 2H, C1″–H); MS (ESI) m/z 423.1 $(M + H)^+$; analysis: calculated for ($C_{27}H_{19}O_2N_2F$) C 76.76, H 4.53, N 6.63%, found C 76.74, H 4.50, N 6.59%.

*N-Allyl-2-(7-bromochromon-3-yl) -4,5-diphenyl-1H-imidazole (**6f**)*

Brownish yellow semisolid, (0.93 g, 62%); IR ($CHCl_3$), ν_{max} (cm^{-1}) 2956, 1645 (C=O), 1469, 1170; ^1H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, 1H, J = 7.4 Hz, C5′–H), 7.53 (d, 1H, J = 7.4 Hz, C6′–H), 7.43-7.13 (m, 12H, Ar–H), 5.78-5.69 (m, 1H, C2″–H), 5.14 (d, $1H_a$, J = 10.8, C3″–H), 4.87 (d, $1H_b$, J = 17.1, C3″–H), 4.47 (dist.d, 2H, J = 2.1, C1″–H); MS (ESI) m/z 484 $(M + H)^+$; analysis: calculated for ($C_{27}H_{19}O_2N_2Br$) C 67.09, H 6.96, N 5.08%, found C 67.14, H 7.01, N 5.15%.

*N-Allyl-2-(8-chlorochromon-3′-yl) -4,5-diphenyl-1H-imidazole (**6g**)*

Brownish yellow semisolid, (0.915 g, 61%); IR (KBr), ν_{max} (cm^{-1}) 2937, 1646 (C=O), 1471, 1167; ^1H NMR (300 MHz, $CDCl_3$) δ 7.74 (dd, 1H, J = 0.8 and 7.2 Hz, C5′–H), 7.53 (m, 1H, C6′–H), 7.47-7.10 (m, 12H, Ar–H), 5.79-5.66 (m, 1H, C2″–H), 5.13 (d, $1H_a$, J = 10.5 Hz, C3″–H), 4.86 (d, $1H_b$, J = 17.1 Hz, C3″–H), 4.46 (dist d, 2H, J = 2.1 Hz, C1″–H); MS (ESI) m/z: analysis 439 $(M + H)^+$; calculated for ($C_{27}H_{19}O_2N_2Cl$) C 73.89, H 4.36, N 6.38% found C 73.93, H 4.40, N 6.42%.

*N-Allyl-2-(6,8-dichlorochromon-3′-yl) -4,5-diphenyl-1H-imidazole (**6h**)*

Light yellowish semisolid, (0.900 g, 60%); IR ($CHCl_3$) ν_{max} (cm^{-1}) 2937, 1646 (C=O), 1471, 1167; ^1H NMR (300 MHz, $CDCl_3$) δ = 8.08 (d, 1H, J = 2.1 Hz, C5′–H), 7.87-7.77 (m, 3H, C2′–H, C7′–H and Ar–H), 7.69-7.21 (m, 9H, Ar–H), 5.75-5.69 (m, 1H, C2″–H), 5.32-4.64 (m, 2H, C3″–H), 4.54-4.32 (m, 2H, C1″–H); MS (ESI) m/z 472 $(M + 1)^+$; analysis: calculated for ($C_{27}H_{18}O_2N_2Cl_2$) C 68.51, H 3.83, N 5.92%, found C 68.49, H 3.81, N 5.88%.

Antibacterial activity

In vitro antibacterial studies of various compounds (**4a**-**h** and **6a**-**h**) were carried out against Gram negative and Gram positive bacterial strains by disc diffusion assay [21]. The Gram positive strains used were *Staphylococcus aureus* (MTCC 96) and *Bacillus subtilis* (MTCC 2451), and Gram negative bacteria were *Escherichia coli* (MTCC 82) and *Pseudomonas aeruginosa* (MTCC 2642). The activity of compounds was determined in comparison to standard antibiotic discs of amoxicillin (5 μg) and ciprofloxacin (10 μg). Pre-warmed Mueller-Hinton agar plates were inoculated with 10^6 CFU/ml of test bacteria. Each compound was dissolved in DMSO (1 mg/ml) and then 30 μL of each was pipetted onto sterile paper discs (6-mm diameter) placed on the surface of inoculated agar plates. Plates were incubated at 37 °C for 24 h. Activity was expressed as the diameter of the inhibition zone (mm) produced by the compounds. DMSO was used as negative control. MIC of compounds exhibiting considerable activity was evaluated by using serial tube dilution method [22]. The initial optical density (OD) of the medium was measured by spectrophotometer at 600 nm. The test strains were incubated in nutrient broth until the OD reached 0.4–0.6. Different concentrations of synthesized compounds (0.78–500 μg/ml) were tested for the inhibition of growth of these microbes, in separate tubes. The 10-ml tubes each containing 5-ml nutrient broth and 1 ml of varied concentrations of compounds was incubated for 24 h with shaking at 180 rpm using a rotary shaker. Each tube corresponding to different concentrations was observed and concentration showing apparently no turbidity was considered to be the MIC of respective compound. Active compounds were further evaluated against bacterial resistant strains such as methecillin resistant *S. aureus* (MRSA), a clinically isolate obtained from PGIEMR-Chandigarh by using disc diffusion assay. Minimum inhibitory concentration (MIC) in milligram per millilitre of

compounds exhibiting activity was determined by using serial tube dilution method.

