Imidazole-thiazole coupled derivatives as novel lanosterol 14-α demethylase inhibitors: ionic liquid mediated synthesis, biological evaluation and molecular docking study

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Anna Pratima G. Nikalje, Shailee V. Tiwari, Aniket P. Sarkate & Kshipra S. Karnik

Abstract

A novel series of imidazole-thiazole coupled derivatives (7a–7q) were synthesized using Green protocol and identified by different spectroscopic techniques. The synthesized derivatives (7a–7q) were evaluated for their in vitro antifungal activity against the six fungi strains. The compounds 7j and 7k exhibited the most promising antifungal activity. The

compound 7k exhibited extremely high antifungal activity against *C. albicans*, *C. glabrata*, *F. oxysporum*, *A. flavus*, *A. niger*, and *C. neoformans* with MIC_{80} values of 0.2, 0.2, 20, 35, 40, and 5 µg/ml respectively. The mode of action of the most promising antifungal compounds 7j and 7k was established by ergosterol extraction and quantitation assay. From the ergosterol extraction and quantitation assay it was found that the compounds 7j and 7k act by inhibition of ergosterol biosynthesis in *C. albicans*. The molecular docking study revealed the high spontaneous binding ability of the tested compounds to the active site of lanosterol 14α-demethylase, which proves that the tested compounds inhibit the synthesis of lanosterol 14α-demethylase. The synthesized compounds were analyzed for ADMET properties to establish oral drug like behavior and shows satisfactory results. To establish the antifungal selectivity and safety, the most active compounds were further tested for cytotoxicity against human cancer cell lines HeLa and K-562 and were found to be non-cytotoxic in nature. The in vivo acute oral toxicity study was performed for the most active compounds and results indicate that the compounds are non-toxic in nature.

Introduction

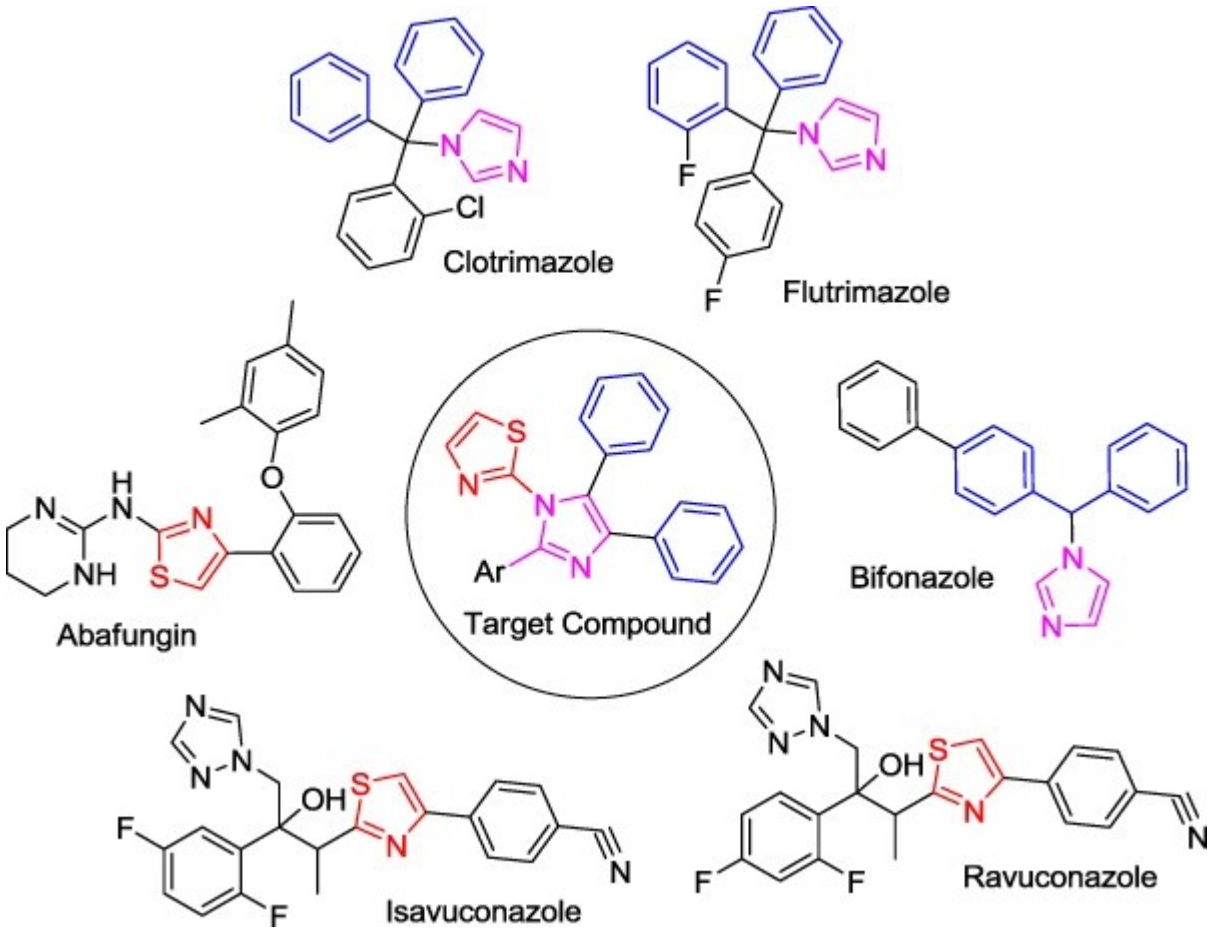
Invasive fungal infections lead to at least 1.5 million human deaths worldwide each year (Pianalto and Alspaugh 2016; Brown et al. 2012). Invasive fungal infections represent a sober threat to the health of the human beings. Fungi such as molds within the genera *Aspergillus*, *Fusarium*, and *Scedosporium*; yeast within the genera *Candida* and *Cryptococcus*, and the members of the order Mucorales are associated with invasive fungal infections in humans. There has been a noteworthy increase in the number of fungal species which are associated with invasive fungal diseases in humans over the past two decades. Factors that have contributed to a significant increase in invasive fungal infections, includes an increase in the number of patients receiving immunosuppressive chemotherapy for malignancies, HIV–AIDS patients and solid organ transplant recipients (Albataineh et al. 2016).

In comparison with the development of new antibacterial drugs, antifungal drug development is more challenging because fungi are eukaryotes and many potential targets for therapy are also found in humans with substantial host toxicity risk (Roemer and Krysan 2014; Denning and Bromley 2015). Therefore, all these observations have emphasized the urgent need for search of new, more effective class of safer antifungal agents. The novel and potent antifungal agents can be obtained by the structural modification or optimization of the existing agents, while minimizing the side effects.

In the search for potential antifungal agents, significant efforts have been focused on the development of scaffolds containing heterocyclic structures as their key structural design. Such heterocyclics, Imidazole is a privileged fragment in modern medicinal chemistry considering its broad spectrum and affinity towards various biological targets (Kathiravan et al. 2012; Zhai and Lin 2011). Imidazoles are the important scaffolds and have been the mainstay of the antifungal armamentarium (Kathiravan et al. 2012; Zhai and Lin 2011). Azole antifungal drugs act by competitive inhibition of cytochrome P450 14α-demethylase, a necessary enzyme which is required in the process of biosynthesis of ergosterol, which is the primary sterol in the cell membrane of the fungi. The structures of the some clinically pivotal imidazole-based antifungal drugs are shown in Fig. 1. Clotrimazole, Flutrimazole, Bifonazole, Miconazole, Ketoconazole, Econazole, Fenticonazole, etc are the antifungal drugs with active imidazole scaffold present in their structure. Abafungin, Isavuconazole, Ravuconazole, etc are

the thiazole scaffold containing clinically pivotal antifungal drugs with good antifungal activity. The widespread use of currently available antifungal drugs and the increasing fungal resistances to the available antifungal drugs have largely undermined their therapeutic effects. Thus, in quest of novel imidazoles which is more effective, less toxic in nature, and fewer resistances remain to be a highly formidable task and has aroused the great interest among the researchers for the search of novel antifungal agents (Zhou and Mi 2009; Lamberth et al. 2013). From the literature survey, it is predictable that, thiazole represents indispensable pharmacophore, and plays a paramount role as a medicinal agent (Turan-Zitouni et al. 2005; Shiradkar et al. 2007; Tsuruoka et al. 1997, 1998). A degree of respectability has been bestowed for thiazole derivatives due to their wide range of biological activities such antifungal, antitumor, anticonvulsant, cardiotonic, analgesic, etc (Gu et al. 1999; Medime and Capan (1994; Franchetti et al. 2005). The designing protocol for the synthesis of target compounds is presented in Fig. 1.

Fig. 1



Designing protocol for the target compound

The amalgamation of two dissimilar bioactive pharmacophores made into a single molecule is a successful and frequently used approach in modern medicinal chemistry for the exploration of the novel and highly active compounds which may have synergistic effect on biological properties (Meunier 2008). Therefore, owing to the importance of Imidazole and thiazole scaffold and our interest in the synthesis of biologically imperative frameworks with medicinal potential (Nikalje et al. 2015; Tiwari et al. 2017a, b; Nimbalkar et al. 2016), the present report explicates the synthesis of a series of 2-(2-(substituted phenyl/heteryl)-4,5-diphenyl-1H-imidazole-1-yl)thiazole derivatives (7a–7q) using triethyl ammonium hydrogen sulfate $[Et_3NH][HSO_4]$, as an ionic liquid catalyst and also as a solvent. Their bioactivity study suggests that these compounds possess moderate to potent antifungal activity. Herein, we describe the structure activity relationship (SAR) of the newly synthesized

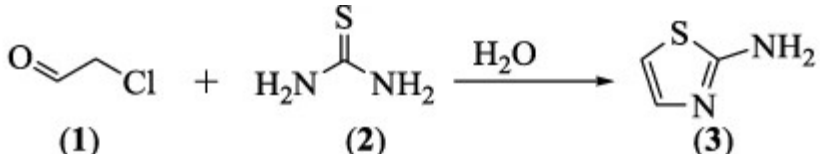
series. The various substitutents on phenyl ring and replacement of phenyl ring with various heteryl rings have been introduced with the aim to improve the overall antifungal activity of the synthesized series. The ergosterol extraction and quantitation assay method and homology modeling study suggested that the compounds 7j and 7k are potent antifungal agents which inhibit ergosterol biosynthesis by inhibiting enzyme cytochrome P450 lanosterol 14α-demethylase of *C. albicans*. In addition to this the in silico ADMET study, in vitro cytotoxicity study, and in vivo acute oral toxicity studies were also performed.