Antifungal activity

The antifungal activity of synthesized compounds against *Aspergillus niger* (MTCC 1344), *Saccharomyces cerevisiae* (MTCC 172), *C. albicans* (MTCC 3018) and *Cryptococcus gastricus* (MTCC 1715) was performed by disc diffusion method [21] by observing the zone of inhibition in comparison to the standard antifungal discs (fluconazole and griseofulvin). Test compounds were dissolved in DMSO to make a stock solution of 1 mg/ml. The fresh sub culture of strains in normal saline was added to the sterile assay medium (Saboraud dextrose agar with chloramphenicol) at 40–45 °C and mixed well. The medium was poured into each of the Petri dishes. Sterile discs of diameter 6 mm were placed on the medium. 20 µl of each test solution was added to the previously marked discs and the media was allowed to stand for 5 min. The petridishes were kept aside for 1 h and then incubated at 28 °C for 48 h. Zone of inhibition was measured and the average of the three readings were calculated; DMSO was also used as negative control. The MIC of active compounds (zone of inhibition) was determined by serial tube dilution method [22]. Different dilutions of test compounds (0.9–500 µg/ml) were made from stock solution and 1-ml nutrient broth was taken in each test tube and 20 µl of standard strains were added to previously marked test tubes.

Results and discussions

Substituted 3-formylchromones (**1a**-**h**) were reacted with benzil (**2**) and ammonium acetate (**3**) in refluxing glacial acetic acid with stirring for 4–5 h, [20] leading to the formation of 2-(chromon-3-yl)-4,5-diphenyl-1*H*-imidazoles (**4a**-**h**, Scheme 1, Table 1), which were purified by column chromatography and characterized spectroscopically. Compounds (**4a**-**h**) were further reacted with allylbromide (**5**) in the presence of base i.e. potassium carbonate (fused K_2CO_3) in dry *N*,*N*-dimethylformamide (DMF) with overnight stirring at room temperature. After completion of reaction (TLC), the reaction mixture was extracted with chloroform and solid allylated products (**6a-h**) were obtained on concentrate the chloroform extract by vacuum distillation. The products were further purified by column chromatography, using neutral silica gel (pH~7, 60–120 mesh) and eluted with 1:10 chloroform in hexane, and characterized by rigorous spectroscopic techniques (IR, NMR and mass) and elemental analysis.

The assigned structures of 2-(chromon-3-yl)imidazoles are based on the detailed spectroscopic analysis. The ^{1}H NMR spectrum of compound (**4a**) showed doublet of doublets of the chromone ring proton C5′–H at δ 8.30 and signal of –NH of imidazole ring appeared as singlet at δ 9.18, which was further corroborated through a sharp band at 3342 cm^{-1} in its IR spectrum. ^{13}C NMR spectrum of **4a** showed resonance at δ 176.2 ppm attributed to carbonyl group of chromone ring, which was further confirmed by IR spectrum through band at 1643 cm^{-1}. The mass spectrum of **4a** showed peak at *m*/*z* 365 $(M + H)^+$. The ^{1}H NMR spectrum of allylated compound (**6a**) showed resonances at δ 5.73–5.63 (m, 1H, C2″–H), 4.95 (d, 1H$_a$, J = 10.5, C3″–H), 4.67 (d, 1H$_b$, J = 17.1, C3″–H) and 4.54 (d, 2H, J = 5.1, C1″–H) for allylic protons. ^{13}C NMR spectrum of compound **6a** showed resonance at δ 174.7 ppm attributed to carbonyl group of chromone ring, which is further confirmed by its IR spectrum through band appeared at 1650 cm^{-1}; its mass spectrum showed the molecular ion peak at *m*/*z* 405.1 $(M + H)^+$, which corresponds to the molecular formula of **6a**.