Materials and methods

Chemistry

All chemicals, unless otherwise specified, were purchased from commercial sources and were used without further purification. The major chemicals were purchased from Sigma Aldrich and Avralabs. The synthetic protocol employed for the synthesis of 2-(2-(substituted phenyl/heteryl)-4,5-diphenyl-1H-imidazole-1-yl)thiazole derivatives (7a–7q) is presented in Scheme 1. The development of reactions was monitored by thin layer chromatography (TLC) analysis on Merck pre-coated silica gel 60 F254 aluminum sheets, visualized by UV light. Infrared (IR) spectra were recorded on JASCO FTIR (PS 4000) using KBr pallet. Melting points were determined in open capillary tubes and are uncorrected. The ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra of synthesized compounds were recorded on Bruker Advance II 400 NMR Spectrometer (Billerica, MA, USA) in deuterated DMSO. Tetramethylsilane was used as an internal standard. Chemical shift values are given in ppm relative to TMS as internal reference and the coupling constant (*J*) in Hertz (Hz). The chemical shifts are reported as NMR spectra δ_{ppm} units. The following abbreviations are used; singlet (s), doublet (d), multiplet (m). Mass spectra were taken with WATERS, Q-TOF MICROMASS (E SI-MS). Elemental analyses were done with a FLASHEA 112 Shimadzu analyzer (Mumbai, Maharashtra, India) and all analyses were consistent (within 0.4%) with theoretical values.

Scheme 1



Ar

7a= Phenyl; **7b**= 4-chlorophenyl; **7c**= 4-fluorophenyl; **7d**=4-bromophenyl;
7e= 4-methoxyphenyl; **7f**= 3,4,5-methoxyphenyl; **7g**= 3,4-methoxyphenyl;
7h= 4-hydroxyphenyl; **7i**= 2-hydroxyphenyl; **7j**= 4-hydroxy-3-methoxyphenyl;
7k= 4-hydroxy-3-ethoxyphenyl; **7l**= 4-nitrophenyl; **7m**= 4-methylphenyl;
7n= 2-methylphenyl; **7o**= pyridine-2-yl; **7p**= furan-2-yl; **7q**=thiophen-2-yl

Synthesis of target compounds (7a–7q)

Synthesis of 2-aminothiazole (3)

The procedure for synthesis of 2-aminothiazole was followed as shown in literature (Yasnitskii and Dolberg 1971).

Synthesis of 2-(2-(substituted phenyl/heteryl)-4,5-diphenyl-1H-imidazole-1-yl)thiazole (7a–7q)

A mixture of 2-aminothiazole (3) (0.01 mol), benzil (4) (0.01 mol), suitable aldehyde (5a–5q) (0.01 mol), ammonium acetate (6) (0.01 mol) and ionic liquid [Et_3NH][HSO_4] (15 mol%) as a catalyst and solvent were stirred at a 100 °C temperature for about 25–35 min. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled to room temperature and was added into ice cold water. The separated solid was suction filtered, washed with water, dried and recrystallized from ethanol to get the corresponding tetra-substituted imidazoles.

The products were obtained in good yield (88–92%). The filtrate containing the ionic liquid was then evaporated to dryness under reduced pressure and the resulting catalyst was reused directly for the next run.

2-Aminothiazole (3)

Cream solid; Yield 84%; m.p.: 90–92 °C (m.p.: 86–89 °C Literature) (https://pubchem.ncbi.nlm.nih.gov/compound/2-aminothiazole); IR: (KBr ν_{max} in cm^{-1}): 3005 (C–H), 1601 (C=C), 1580 (C=N); ^{1}H NMR (400 MHz, DMSO, δ_H ppm): 6.55 (d, 1H), 7.55 (d, 1H), 10.51 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO, δ_C ppm): 111.2 (C-5), 137.5 (C-4), 171.3 (C-2); MS: *m/z* 101.14 (100%) $[M+1]^+$; Anal. Calcd. for $C_3H_4N_2S$: C, 35.98; H, 4.03; N, 27.97. Found: C, 35.99; H, 4.06; N, 27.95.

2-(2,4,5-Triphenyl-1H-imidazol-1-yl)thiazole (7a)

Cream solid; Yield 88%; m.p.: 262–264 °C (m.p.: 268–270 °C Literature) (Zhao et al. 2013); IR: (KBr ν_{max} in cm^{-1}): 3005 (C–H), 1601 (C=C), 1580 (C=N); ^{1}H NMR (400 MHz, DMSO, δ_H ppm): 6.66 (d, *J* = 3.07 Hz, 1H), 7.16 (d, *J* = 3.07 Hz, 1H), 7.34–7.47 (m, 7H), 7.53 (t, *J* = 7.44 Hz, 2H), 7.85–7.89 (m, 4H), 8.37–8.41 (m, 2H); ^{13}C NMR (100 MHz, DMSO, δ_C ppm): 111.6 (C-5), 127.2 (C-12 and C-16), 128.0 (C-18 and C-22), 128.1 (C-24 and C-28), 128.7(C-14 and C-20), 129.3 (C-8), 129.1 (C-13 and C-15), 129.5 (C-19 and C-21), 129.6 (C-25 and C-27), 129.7 (C-11), 129.8 (C-26), 130.9 (C-9), 131.4 (C-17), 132.0 (C-4), 136.3 (C-23), 145.5 (C-6), 161.3 (C-2); MS: *m/z* 380.46 (25%) $[M+1]^+$; Anal. Calcd. for $C_{24}H_{17}N_3S$: C, 75.96; H, 4.52; N, 11.07. Found: C, 75.98; H, 4.55; N, 11.05.

2-(2-(4-Chlorophenyl)-4,5-diphenyl-1H-imidazol-1-yl)thiazole (7b)

White solid; Yield 89%; m.p.: 136–138 °C [m.p.: 134–136 °C Literature (Zhao et al. 2013)]; IR: (KBr ν_{max} in cm^{-1}): 3000 (C–H), 1600 (C=C), 1580 (C=N), 755 (C-Cl); ^{1}H NMR (400 MHz, DMSO, δ_H ppm): 6.64 (d, *J* = 3.07 Hz, 1H), 7.14 (d, *J* = 3.07 Hz, 1H), 7.32–7.45 (m, 6H), 7.53 (d, *J* = 7.40 Hz, 2H), 7.83–7.87 (m, 4H), 8.08 (d, *J* = 7.90 Hz, 2H); ^{13}C NMR (100 MHz, DMSO, δ_C ppm): 111.6 (C-5), 125.3 (C-12 and C-16), 127.8 (C-18 and C-22), 128.0 (C-24 and C-28), 128.1 (C-14 and C-20), 128.7 (C-8), 129.1 (C-13 and C-15), 129.6 (C-19 and C-21), 129.7 (C-25 and C-27), 129.8 (C-11), 130.9 (C-26), 131.4 (C-9), 133.2 (C-17), 136.3 (C-4), 138.9 (C-23), 145.5 (C-6), 161.3 (C-2); MS *m/z* 415.46 (36%) $[M+2]^+$; Anal. Calcd. for $C_{24}H_{16}ClN_3S$: C, 69.64; H, 3.90; N, 10.15. Found: C, 69.67; H, 3.94; N, 10.13.

2-(2-(4-Fluorophenyl)-4,5-diphenyl-1H-imidazol-1-yl)thiazole

(7c)

White solid; Yield 92%; m.p.: 150–152 °C; IR: (KBr ν_{max} in cm^{-1}): 3000 (C–H), 1600 (C=C), 1580 (C=N), 1193 (C-F); ^1H NMR (400 MHz, DMSO, δ_H ppm): 6.66 (d, *J* = 3.07 Hz, 1H), 7.16 (d, *J* = 3.07 Hz, 1H), 7.28–7.38 (m, 4H), 7.42–7.47 (m, 4H), 7.85–7.91 (m, 6H); ^{13}C NMR (100 MHz, DMSO, δ_C ppm): 111.6 (C-5), 115.7 (C-25 and C-27), 125.3 (C-12 and C-16), 128.0 (C-18 and C-22), 128.1 (C-14 and C-20), 128.7 (C-8), 128.8 (C-24), 128.8 (C-28), 129.1 (C-13 and C-15), 129.6 (C-19 and C-21), 129.7 (C-25 and C-27), 129.8 (C-11), 129.8 (C-26), 130.9 (C-9), 131.4 (C-17), 136.3 (C-4), 138.9 (C-23), 145.5 (C-6), 161.3 (C-2), 164.8 (C-26); MS *m/z* 398.10 (10%) $[M+1]^+$; Anal. Calcd. for $C_{24}H_{16}FN_3S$: C, 72.52; H, 4.06; N, 10.57. Found: C, 72.54; H, 4.08; N, 10.54.