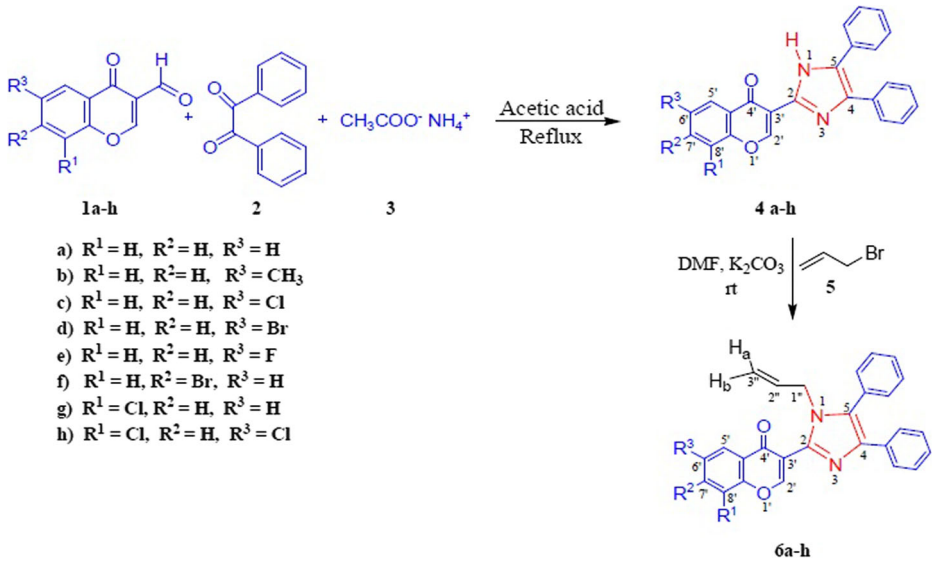
Antimicrobial activity

Antibacterial activity

In vitro antibacterial studies of various compounds (**4a**-**h** and **6a**-**h**) were carried out against Gram negative and Gram positive bacterial strains by disc diffusion assay [21]. The Gram positive strains used were *S. aureus* (MTCC 96) and *B. subtilis* (MTCC 2451) and Gram negative bacteria were *P. aeruginosa* (MTCC 2642) and *E. coli* (MTCC 82). The minimum inhibitory concentration (MIC, µg/ml) is the lowest concentration of a chemical that prevents visible growth of a bacterium in microgram per millilitre of active compounds (Table 2) was determined by using serial tube dilution method [22].

Almost all synthesized compounds (**4a**-**h** and **6a**-**h**) were found to be active against all investigated pathogenic bacterial strains. In the case of *S. aureus*, compound **6a** exhibits highest antibacterial potential with MIC 1.32, followed by compound **4c** and **6d** with MIC 2.25 and compound **4e** showed MIC 4.5. Compound **6a** displayed significant inhibitory potential with MIC 0.98 against *B. subtilis*, followed by compound **4c**,**e** and **6b**,**e**,**g**, which displayed MIC <5. In the case of *P. aeruginosa* compound **4c** and **6c** displayed valuable antibacterial activity with MIC 3.5 and 3.25, respectively. Compound **6a** and **6c** showed promising inhibitory activity against *E. coli* with MIC 1.12 and 1.5, respectively, followed by compound **4e** and **6g**, which showed MIC 3.6. Active compounds **4c**, **4e**, **4h**, **6a**-**e** and **6g** were further evaluated against methicillin resistant *S. aureus* (MRSA), a clinical isolate obtained from PGIEMR-Chandigarh; by using disc diffusion assay. Compounds were found to be active against methicillin resistant *S. aureus* (MRSA). Minimum inhibitory concentration (MIC) in milligram per millilitre of active compounds (Table 3) was determined by using serial tube dilution method [22].

Scheme 1 Synthesis of 2-(chromon-3-yl)imidazoles **4a-h** and **6a-h**



a) $R^1 = H$, $R^2 = H$, $R^3 = H$
b) $R^1 = H$, $R^2 = H$, $R^3 = CH_3$
c) $R^1 = H$, $R^2 = H$, $R^3 = Cl$
d) $R^1 = H$, $R^2 = H$, $R^3 = Br$
e) $R^1 = H$, $R^2 = H$, $R^3 = F$
f) $R^1 = H$, $R^2 = Br$, $R^3 = H$
g) $R^1 = Cl$, $R^2 = H$, $R^3 = H$
h) $R^1 = Cl$, $R^2 = H$, $R^3 = Cl$

Antifungal activity

All synthesized compounds (**4a-h** and **6a-h**) were tested against four reference fungal strains such as *S. cerevisiae (*MTCC 172), *Cryptococcus gastricus* (MTCC 1715), *Candida albicans* (MTCC 3018) and *A. niger* (MTCC 1344), by disc diffusion method [21]. Minimum inhibitory concentration (MIC) in microgram per millilitre of compounds exhibiting activity (Table 4) was determined by using serial tube dilution method [22].