2-(2-(4-Bromophenyl)-4,5-diphenyl-1H-imidazol-1-yl)thiazole (7d)

White solid; Yield 90%; m.p.: 154–156 °C; IR: (KBr ν_{max} in cm^{-1}): 3000 (C–H), 1607 (C=C), 1580 (C=N), 680 (C-Br); ^1H NMR (400 MHz, DMSO, δ_H ppm): 6.67 (d, *J* = 3.07 Hz, 1H), 7.17 (d, *J* = 3.07 Hz, 1H), 7.34–7.48 (m, 6H), 7.72–7.76 (m, 2H), 7.86–7.90 (m, 4H), 8.07–8.11 (m, 2H); ^{13}C NMR (100 MHz, DMSO, δ_C ppm): 111.6 (C-5), 123.6 (C-26), 125.3 (C-12 and C-16), 128.0 (C-18 and C-22), 128.1 (C-14 and C-20), 128.7 (C-8), 129.1 (C-13 and C-15), 129.6 (C-19 and C-21), 129.7 (C-25 and C-27), 129.8 (C-11), 129.8 (C-26), 130.9 (C-9), 131.3 (C-24), 131.4 (C-28), 133.9 (C-17), 136.3 (C-4), 138.9 (C-23), 145.5 (C-6), 161.3 (C-2); MS *m/z* 461.02 (25%) $[M+2]^+$; Anal. Calcd. for $C_{24}H_{16}BrN_3S$: C, 62.89; H, 3.52; N, 9.17. Found: C, 62.93; H, 3.55; N, 9.14.

2-(2-(4-Methoxyphenyl)-4,5-diphenyl-1H-imidazol-1-yl)thiazole (7e)

White solid; Yield 89%; m.p.: 210–212 °C (m.p.: 205–207 °C Literature) (Zhao et al. 2013); IR: (KBr ν_{max} in cm^{-1}): 3107 (C–H), 1605 (C=C), 1580 (C=N); ^1H NMR (400 MHz, DMSO, δ_H ppm): 3.68 (s, 3H) 6.64 (d, J = 3.07 Hz, 1H), 7.02–7.06 (m, 2H), 7.14 (d, J = 3.07 Hz, 1H), 7.31–7.36 (m, 2H), 7.40–7.45 (m, 4H), 7.83–7.87 (m, 4H), 7.91–7.95 (m, 2H); ^{13}C NMR (100 MHz, DMSO, δ_C ppm): 55.3 (C-30), 111.6 (C-5), 114.3 (C-25 and C-27), 125.3 (C-12 and C-16), 128.0 (C-18 and C-22), 128.1 (C-14 and C-20), 128.7 (C-8), 129.1 (C-13 and C-15), 129.6 (C-19 and C-21), 129.7 (C-25 and C-27), 129.8 (C-11), 129.8 (C-26), 130.9 (C-9), 131.4 (C-28 and C-24),

133.9 (C-17), 136.3 (C-4), 138.9 (C-23), 145.5 (C-6), 161.1 (C-26), 161.3 (C-2); MS *m/z* 410.12 (48%) $[M+1]^+$; Anal. Calcd. for $C_{25}H_{19}N_3OS$: C, 73.32; H, 4.68; N, 10.26. Found: C, 73.34; H, 4.70; N, 10.25.

2-(4,5-Diphenyl-2-(3,4,5-trimethoxyphenyl)-1H-imidazol-1-yl)thiazole (7f)

White solid; Yield 90%; m.p.: 266–268 °C; IR: (KBr ν_{max} in cm^{-1}): 3100 (C–H), 1600 (C = C), 1583 (C = N); ^1H NMR (400 MHz, DMSO, δ_H ppm): 3.86 (s, 6H, OCH_3), 3.92 (s, 3H, OCH_3), 6.67 (d, *J* = 3.07 Hz, 1H) 7.16–7.21 (m, 3H) 7.34–7.39 (m, 2H) 7.43–7.48 (m, 4H), 7.86–7.90 (m, 4H); ^{13}C NMR (100 MHz, DMSO, δ_C ppm): 56.4 (C-18 and C-22), 60.7 (C-20), 111.6 (C-5), 111.7 (C-12 and C-16), 124.9 (C-11), 125.3 (C-24 and C-28), 127.1 (C-30 and C-34), 128.0 (C-26 and C-32), 128.1 (C-25 and C-27), 128.7 (C-31 and C-33), 129.1 (C-8), 129.7 (C-26 and C-32), 129.8 (C-23), 130.9 (C-9), 136.3 (C-4), 138.9 (29), 142.6 (C-14), 145.5 (C-6), 151.7 (C-13 and C-15), 160.4 (C-2); MS m/z 470.15 (15%) $[M+1]^+$; Anal. Calcd. for $C_{27}H_{23}N_3O_3S$: C, 69.06; H, 4.94; N, 8.95. Found: C, 69.09; H, 4.97; N, 8.91.

2-(2-(3,4-Dimethoxyphenyl)-4,5-diphenyl-1H-imidazol-1-yl)thiazole (7g)

White solid; Yield 87%; m.p.: 218–220 °C; IR: (KBr ν_{max} in cm^{-1}): 3000 (C–H), 1600 (C=C), 1580 (C=N); ^1H NMR (400 MHz, DMSO, δ_H ppm): 3.95 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 6.64 (d, *J* = 3.07 Hz, 1H), 6.98 (d, *J* = 8.21 Hz, 1H), 7.14 (d, *J* = 3.07 Hz, 1H), 7.31–7.36 (m, 2H), 7.40–7.45 (m, 5H), 7.83–7.91 (m, 5H); ^{13}C NMR (100 MHz, DMSO, δ_C ppm): 55.9 (C-32), 56.0(C-30), 111.6 (C-5), 112.0 (C-27), 112.8 (C-24), 125.3 (C-28), 126.7 (C-23), 127.5 (C-12 and C-16), 128.0 (C-18 and C-22), 128.1 (C-20 and C-14), 128.7 (C-13 and C-15), 129.1 (C-21 and C-19), 129.6 (C-8), 129.7 (C-11), 129.8 (C-4), 130.9 (C-9), 131.4 (C-17), 136.3 (C-4), 145.5 (C-6), 149.9 (C-25), 152.3 (C-26), 160.42 (C-2); MS *m/z* 440.14 (28%) $[M+1]^+$; Anal. Calcd. for $C_{26}H_{21}N_3O_2S$: C, 71.05; H, 4.82; N, 9.56. Found: C, 71.09; H, 4.87; N, 9.53.

2-(4,5-Diphenyl-1-(thiazol-2-yl)-1H-imidazol-2-yl)phenol (7h)

Cream solid; Yield 89%; m.p.: 200–202 °C; IR: (KBr ν_{max} in cm^{-1}): 3500 (–OH), 3003 (C–H), 1600 (C=C), 1581 (C=N); ^1H NMR (400 MHz, DMSO, δ_H ppm): 6.64 (d, *J* = 3.07 Hz, 1H), 7.06–7.15 (m, 3H), 7.32–7.45 (m, 7H), 7.83–7.91 (m, 5H), 10.85 (s, 1H, OH); ^{13}C NMR (100 MHz,

DMSO, δ_C ppm): 111.6 (C-17), 116.2 (C-6), 117.2 (C-2), 124.0 (C-4), 125.3 (C-19 and C-23), 128.0 (C-25 and C-29), 128.1 (C-21 and C-27), 128.2 (C-20 and C-22), 128.7 (C-26 and C-28), 129.1 (C-10), 129.6 (C-5), 129.7 (C-3), 129.8 (C-18), 130.9 (C-11), 131.4 (C-24), 136.3 (C-16), 143.5 (C-8), 145.5 (C-1), 161.3 (C-14); MS *m/z* 396.11 (27%) $[M+1]^+$; Anal. Calcd. for $C_{24}H_{17}N_3OS$: C, 72.89; H, 4.33; N, 10.63. Found: C, 72.92; H, 4.37; N, 10.61.

4-(4,5-Diphenyl-1-(thiazol-2-yl)-1H-imidazol-2-yl)phenol (7i)

Cream solid; Yield 90%; m.p.: 208–210 °C; IR: (KBr ν_{max} in cm^{-1}): 3500 (–OH), 3000 (C–H), 1605 (C=C), 1580 (C=N); ^1H NMR (400 MHz, DMSO, δ_H ppm): 6.64 (d, *J* = 3.08 Hz, 1H), 6.81–6.91 (m, 2H), 7.14 (d, *J* = 3.08 Hz, 1H), 7.31–7.36 (m, 2H), 7.40–7.45 (m, 4H), 7.83–7.87 (m, 4H), 7.93–7.97 (m,2H), 9.85 (s, 1H, OH); ^{13}C NMR (100 MHz, DMSO, δ_C ppm): 111.6 (C-17), 115.2 (C-2 and C-6), 125.3 (C-25 and C-29), 128.0 (C-19 and C-23), 128.1 (C-27), 128.7 (C-21), 129.0 (C-26 and C-28), 129.1 (C-20 and C-22), 129.6 (C-10), 129.7 (C-3 and C-5), 129.8 (C-18), 130.9 (C-4), 131.4 (C-11), 136.3 (C-24), 136.4 (C-16), 145.5 (C-8), 157.8 (C-14), 163.7 (C-1); MS *m/z* 396.11 (27%) $[M+1]^+$; Anal. Calcd. for $C_{24}H_{17}N_3OS$: C, 72.89; H, 4.33; N, 10.63. Found: C, 72.91; H, 4.37; N, 10.61.