The compounds evaluated were found to exert prominent antifungal activity against pathogenic fungal strains. Compound **4f** showed significant inhibitory activity against *S. cerevisiae* with MIC 1.4, whereas, compounds **4d** displayed inhibitory activity with MIC 4.5, followed by compound **6a** with MIC 6.5. In the case of *C. gastricus*, compounds **4d** and **4f** showed good activity with MIC 11.2 and 11.7, respectively. Compound **4g** exhibits potent inhibitory potential against *C. albicans* with MIC 1.5, followed by compound **4e** with MIC 6.6. In the case of *A. niger*, compounds **4f, 6h** and **6a** showed maximum inhibitory activity with MIC 2.5, 2.8 and 3.4, respectively, followed by **6b** with MIC 6.6. From the obtained in vitro antimicrobial results, it was observed that substitution of electron withdrawing groups at C-6 position of chromone ring leads to increase in both antifungal and antibacterial activity and substitution at other than C-6 of the chromone ring

Table 1 Reaction time (h) and yield (%) of various purified products (**4a-h** and **6a-h**)

Comp. no.	Time (h)	Yield (%)	Comp. no.	Time (h)	Yield (%)
4a	4	79	6a	24	60
4b	4	78	6b	24	50
4c	5	80	6c	24	54
4d	5	82	6d	24	59
4e	4	83	6e	24	57
4f	4	80	6f	24	62
4 g	4	81	6 g	24	61
4 h	4	83	6 h	24	60

Table 2 MIC (μg/ml) of compounds **4a-h** and **6a-h** against various bacterial strains

Comp. no	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*E. coli*
4a	17	25	13	19
4b	78	>100	56	>100
4c	2.25	1.23	3.5	5.54
4d	25.5	45	>100	>100
4e	4.5	2.33	6.3	3.6
4f	–	25	–	>100
4g	>100	35	68	–
4h	25	3.12	12.5	6.25
6a	1.32	0.98	5.5	1.12
6b	12.2	2.5	>100	7.5
6c	7.25	4.52	3.24	1.5
6d	2.25	10.4	5.4	7.6
6e	6.7	2.7	19	5.5
6f	–	>100	45	67
6g	5.7	2.5	6.7	3.6
6h	24	18	16.4	65
Amoxicillin	0.6	0.4	1.1	0.11
Ciprofloxacin	1.1	0.78	1.6	0.8

Table 3 MIC values (mg/ml) of active compounds against methicillin resistant *Staphylococcus aureus* (MRSA)

Compound no.	4c	4e	4h	6a	6b	6c	6d	6e	6g
MIC	30	15	12	20	5	10	10	2	20

showed good antifungal but decreased antibacterial activity. Chromone moiety has also been substituted with electron donating group, i.e. $–CH_3$, which resulted in less inhibitory potential against both bacterial and fungal strains. The observed antimicrobial results also revealed compounds **4a**-**h** having –NH group replaced by *N*-allyl do not give any significant results against fungal strains vis-à-vis against bacterial strains.

Time kill assay

In vitro time kill assay determines the rate and extent of antimicrobial activity and provides more accurate description of antimicrobial agents than the MIC. Such studies also elucidate the microbicidal or microstatic action of antimicrobials and provide a relative indication of the potential in vivo activity of new agents, which can be further useful pharmaceutically. In the present study, the time kill analysis for most active compound **6a** was performed by viable cell count method (VCC) [23]. The 4h grown inoculums were adjusted to 0.5 McFarland standards and serially diluted to 10^{-3} with suitable double strength broth medium. Each diluted inoculums of 0.1 ml was mixed with equal volume of compound **6a** (stock solution 1 mg/ml of compound **6a** was prepared) and incubated at respective temperature of 25 °C for yeast and 37 °C for bacteria. At different time intervals viz. 0, 1, 2, 3, 4…24 h, 0.1 ml of the mixed suspension was spread on suitable agar plates in duplicate and incubated for 24 h at suitable temperature. The mean number of colonies were determined and compared with control, whereas the compound was replaced with DMSO [24].

The results of time kill assay (Fig. 1) revealed that compound **6a** shows complete killing of *E. coli* at 0 h of incubation, whereas *C. albicans* and MRSA got completely killed at 2 h. However, *S. aureus*, *B. subtilis* and *C. gastricus* took 10 h for complete killing. Similarly, killing of *S. cerevisiae* took 8 h and *P. aeruginosa* 12 h of incubation. The microbicidal nature was further confirmed as no re-growth occurred in any of the microorganisms even after 24 h of incubation.

Table 4 MIC (μg/ml) of compounds **4a**-**h** and **6a**-**h** against various fungal strains

Comp. no	*S. cerevisiae*	*C. gastricus*	*C. albicans*	*A. niger*
4a	45	54	12.5	34
4b	>100	–	89	–
4c	26.6	57	19.5	14.2
4d	4.5	11.2	17.6	12.4
4e	10.9	–	6.6	–
4f	1.4	11.7	8.2	2.5
4g	22.5	45	1.5	13.5
4h	68.5	34.6	22.4	71.7
6a	6.5	12.6	11.2	3.4
6b	10.9	54	31.2	6.6
6c	>100	>100	47.4	–
6d	78.5	85	57	>100
6e	34	>100	22	15.9
6f	>100	58.5	67	71.4
6g	>100	>100	61.5	34
6h	6.7	13.6	11.7	2.8
Fluconazole	1.8	3.1	3.7	1.7
Griseofulvin	1.9	1.8	1.8	1.9

Molecular docking studies

Molecular docking studies are routinely used to understand the atomistic level details of plausible protein-ligand interactions and to identify potential ligands against disease target of interest. As discussed above, the chromone-based dithiazoles showed good antifungal activity [11], and in the present work, the dithiazole ring has been replaced with imidazole. To gain the better understanding on potency of active compounds, molecular docking studies were carried out to check the possible interactions of designed molecules with molecular target 'lanosterol 14α-demethylase' (CYP51) which is the same target for clinically used azoles such as clotrimazole, miconazole and econazole containing imidazole nucleus [12]. Therefore, we have predicted the binding mode of best three compounds **4f**, **6a** and **D** to in silico modelled 3D structure of *S. cerevisiae* (CYP51, PDP ID 4ZE1) by using Schrödinger software

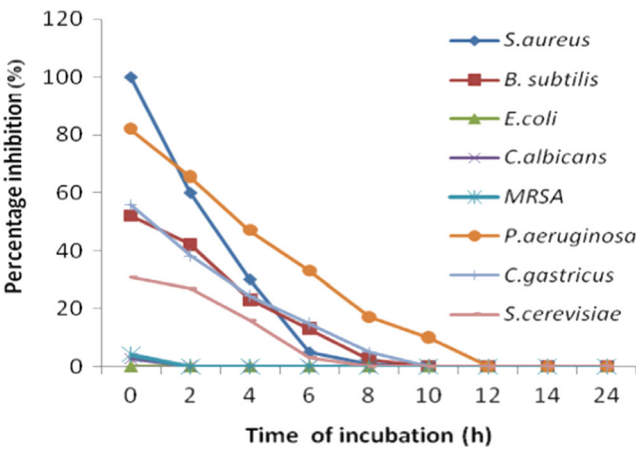


Fig. 1 Time kill assay curve of compound **6a**

Table 5 Docking study reports of the synthesized compounds on *Saccharomyces cerevisiae* (CYP51)

S. No	Ligand name	Dock score (binding affinity)[a]	MM-GBSA score (binding energy)[a]
1	4f	−4.132	−94.559
2	6a	−3.165	−90.576
3	D	−8.684	−81.969

[a] Binding affinity and binding energy are in kcal/mol

package [25–30] and docking studies carried out by using GLIDE module. The target protein crystal structures were retrieved from Protein data bank (ID: 4ZE1) and subjected to protein preparation wizard facility under default conditions implemented in Maestro v10.4 and Impact subprogram v5.5 (Schrodinger, Inc., New York, NY, 2009). Further, the complex was subjected to minimization by OPLS force fields 2005. The prepared protein was further utilized to construct grid file by targeting the active site residues involved in binding of the co-crystallized ligand as centroid of grid box. The ligands were initially sketched in Maestro using 2D-sketcher and were prepared using LigPrep. The prepared ligands were subjected to Docking into respective protein grid using XP docking mode. After XP docking, the protein-ligand complexes were subjected to free energy analysis of the binding (ΔG) which was calculated using Prime MMGB/SA function.

Based on insights provided by molecular modelling and biological studies, it was found that compound **4f** which is having MIC value of 1.4 μg/ml better than fluconazole possessed high affinity towards 'lanosterol 14α-demethylase' (CYP51) because of compound **4f** suitably bound to active site with favourable interactions of HEM 601 and TYR126 (휋-stacking) with binding energy −94.554 kcal/mol (Table 5). The allylated compound **6a** showed similar 휋- interactions with HEM601 and PHE236 instead of TYR126; this may be due to the protection of *N* with *allyl* group which may hinder the interaction with TYR126. Chromone-based dithiazole (**D**) possessed significant interaction with TYR126 instead of HEM601 and has the binding energy −81.969 kcal/mol. Observations from this binding model revealed that residues in the active sites play important role; interaction with both TYR126 and HEM601 showed effective 'lanosterol 14α-demethylase'(CYP51) inhibition as in compound **4f** (Figs.2, 3).