4-(4,5-Diphenyl-1-(thiazol-2-yl)-1H-imidazol-2-yl)-2-methoxyphenol (7j)

White solid; Yield 86%; m.p.: 232–234 °C; IR: (KBr ν_{max} in cm^{-1}): 3500 (–OH), 3001 (C–H), 1600 (C=C), 1585 (C=N); ^1H NMR (400 MHz, DMSO, δ_H ppm): 3.83 (s, 3H, OCH_3), 6.69 (d, *J* = 3.07 Hz, 1H), 6.96 (d, *J* = 8.21 Hz, 1H), 7.19 (d, *J* = 3.07 Hz, 1H), 7.36–7.41 (m, 2H), 7.45–7.50 (m, 5H), 7.83 (s, 1H, OH), 7.88–7.92 (m, 4H), 7.97 (dd, *J* = 8.26, 1.01 Hz, 1H); ^{13}C NMR (100 MHz, DMSO, δ_C ppm): 56.0 (C-9), 111.6 (C-19), 113.4 (C-3), 114.4 (C-6), 124.3 (C-5), 125.3 (C-4), 127.8 (C-21 and C-25), 128.0 (C-27 and C-31), 128.1 (C-23), 128.7 (C-29), 129.1 (C-22 and C-24), 129.6 (C-28 and C-30), 129.7 (C-12), 129.8 (C-20), 130.9 (C-13), 131.4 (C-26), 136.3 (C-18), 145.5 (C-10), 148.6 (C-2), 149.3 (C-1), 160.4 (C-16); MS *m/z* 426.12 (29%) $[M+1]^+$; Anal. Calcd. for $C_{25}H_{19}N_3O_2S$: C, 70.57; H, 4.50; N, 9.88. Found: C, 70.59; H, 4.53; N, 9.84.

4-(4,5-Diphenyl-1-(thiazol-2-yl)-1H-imidazol-2-yl)-2-ethoxyphenol (7k)

White solid; Yield 88%; m.p.: 240–242 °C; IR: (KBr ν_{max} in cm^{-1}): 3500, (aromatic OH), 3000

(C−H), 1602 (C=C), 1580 (C=N); ^{1}H NMR (400 MHz, DMSO, δ_H ppm): 1.48 (t, *J* = 6.96 Hz, 3H, OCH_2CH_3), 4.16 (q, *J* = 6.98 Hz, 2H, OCH_2CH_3), 6.71 (d, *J* = 3.07 Hz, 1H), 6.95–7.02 (m, 2H), 7.20 (d, *J* = 3.07 Hz, 1H), 7.38–7.43 (m, 2H), 7.46–7.51 (m, 5H), 7.89–7.94 (m, 4H), 7.98 (dd, *J* = 8.26, 1.01 Hz, 1H); ^{13}C NMR (100 MHz, DMSO, δ_C ppm): 14.7 (C-10), 63.9 (C-9), 111.6 (C-20), 114.0 (C-3), 114.0 (C-6), 121.1 (C-5), 125.3 (C-4), 127.3 (C-22 and C-26), 128.0 (C-28 and C-32), 128.1 (C-30), 128.7 (C-24), 129.1 (C-23 and C-25), 129.6 (C-29 and C-31), 129.7 (C-13), 129.8 (C-21), 130.9 (C-14), 131.4 (C-27), 136.3 (C-19), 145.5 (C-11), 149.9 (C-2), 150.1 (C-1), 160.4 (C-17); MS *m/z* 440.14 (30%) $[M+1]^+$; Anal. Calcd. for $C_{26}H_{21}N_3O_2S$: C, 71.05; H, 4.82; N, 9.56. Found: C, 71.09; H, 4.85; N, 9.52.

2-(2-(4-Nitrophenyl)-4,5-diphenyl-1H-imidazol-1-yl)thiazole (7l)

White solid; Yield 89%; m.p.: 246–248 °C; IR: (KBr ν_{max} in cm^{-1}): 3002 (C−H), 1605 (C=C), 1581 (C=N); ^{1}H NMR (400 MHz, DMSO, δ_H ppm): 6.64 (d, *J* = 3.07 Hz, 1H), 7.14 (d, *J* = 3.07 Hz, 1H), 7.32–7.45 (m, 6H), 7.83–7.87 (m, 4H), 8.40–8.48 (m, 4H); ^{13}C NMR (100 MHz, DMSO, δ_C ppm): 111.6 (C-5), 124.6 (C-25 and C-27), 125.3 (C-24 and C-28), 127.7 (C-12 and C-16), 128.0 (C-18 and C-22), 128.1 (C-14), 128.7 (C-20), 129.1 (C-13 and C-15), 129.6 (C-19 and C-21), 129.7 (C-8), 129.8 (C-11), 130.9 (C-9), 131.4 (C-17), 135.0 (C-4), 136.3 (C-6), 145.5 (C-26), 149.3 (C-23), 161.3 (C-2); MS *m/z* 425.10 (28%) $[M+1]^+$; Anal. Calcd. for $C_{24}H_{16}N_4O_2S$: C, 67.91; H, 3.80; N, 13.20. Found: C, 67.94; H, 3.84; N, 13.17.

2-(4,5-Diphenyl-2-p-tolyl-1H-imidazol-1-yl)thiazole (7m)

White solid; Yield 88%; m.p.: 210–212 °C; IR: (KBr ν_{max} in cm^{-1}): 3000 (C−H), 1604 (C=C), 1581 (C=N); ^{1}H NMR (400 MHz, DMSO, δ_H ppm): 2.42 (s, 3H, CH_3), 6.67 (d, *J* = 3.07 Hz, 1H), 7.16 (d, *J* = 3.07 Hz, 1H), 7.31–7.39 (m, 4H), 7.43–7.46 (m, 4H), 7.86–7.92 (m, 6H); ^{13}C NMR (100 MHz, DMSO, δ_C ppm): 21.5 (C-17), 111.6 (C-5), 125.3 (C-12 and C-14), 127.5 (C-19 and C-23), 128.0 (C-25 and C-29), 128.1 (C-21), 128.7 (C-27), 129.1 (C-20 and C-22), 129.6 (C-26 and C-28), 129.7 (C-8), 129.8 (C-15 and C-16), 130.1 (C-13), 131.2 (C-18), 136.3 (C-9), 136.9 (C-24), 140.9 (C-11), 145.5 (C-6), 161.3 (C-2); MS *m/z* 394.13 (12%) $[M+1]^+$; Anal. Calcd. for $C_{25}H_{19}N_3S$: C, 76.31; H, 4.87; N, 10.68. Found: C, 76.33; H, 4.89; N, 10.64.

2-(4,5-Diphenyl-2-o-tolyl-1H-imidazol-1-yl)thiazole (7n)

White solid; Yield 87%; m.p.: 214–216 °C; IR: (KBr ν_{max} in cm^{-1}): 3000 (C–H), 1602 (C=C), 1580 (C=N); ^1H NMR (400 MHz, DMSO, δ_H ppm): 2.47 (s, 3H, CH_3), 6.64 (d, *J* = 3.07 Hz, 1H), 7.14 (d, *J* = 5.08 Hz, 2H), 7.26 (d, *J* = 1.25 Hz, 1H), 7.31–7.36 (m, 2H), 7.40–7.45 (m, 4H), 7.83–7.91 (m, 5H), 8.10 (dd, *J* = 7.63, 1.20 Hz, 1H); ^{13}C NMR (100 MHz, DMSO, δ_C ppm): 19.6 (C-17), 111.6 (C-5), 125.3 (C-25 and C-29), 126.7 (C-19 and C-23), 128.0 (C-15), 128.0 (C-16), 128.1 (C-21), 128.3 (C-27), 128.4 (C-13), 128.7 (C-26 and C-28), 129.1 (C-20 and C-22), 129.6 (C-8), 129.7 (C-14), 129.8 (C-18), 130.9 (C-12), 131.4 (C-9), 133.6 (C-24), 133.9 (C-4), 136.3 (C-11), 145.5 (C-6), 155.4 (C-2); MS *m/z* 394.13 (10%) $[M + 1]^+$; Anal. Calcd. for $C_{25}H_{19}N_3S$: C, 76.31; H, 4.87; N, 10.68. Found: C, 76.34; H, 4.90; N, 10.65.

2-(4,5-Diphenyl-2-(pyridin-2-yl)-1H-imidazol-1-yl)thiazole (7o)

Dark brown solid; Yield 90%; m.p.: 210–212 °C; IR: (KBr ν_{max} in cm^{-1}): 3005 (C–H), 1602 (C=C), 1580 (C=N); ^1H NMR (400 MHz, DMSO, δ_H ppm): 6.64 (d, *J* = 3.07 Hz, 1H), 7.14 (d, *J* = 3.07 Hz, 1H), 7.32–7.45 (m, 7H), 7.83–7.89 (m, 5H), 8.34 (dd, *J* = 7.85, 1.18 Hz, 1H), 8.54 (dd, *J* = 4.80, 1.73 Hz, 1H); ^{13}C NMR (100 MHz, DMSO, δ_C ppm): 111.6 (C-5), 123.6 (C-14), 125.3 (C-12), 125.6 (C-18 and C-22), 128.0 (C-24 and C-28), 128.1 (C-20), 128.7 (C-26), 129.1(C-19 and C-21), 129.6 (C-25 and C-27), 129.7 (C-8), 129.8 (C-17), 130.9 (C-13), 131.4 (C-9), 136.3 (C-23), 139.0 (C-4), 145.5 (C-15), 146.4 (C-11), 149.1 (C-6), 161.0 (C-2); MS *m/z* 381.11 (27%) $[M + 1]^+$; Anal. Calcd. for $C_{23}H_{16}N_4S$: C, 72.61; H, 4.24; N, 14.73. Found: C, 72.64; H, 4.27; N, 14.70.

2-(2-(Furan-2-yl)-4,5-diphenyl-1H-imidazol-1-yl)thiazole (7p)

Dark brown solid; Yield 89%; m.p.: 206–208 °C; IR: (KBr ν_{max} in cm^{-1}): 3000 (C–H), 1600 (C=C), 1580 (C=N); ^1H NMR (400 MHz, DMSO, δ_H ppm): 6.61 (dd, *J* = 3.26, 1.97 Hz, 1H), 6.64 (d, *J* = 3.07 Hz, 1H), 7.14 (d, *J* = 3.07 Hz, 1H), 7.18 (dd, *J* = 3.26, 1.15 Hz, 1H), 7.32–7.45 (m, 6H), 7.68 (dd, *J* = 1.87, 1.10 Hz, 1H), 7.83–7.87 (m, 4H); ^{13}C NMR (100 MHz, DMSO, δ_C ppm): 111.6 (C-5), 112.4 (C-25), 117.3 (C-26), 125.3 (C-12 and C-16), 127.0 (C-18 and C-22), 128.0 (C-14), 128.7 (C-20), 129.1 (C-13 and C-15), 129.6 (C-19 and C-21), 129.7 (C-8), 129.8 (C-11), 130.9 (C-9), 131.4 (C-17), 135.2 (C-4), 144.4 (C-6), 146.5 (C-27), 147.2 (C-24), 158.8 (C-2); MS *m/z* 370.09 (15%) $[M + 1]^+$; Anal. Calcd. for $C_{22}H_{15}N_3OS$: C, 71.52; H, 4.09; N, 11.37. Found: C, 71.54; H, 4.12; N, 11.35.

2-(4,5-Diphenyl-2-(thiophen-2-yl)-1H-imidazol-1-yl)thiazole (7q)

Dark brown solid; Yield 88%; m.p.: 202–204 °C; IR: (KBr ν_{max} in cm^{-1}): 3000 (C–H), 1605 (C=C), 1582 (C=N); ^{1}H NMR (400 MHz, DMSO, δ_H pm): 6.64 (d, J = 3.07 Hz, 1H), 7.17 (d, J = 3.07 Hz, 1H), 7.24 (dd, J = 4.85 Hz, 1H), 7.35–7.48 (m, 6H), 7.67 (dd, J = 4.85 Hz, 1H), 7.76 (dd, J = 3.84 Hz, 1H), 7.86–7.90 (m, 4H); ^{13}C NMR (100 MHz, DMSO, δ_C ppm): 111.6 (C-5), 125.3 (C-23 and C-27), 127.0 (C-17 and C-21), 127.9 (C-14), 128.0 (C-13), 128.1(C-19), 128.7(C-25), 129.1 (C-12), 129.3 (C-18 and C-20), 129.6 (C-24 and C-26), 129.7 (C-8), 129.8 (C-16), 130.9 (C-9), 131.4 (C-22), 135.2 (C-4), 136.6 (C-6), 146.5 (C-11), 158.6 (C-2); MS *m/z* 386.07 (18%) $[M+1]^+$; Anal. Calcd. for $C_{22}H_{15}N_3S_2$: C, 68.54; H, 3.92; N, 10.90. Found: C, 68.56; H, 3.96; N, 10.88.

Biological activity

In vitro antifungal activity

All the synthesized compounds were screened for their in vitro antifungal activity. The antifungal activity was evaluated against six human pathogenic fungal strains, such as *Candida albicans* (NCIM3471), *Candida glabrata (NCYC 388), Fusarium oxysporum* (NCIM1332), *Aspergillu sflavus* (NCIM539), *Aspergillus niger* (NCIM1196), *Cryptococcus neoformans* (NCIM576), which are often encountered clinically. Miconazole and Fluconazole were used as standard drugs as shown in Table 1. The in vitro minimal inhibitory concentrations (MIC_{80}) of the compounds were determined by the micro-broth dilution method in 96-well microtest plates according to the methods defined by the National Committee for Clinical Laboratory Standards (NCCLS) (National Committee for Clinical Laboratory Standards 2002). The MIC_{80} was defined as the first well with an approximate 80% reduction in growth compared to the growth of the drug-free well. The data are the mean of three replicate tests with each antifungal compound.

Table 1 In vitro antifungal evaluation of the synthesized compounds (7a–7q)

Ergosterol extraction and quantitation Assay

A single *Candida albicans* (NCIM3471) colony from an overnight Sabouraud dextrose agar plate culture was used to inoculate 50 ml of Sabouraud dextrose broth for control and for various concentrations of molecules. The cultures were incubated for 16 h and harvested by centrifugation at 2700 rpm (856×*g*) for 5 min. The net weight of the cell pellet was determined. Three milliliters of 25% alcoholic potassium hydroxide solution were added to each pellet and vortex mixed for one min. Cell suspensions were transferred to sterile borosilicate glass screw-cap tubes and were incubated in an 85 °C water bath for 1 h. Following incubation, the tubes were allowed to cool. Sterols were then extracted by the addition of a mixture of 1 ml of sterile distilled water and 3 ml of n-heptane followed by vigorous vortex mixing for 3 min. The heptane layer was transferred to a clean borosilicate glass screw-cap tube and stored at -20 °C. Prior to analysis, 0.6 ml aliquot of sterol extract was diluted fivefold in 100% ethanol and scanned spectrophotometrically between 240 nm and 300 nm with a spectrophotometer (UV–Visible Spectrophotometer 2100 thermo Fischer scientific). The presence of ergosterol and the late sterol intermediate 24(28) dehydroergosterol (DHE) in the extracted sample resulted in a characteristic four-peaked curve. The absence of detectable ergosterol in the extracts was indicated by a flat line. A dose-dependent decrease in the height of the absorbance peaks was evident and corresponded to the decreased ergosterol concentration (Arthington-Skaggs et al. 2000).

Computational studies

Homology modeling

The 3D model structure of cytochrome P450 lanosterol 14-α demethylase of *C. albicans* was built using homology modeling (Sangshetti et al. 2011). Molecular docking studies were performed using Glide v6.8 (Schrodinger, LLC, New York, NY, 2015) into a homology model of cytochrome P450 lanosterol 14α-demethylase of *C. albicans*.

In silico ADMET prediction

A computational study of the synthesized compounds (7a–7q) was performed for prediction of ADMET properties. The absorption, distribution, metabolism, excretion, and toxicity

(ADMET) properties of all the compounds were predicted using Qikprop v3.5 (Schrödinger LLC, New York, 2015). In the present study, we have calculated the molecular volume (MV), molecular weight (MW), Predicted octanol–water partition coefficient (log Po/w), number of hydrogen bond acceptors (n-ON), number of hydrogen bonds donors (n-OHNH), Percentage human oral absorption (% ABS), Van Der Waals surface area of polar nitrogen and oxygen atoms (Polar Surface Area), Log S (water solubility), BIP_{Caco-2} (apparent Caco-2 cell permeability), Log Khsa (binding to human serum albumin) and Log HERG (toxicity study).

In vitro cytotoxicity studies

To study the safety profile and to explore the selective antimicrobial activity of the most active compounds 7j and 7k, in vitro cytoxocity study was performed. This study proves that the synthesized compounds 7j and 7k show only antimicrobial activity at their MIC values and do not kill the human cell lines indicating their safety profile and selectivity towards antimicrobial activity. To explore the selective antifungal activity of the synthesized compounds in vitro cytoxocity study of the most active synthesized compounds 7j and 7k was performed against HeLa (Human cervical cancer cell line) and K-562 (Human Leukemia cancer cell line) by Sulforhodamine B (SRB) assay using Adriamycin as positive control (Vichai and Kirtikara 2006).

In vivo acute oral toxicity study

The acute oral toxicity study for the most active synthesized compounds 7j and 7k was carried out by following the OECD guideline no. 425 using Swiss albino mice (18–22 g weight) quarantined at animal house at Y.B. Chavan College of Pharmacy, Aurangabad IAEC approval number CPCSEA/IAEC/P'col-52/2015-16/115. Each group consisting of 6 mice (overnight fasted) and kept in colony cage at 25 ± 2 °C with 55% relative humidity and 12 h of light and dark cycle. A specified dose of 100, 250, 500, 750, 1000, 1500, and 2000 mg/kg body weight of mice was administered orally as a single dose. The acute toxic symptoms and the behavioral changes produced by the test compounds were observed continuously for 4 h periods at 8, 12, and 24 h on set of toxic symptoms and the gross behavioral changes were also recorded. These animals were maintained for further 10 days with observation made daily. In case the animal appeared moribund (dying) the animal was sacrificed in a humane way and it is considered to

have died because of toxicity.

Results and discussion

Chemistry

The synthesis of 2-(2-(substituted phenyl/heteryl)-4,5-diphenyl-1H-imidazole-1-yl)thiazole derivatives (7a–7q) using triethyl ammonium hydrogen sulphate $[Et_3NH][HSO_4]$, as an ionic liquid, which acts as an efficient Green catalyst and solvent is as shown in Scheme 1. 2-aminothiazole (3) was obtained by reaction of 2-chloroacetaldehyde (1) (0.01 mol) with thiourea (2) (0.01 mol) in water by stirring at room temperature (Yasnitskii and Dolberg 1971). The final derivatives were obtained by one pot reaction of 2-aminothiazole (3) (0.01 mol), benzil (4) (0.01 mol), suitable aldehyde (5a–5q) (0.01 mol), ammonium acetate (6) (0.01 mol) and ionic liquid $[Et_3NH][HSO_4]$ (15 mol%) as a solvent and reusable catalyst.

To optimize the reaction conditions, as a model, the reaction of benzil (0.01 mol), 4-fluoro-benzaldehyde (0.01 mol), 2-aminothiazole (0.01 mol) and ammonium acetate (0.01 mol) was examined in the presence of various concentrations of $[Et_3NH][HSO_4]$ at a temperature range from 80–110 °C in solvent-free conditions. The results are summarized in Table S1. As it can be seen in Table S1, the best results were obtained when the reaction was carried out using 15 mol% of $[Et_3NH][HSO_4]$ at 100 °C. Therefore, 15 mol% of the $[Et_3NH][HSO_4]$ ionic liquid as a catalyst and solvent was considered to ensure the best yield (92%) in short reaction time (30 min) at 100 °C (Entry 3 of Table S1 in the supporting information). These observations make the process under study more attractive and economic, safe, eco-friendly and simple. The reusability of the catalyst was studied and it was observed that the catalytic activity of the catalyst was restored within the limits of the experimental errors for four successive runs as shown in Table S2.

Total 17 derivatives were synthesized following this synthetic protocol. The reactions were completed in about 25–35 min (monitored by TLC). The yields of synthesized novel compounds were in the range of 88–92%. Melting points were determined in open capillary tubes and are uncorrected. The formation of the synthesized compounds (7a–7q) was confirmed by IR, ^{1}H NMR, ^{13}C NMR, mass spectral analyses, and elemental analyses.