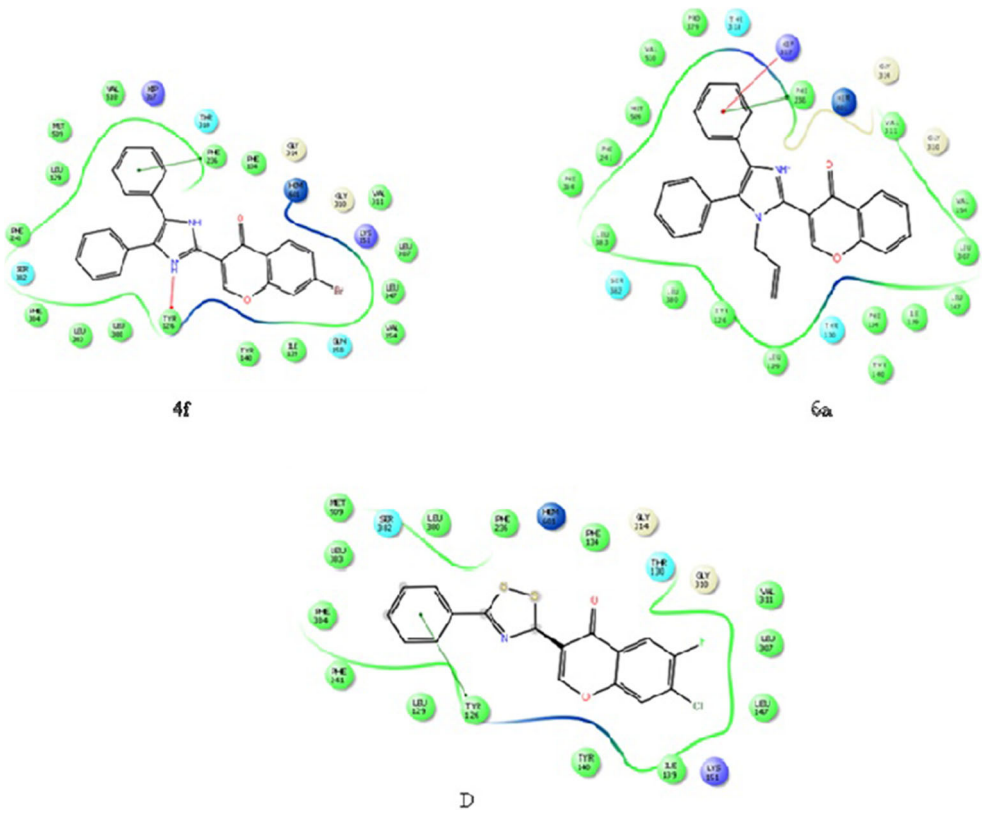


Fig. 2 2D models of compounds in active site *Saccharomyces cerevisiae* (CYP51): **4f**, **6a** and **D** (chromone-based dithiazole)[11]