Yasnitskii has synthesized 2-Amino-thiazole (3) in the year 1971 (Yasnitskii and Dolberg 1971). Zhao et al. 2013 has synthesized 7a, 7b, and 7e in the year 2013 (Zhao et al. 2013). The synthesized compounds 7c, 7d, 7f, 7g, 7h, 7i, 7j, 7k, 7l, 7m, 7n, 7o, 7p, and 7q are novel compounds.

Biological activity

Evaluation of in vitro antifungal activity

The synthesized imidazole-thiazole coupled hybrid derivatives (7a–7q) were screened for their in vitro antifungal activity against six fungal strains *Candida albicans* (NCIM3471), *Candida glabrata* (NCYC388), *Fusarium oxysporum* (NCIM1332), *Aspergillus flavus* (NCIM539), *Aspergillus niger* (NCIM1196), *Cryptococcus neoformans* (NCIM576), which are often encountered clinically. Miconazole and Fluconazole were used as standard drugs. The MIC_{80} of the synthesized compounds against six fungal strains were reported in Table 1.

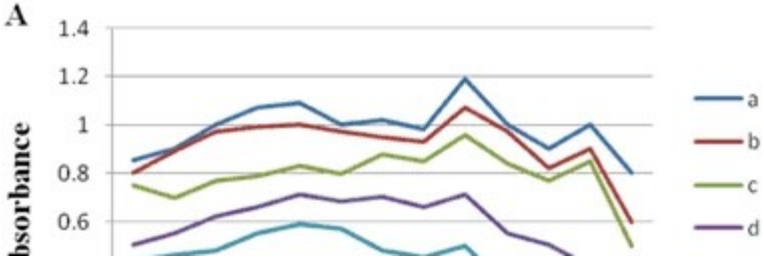
The antifungal activity screening study of the synthesized compounds (7a–7q) reveals that most of the synthesized compounds (7a–7q) had shown good antifungal activity. The compound 7k bearing 3-OC_2H_5-4-OH group on the phenyl ring was found to exhibit extremely high antifungal activity against *C. albicans*, *C. glabrata, F. oxysporum*, *A. flavus*, *A. niger*, and *C. neoformans* with MIC_{80} values of 0.2, 0.2, 20, 35, 40, and 5 µg/ml respectively compared to standard drugs Miconazole and Fluconazole. The MIC_{80} values of compound 7k were found to be 2.5 times lower than that of standard drugs Miconazole and Fluconazole against *C. albicans* and *C. glabrata* in vitro. The MIC_{80} values of compounds 7i and 7h are 1.5 times lower than that of standard drugs Miconazole and Fluconazole against *C. albicans* and *C. glabrata* in vitro. The compound 7l bearing 4-NO_2 group on the phenyl ring was found to exhibit equipotent antifungal activity when compared with the standard drugs. The compound 7l exhibited MIC_{80} values as follows: 0.5 µg/ml for *C. albicans*, 0.5 µg/ml *C. glabrata*, 35 µg/ml for *F. oxysporum*, 50 µg/ml for *A. flavus*, 45 µg/ml for *A. niger*, 8 µg/ml for *C. neoformans* in vitro. The remaining compounds 7a, 7b, 7c, 7d, 7e, 7f, 7g, 7o, 7p, and 7q had shown moderate antifungal activity, as compared to the standard drugs Miconazole and Fluconazole. The compounds 7m and 7n were found to be less active among the synthesized series.

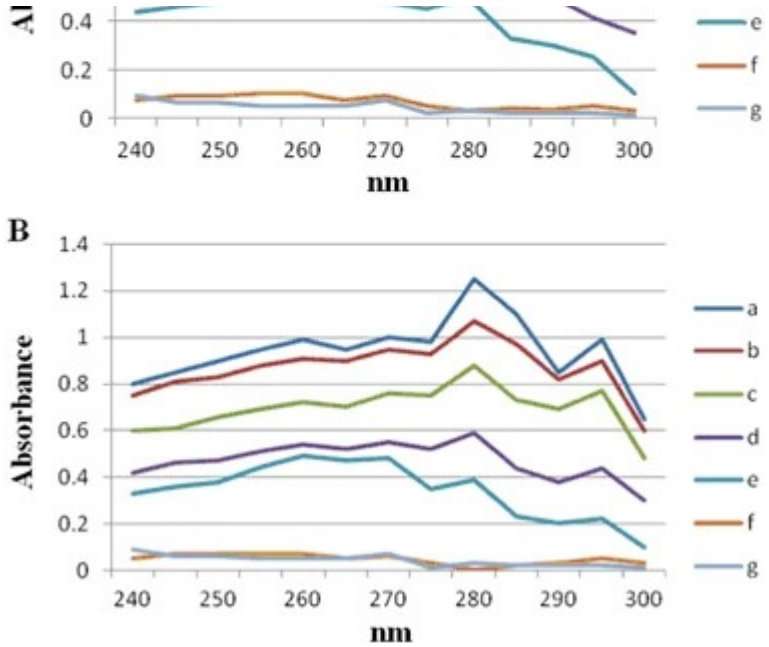
Structure activity relationship (SAR) revealed that the scaffolds 4,5diphenyl imidazole and thiazole are responsible for antifungal activity. Modification of the parent compounds with various substituents on the phenyl ring such as halogen, hydroxyl, methyl, and methoxyl, were performed to explore the SAR of these synthesized compounds (7a–7n). Also, the phenyl ring was replaced by heterocyclic rings like pyridine, furan, and thiophene in order to explore the SAR of these synthesized compounds 7o, 7p, and 7q, respectively. The activity varies depending on the various substituents present on the phenyl rings. Introduction of halogen groups at para position, i.e 4-Cl 7b, 4-F 7c, and 4-Br 7d, group on the phenyl ring showed increased antifungal activity compared to 7a non-substituted derivative. Introduction of electron donating polar, −OH group on the 2/4 position of phenyl ring as shown in 7h and 7i respectively, increased antifungal activity. Introduction of the 3-OC2H5-4-OH group on the phenyl ring has shown significant enhancement in antifungal activity and found to be the most promising antifungal derivative 7k from the synthesized series. Replacement of phenyl ring with heterocyclic rings such as furan ring, in compound 7p and with a thiophene ring as in 7q did not alter the antifungal activity and were found to have good antifungal activity.

Ergosterol extraction and quantitation assay

Considering ergosterol as an important fungal cell membrane lipid, changes in its biosynthetic pathway may also cause damage to the fungal cell, preventing growth in a way similar to azole compounds such as Miconazole, Fluconazole, etc. (Arthington-Skaggs et al. 2000). To reveal the antifungal mechanism of the most potent synthesized compounds 7j and 7k, its influence on the sterol composition on the *C. albicans* membrane was monitored by analyzing the changes in sterol composition in the cells of *C. albicans* by U.V. analysis. The assay was performed at various concentrations of the most potent synthesized compounds 7j and 7k such as $MIC_{80}/16$, $MIC_{80}/8$, $MIC_{80}/4$, $MIC_{80}/2$, and MIC_{80} value to quantify the content of sterol produced by *C. albicans* as shown in Fig. 2a, b respectively.

Fig. 2





UV spectrophotometric sterol profile of *C. albicans* (NCIM3471) treated with, 0 (curve a), 0.015 (curve b), 0.03 (curve c), 0.06 (curve d), 0.12 (curve e) and 0.25 (curve f) µg/ml of synthesized compound 7j (a); 0 (curve a), 0.012 (curve b), 0.025 (curve c), 0.05 (curve d), 0.1 (curve e) and 0.2 (curve f) µg/ml of synthesized compound 7k (b); 0.5 (curve g) µg/ml of Fluconazole in a and b, sterols were extracted from cells, and spectral profiles between 240 and 300 nm were determined

The "curve a" in Fig. 2a, b represents negative control (no compound). The absorption of sterols extracted from fungal culture at the wavelengths of 230 nm, and 281.5 nm was analyzed and the results obtained are shown in Fig. 2 a, b for the most potent synthesized compounds 7j and 7k respectively. Ergosterol and an intermediate of the metabolic pathway of ergosterol–24(28) dehydroergosterol (DHE) absorb energy at 281.5 nm, but DHE alone shows intense absorption at 230 nm. Changes in this pattern of absorption are indicative of interference in the synthetic pathway of ergosterol (Lima et al. 2012). There was a change in the absorption pattern for the synthesized compounds 7j and 7k as shown in Fig. 2a, b respectively, which proves that the synthesized compounds 7j and 7k inhibits ergosterol biosynthesis by inhibiting enzyme cytochrome P450 lanosterol 14α-demethylase of *C. albicans*. The "curve a" shows an intense peak at 281.5 nm of ergosterol indicating presences of ergosterol. The "curve g" in Fig. 2a, b represents the inhibition of ergosterol by Fluconazole (standard drug) at its MIC_{80} value. As the concentration of synthesized compounds 7j and 7k increases the intensity of the ergosterol peak at 281.5 nm decreases, which indicated decrease

in concentration of ergosterol in the culture medium. At the MIC_{80} value of the synthesized compounds 7j and 7k there is almost a flat peak similar to that of Fluconazole at its MIC_{80} value as shown in Fig. 2. These results suggest that the compound might inhibit fungal lanosterol 14α-demethylase similar to that accepted mechanism of Fluconazole.

Computational method

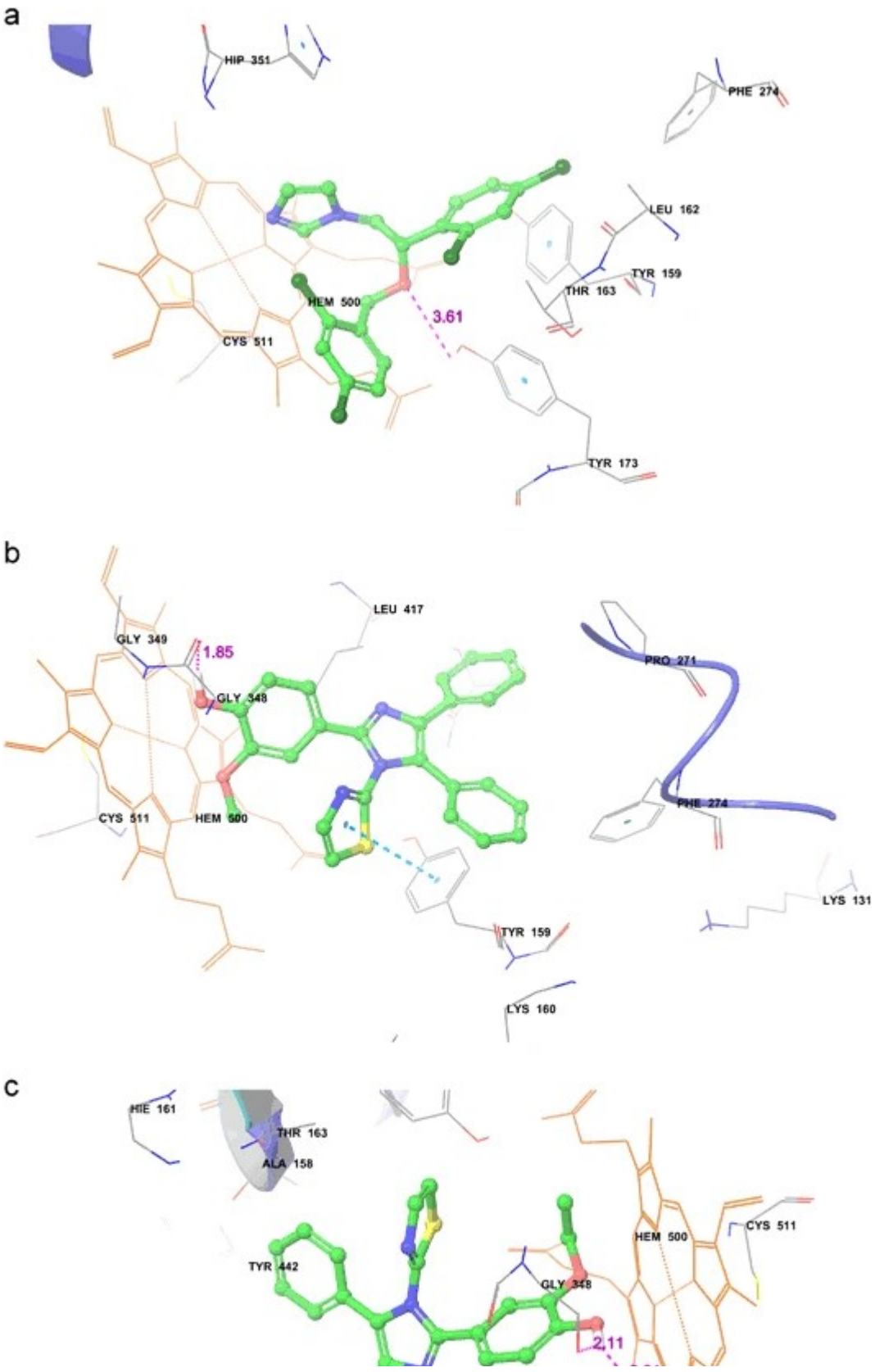
Homology modeling

In order to give an explanation and understanding of the mechanism of action of the most potent synthesized compounds against fungal strains, docking studies were performed using Glide v6.8 (Schrodinger, LLC, New York, NY, 2015) into a homology model of cytochrome P450 lanosterol 14α-demethylase of *C. albicans* (Sangshetti et al. 2011). It is reported in the literature that the azole antifungal agents such as Miconazole, Fluconazole, and Ketoconazole act by a mechanism in which heterocyclic nitrogen atom of azole compound binds to heme iron atom in the active site of the enzyme (Strushkevich et al. 2010). Therefore, we predicted the binding pose of our docked compounds and measured the distance between hetero atom of heterocyclic ring and heme iron and then compared with the reference standard, Miconazole. It is seen from the docking analyses that the novel imidazole-thiazole compounds fit well into the hydrophobic binding cavity containing the heme group and meet the essential interaction through imidazole-thiazole nucleus and the substituted phenyl ring. It is also noted that the "enzyme-ligand" complex is stabilized by hydrogen bonds and π−π interaction involving amino acid like Tyr159, Gly348, and Thr352.

Figure 3a represents the docking pose of standard drug Miconazole in the active pocket of cytochrome P450 lanosterol 14α-demethylase of *C. albicans*. According to the docking study, there is π−π stacking interaction between thiazole nucleus of compound 7j and phenyl ring of Tyr159 amino acid residue as shown in Fig. 3b. The compound 7j shows a hydrogen bonding between the hydroxyl group of the structure and carbonyl group of amino acid residue of Gly348. The Compound 7k shows a hydrogen bond interaction between hydroxyl group and the amino acid residues like Gly348 and Thr352 respectively as shown in Fig. 3c. The docking studies revealed that the thiazole ring along with an imidazole ring plays an important role in binding affinity of docked compounds against cytochrome P450 lanosterol 14α-demethylase of *C. albicans*. Hence, on the basis of docking analysis, it is observed that the novel synthesized

imidazole–thiazole derivatives could have a probable binding affinity towards lanosterol 14 α-demethylase enzyme.

Fig. 3



a Docking Pose of Miconazole in the active site of cytochrome P450 lanosterol 14α-demethylase of *C. albicans*. b Docking pose of compound 7j in the active site of cytochrome P450 lanosterol 14α-demethylase of *C. albicans*. c Docking pose of compound 7k in the active site of cytochrome P450 lanosterol 14α-demethylase of *C. albicans*

In silico ADMET prediction

If the pharmacokinetics properties of the new developing drugs are found to be poor during the last phase of research, it may lead to the gigantic loss of the money, manpower, etc. Therefore, initial evaluation of ADMET (Adsorption, Distribution, Metabolism, Excretion, and Toxicity) is the best practice for an effective drug development process. 2D structures of all the synthesized derivatives (7a–7q) were subjected for in silico pharmacokinetics screening using Qikprop v3. 5 (Schrödinger LLC, New York, 2015) to study compliance with the Lipinski rule of five and Jorgensen's rules of three.

The synthesized derivatives (7a–7q) were tested for their in silico physicochemical pharmacokinetics parameters which are important for good oral bioavailability and the result obtained is as shown in Tables S3 and S4. It was observed that none of the synthesized compounds (7a–7q) has violated a Lipinski rule of five and Jorgensen's rule of three as shown in Tables S3 and S4, respectively.

The synthesized compounds (7a–7q) had shown the PSA value ranges between 24.4 to 70.9 Å, which indicates the good cell permeability property of the compounds (Kumar et al. 2015). Aqueous solubility of a compound significantly affects its absorption and distribution characteristics. All synthesized compounds (7a–7q) have solubility values within the desired range (−6.5 to −0.5) as shown in Table S3. The synthesized compounds had shown BIP_{Caco-2} value ranging from 763.6 to 6798.8, which indicates good in silico intestinal absorption. The

synthesized compounds showed MDCK value which ranges from 533.4 to 10,000 indicating good in silico oral absorption (Veber et al. 2002). The synthesized compounds showed Log Khsa value ranges between 0.83 to 1.2 (Table S4) this is an indication that a significant proportion of the compounds are likely to circulate freely in the blood stream and hence reach the drug target sites. The most active antifungal compounds 7i, 7j, and 7k showed 99%, 100%, and 100%, absorption respectively. The HERG K^+ channel, which is best known for its contribution to the electrical activity of the heart that coordinates the heart's beating, appears to be the molecular target responsible for the cardiac toxicity of a wide range of therapeutic drugs (Vandenberg et al. 2001). From Log HERG value it is observed that all the synthesized compounds (7a–7q) are non-toxic in nature as shown in Table S4.