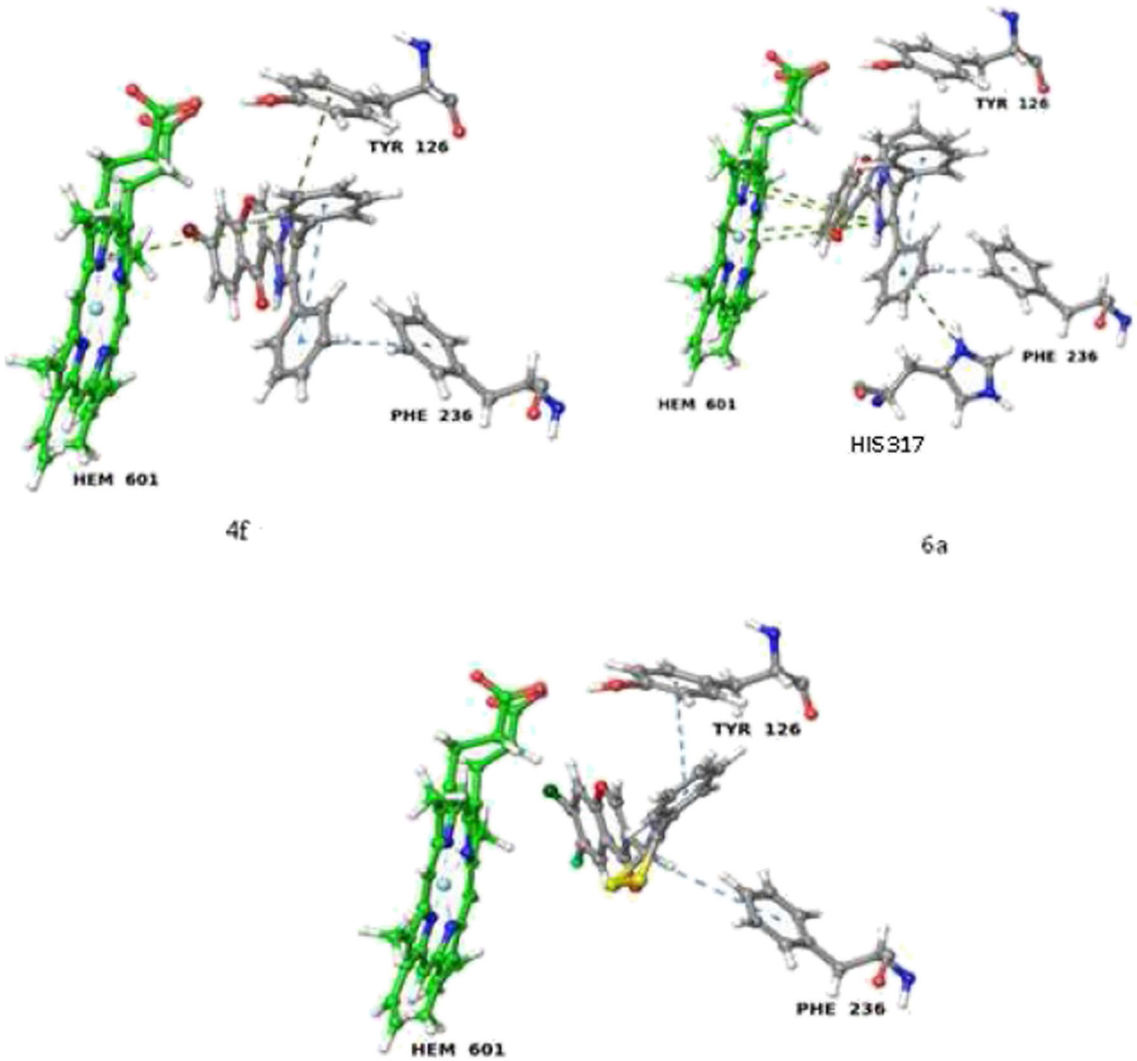


Fig. 3 3D models of compounds **4f**, **6a** and chromone-based dithiazole (**D**) in active site of *Saccharomyces cerevisiae* (CYP51)

Conclusions

A series of 2-(chromon-3-yl)imidazoles (**4a**-**h** and **6a**-**h**) were synthesized and characterized by spectroscopic techniques. The synthesized compounds were evaluated for in vitro antimicrobial activity against various pathogenic bacterial and fungal strains. Compounds **6a** and **4c** exhibit excellent inhibitory activity against *B. subtilis* with MIC 0.98 and 1.23, respectively, whereas compound **6a** displays significant inhibitory activity against *E. coli* with MIC 1.12 and followed by **6c** with MIC 1.5. In the case of antifungal activity, compounds such as **4f** and **4g** display more potent inhibitory activity against *S. cerevisiae* and *C. albicans* as compared to the standard drugs with MIC 1.4 and 1.5. Antimicrobial activities also revealed that compounds bearing electron withdrawing groups are more potent than compounds bearing electron releasing group. The results of time kill analysis of most active compound **6a** showed the microbicidal nature of 2-chromonylimidazoles. From binding mode of these derivatives, it was concluded that the role of TYR126 and HEM601 interactions may be considered for effective inhibition of 'lanosterol 14α-demethylase'(CYP51). These 'lead' compounds can be considered for investigation of their mode of action and for further development.

Acknowledgements We sincerely express our thanks to the University Grant Commission (UGC) for providing support and funds to our university to generate necessary research facilities.

References

1. Smith HJ (2006) Introduction to the principles of drug design and action. fourth ed. Taylor Francis,Boca Raton, pp 567−614
2. Goodman LS, Gilman AG (2001) The pharmacological basis of therapeutic, tenth ed. McGraw-Hill, New York, Sec. VIII, pp 1141–1310
3. Laura G, Marinella R, Daniela P (2007) Quinones: a class of potential selective antitumor agents. Mini Rev Med Chem 7:481−489

4. Mascaretti OA (2003) Bacteria versus antibacterial agents: an integrated approach, ASM Press, Washington, p 321
5. Bakavoli M, Nikapour M, Rahimizadeh M, Saberi MR, Sadeghian H (2007) Design and synthesis of pyrimido[4,5-b][1,4]benzothiazine derivatives, as potent 15-lipoxygenase inhibitors. Bioorg Med Chem 15:2120−2126
6. Misono M (2001) Unique acid catalysis of heteropoly compounds (heteropolyoxometalates) in the solid state. Chem Commun 1141−1152.
7. Black JW, Durant GJ, Emmett JC, Ganellin CR (1974) Sulphur-methylene isosterism in the developent of metiamide, a new histamine H2-receptor antagonist. Nature 248:65−67
8. Antolini M, Bozzoli A, Ghiron C, Kennedy G, Rossi T, Ursini A (1999) Analogues of 4,5-bis(3,5-dichlorophenyl)-2-trifluoromethyl-1H-imidazole as potential antibacterial agents. Bioorg Med Chem Lett 9:1023−1028
9. Ucucu U, Karaburun NG, Iskdag II (2001) Synthesis and analgesic activity of some 1-benzyl-2-substituted-4,5-diphenyl-1H-imidazole derivatives. Farmaco 56: 285−290
10. Kathiravan MK, Salake AB, Chothe AS, Dudhe PB, Watode RP, Mukta MS, Gadhwe S (2012) The biology and chemistry of antifungal agents: A review. Bioorg Med Chem 20:5678−5698
11. Raj T, Bhatia RK, Sharma RK, Gupta V, Sharma D, Ishar MPS. (2009) Mechanism of unusual formation of 3-(5-phenyl-3H-[1,2,4]dithiazol-3-yl)chromen-4-ones and 4-oxo-4H-chromene-3-carbothioic acid N-phenylamides and their antimicrobial evaluation. Eur J Med Chem 22:3209−3216
12. Mohan H (2010) Medicinal chemistry textbook, 10th ed. pp 519−521
13. Vijesh AM, Isloor AM, Telkar S, Peethambar SK, Rai S, Isloor N (2011) Synthesis, characterization and antimicrobial studies of some new pyrazole incorporated imidazole derivatives. Eur J Med Chem 46:3531−3536
14. Padmavathi V, kumara CP, Venkatesh BC, Padmaja A (2011) Synthesis and antimicrobial activity of amido linked pyrrolyl and pyrazolyl-oxazoles, thiazoles and imidazoles. Eur J Med Chem 46: 5317−5326
15. Ramachandran R, Rani M, Senthan S, Jeong YT, Kabilan S (2011) Synthesis, spectral, crystal structure and in vitro antimicrobial evaluation of imidazole/benzotriazole substituted piperidin-4-one derivatives. Eur J Med Chem 46:1926−1934.
16. Turner WB, Aldridge DC (1983) Fungal metabolites–II, Acedamic Press Inc., New York, p 152
17. Alam S (2004) Synthesis, antibacterial and antifungal activity of some derivatives of 2-phenylchromen-4-one. J Chem Sci 116:325−331
18. Prakash O, Kumar R, Parkash V (2008) Synthesis and antifungal activity of some new 3-hydroxy-2-(1-phenyl-3-aryl-4-pyrazolyl) chromones. Eur J Med Chem 43:435−440
19. Raj T, Bhatia RK, Kapur A, Sharma M, Saxena AK, Ishar MPS (2010) Cytotoxic activity of 3-(5-phenyl-3H-[1,2,4]dithiazol-3-yl)chromen-4-ones and 4-oxo-4H-chromene-3-carbothioic acid Nphenylamides. Eur J Med Chem 45:790−794
20. (a) Wang J, Mason R, Vanderveer D, Feng K, Bu XR (2003) Convenient preparation of a novel class of Imidazo[1,5-a]pyridines: cecisive role by ammonium acetate in chemoselectivity. J Org Chem 68:5415-5418 (b) Ingle VN, Hatzade KM, Taile VS, Gaidhane PK, Kharche ST (2007) Synthesis of O-β-d-glucopyranosides of 7-hydroxy-3-(imidazol-2-yl)-4H-chromen-4-ones. J Carbohydr Chem 26:107−123
21. Mitic-Ćulafic D, Vukovic-Gacic B, Knezevic-Vukcevic J, Stankovic S (2005) Comparative study on the antibacterial activity of volatiles from sage (Salvia officinalis L.). Arch Biol Sci 57:173−178
22. Hindler J, Mahon CC, Manuselis G (Eds.) (1995) Textbook of diagnostic micro-biology special antimicrobial susceptibility tests 89
23. Kaur GJ, Arora DS (2008) Emergence of highly fluoroquinolone-resistant Salmonella enterica serovar Typhi in a community-based fever surveillance from Kolkata. Int J Antimicrob Ag 31:380−399
24. Arora DS, Onsare JG (2014) In vitro antimicrobial evaluation and phytoconstituents of Moringa oleifera pod husks. Industrial Crops and Prod 52:125−135
25. (a) Pore VS, Jagtap MA, Agalave SG, Pandey AK, Siddiqi MI, Kumar V, Shukla PK (2012) Synthesis and antifungal activity of 1,5-disubstituted-1,2,3-triazole containing fluconazole analogues. Med Chem Commun 3:484-488. (b) Sagatova AA, Keniya MV, Wilson RK, Sabherwal M, Tyndall JD, Monk, BC (2016) Triazole resistance mediated by mutations of a conserved active site tyrosine in fungal lanosterol 14 alpha-demethylase. Sci Rep 6: 26213−26213
26. Schrodinger, Schrodinger Release 2015−4: Schrodinger Suite2015 Protein Preparation Wizard, Epik version 2.5, Schrodinger, LLC, New York, NY, 2013; Impact version 6.0, Schrodinger, LLC, NewYork, NY 2015, Prime version 3.3, Schrodinger, LLC, New York, NY, 2015
27. Jorgensen WL, Maxwell DS, TiradoRives J (1996) Development and testing of the OPLS all-atom force field on conformational energetics and properties of organic liquids. J Am Chem Soc 118: 11225−11236
28. Schrodinger, Schrödinger Release 2015−4: Maestro, version 10.4, Schrödinger, LLC, New York, NY, 2015
29. Schrödinger, Schrödinger Release 2015−4: LigPrep, version 2.7, Schrödinger, LLC, New York, NY, 2015
30. Friesner R A, Murphy RB, Repasky MP, Frye LL, Greenwood JR, Halgren TA, Sanschagrin PC, Mainz, DT (2006) Extra precision glide: docking and scoring incorporating a model of hydrophobic enclosure for protein−ligand complexes. J Med Chem. 49:6177−6196