In vitro cytotoxicity study

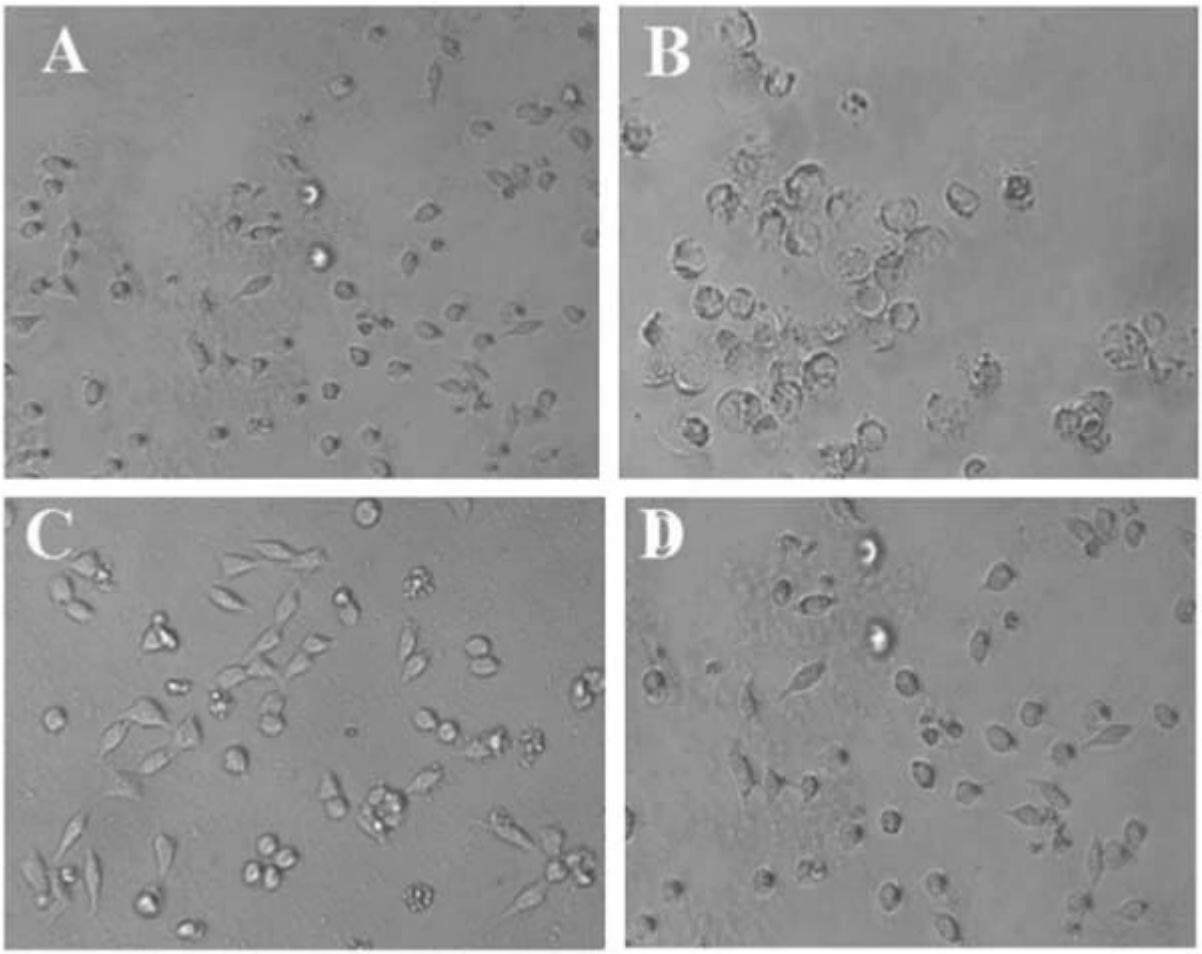
To examine the safety of the most potent synthesized compounds 7j and 7k, these newly synthesized compounds were evaluated for their toxicity against the two human cancer cell lines, HeLa (Human cervical cancer cell line) and K-562 (Human Leukemia cancer cell line) by Sulforhodamine B (SRB) assay using Adriamycin as positive control. The toxicity study of the synthesized compounds at the early stage of research, simplifying the path to clinical trials and reduces the failure of potential therapeutics at later stages of testing. Therefore, the cytotoxicity of the most promising compounds 7j and 7k was evaluated. The observed results are summarized in Table 2. The results indicated that, in SRB cytotoxicity studies, most active compounds 7j and 7k can be considered as leads antimicrobials having no significant cell toxicity against HeLa and K-562 cell lines at the maximum concentration evaluated. The disparity between the cytotoxicity and the antifungal activities of the synthesized compounds 7j and 7k suggested that these compounds exhibited high in vitro antifungal activities at non cytotoxic concentrations.

Table 2 In vitro Cytotoxicity profile of the synthesized compounds 7j and 7k

HeLa (Human cervical cancer cell line) and K-562 (Human Leukemia cancer cell line).

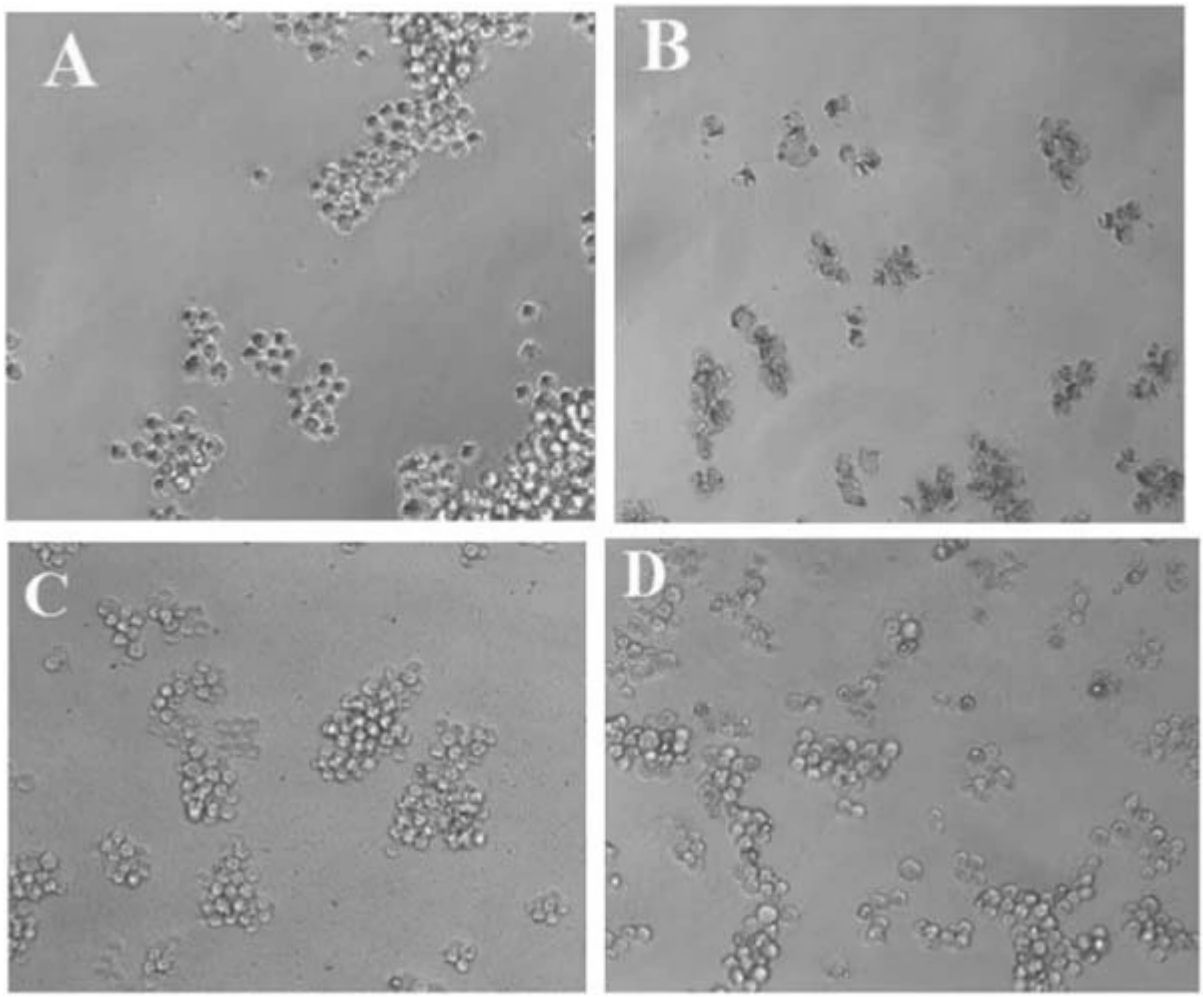
The cytotoxic effect was checked using the concentration range from 50 to 0.10 µg/ml to find the 50% growth inhibition value (GI_{50}) of compounds 7j and 7k on HeLa (Human cervical cancer cell line) and K-562 (Human Leukemia cancer cell line) by Sulforhodamine B (SRB) assay. The results indicated that, in SRB cytotoxicity studies, the synthesized compound 7j and 7k have shown no significant cell toxicity against HeLa and K-562 cell lines hence can be developed as safer antifungal agents. Figures 4 and 5 show that the cell inhibition did not take place even upto 50 µg/ml concentration of the synthesized compounds 7j and 7k and hence are not cytotoxic in nature.

Fig. 4



Images of in vitro cytotoxicity screening against HeLa (Human cervical cancer cell line) for control (a), positive control (b), compound 7j (c) at 50 µg/ml concentration, compound 7k (d) at 50 µg/ml concentration

Fig. 5



Images of in vitro cytotoxicity screening against K-562 (Human Leukemia cancer cell line) for control (a), positive control (b), compound 7j (c) at 50 µg/ml concentration, compound 7k (d) at 50 µg/ml concentration

Structure activity relationship (SAR) revealed that the scaffolds 4,5diphenyl imidazole and thiazole showed negligible cytotoxicity. Modification of the parent compounds with various substituents on the phenyl ring such as methoxy, ethoxy were performed. The synthesized compounds 7j bearing 3-OCH_3-4-OH group on the phenyl ring and 7k bearing 3-OC_2H_5-4-OH group on the phenyl ring were found to be non cytotoxic in nature when compared with the standard drug Adriamycin.

In vivo acute oral toxicity study

Animals treated with the most potent synthesized compounds 7j and 7k were free of any toxicity, as per the acceptable range given by the OECD guideline no. 425 and no mortality was found up to 2000 mg/kg, which indicates that the lethal dose of the compounds is above 2000 mg/kg body weight in mice and that the compounds can be considered to be safe and can be developed in future as good antifungal agents.

Conclusion

In conclusion, a novel series of 2-(2-(substituted phenyl/heteryl)-4,5-diphenyl-1H-imidazole-1-yl)thiazole derivatives (7a–7q) was obtained using Green synthetic protocol using $[Et_3NH][HSO_4]$ as an ionic liquid. Use of green method, i.e. use of ionic liquid helped us in the synthesis of excepted derivatives in good yield and proving its advantage by avoiding pollution in the environment caused by hazardous chemicals. The mild reaction conditions, excellent yields in shorter reaction time and evasion of cumbersome work-up procedures make this process economically lucrative for industrial application with the advantage of reusability of catalyst. The synthesized compounds were evaluated for their in vitro antifungal activity. The compound 7h, 7i, 7j, 7k, and 7l were found to be potent antifungal agents from the synthesized series. The ergosterol extraction and quantitation assay method and the docking study prove that the synthesized compounds 7j and 7k act by the inhibition of the ergosterol biosynthesis by inhibiting lanosterol 14α-demethylase enzyme. Further, in silico ADMET prediction results showed that compounds could be exploited as an oral drug candidate. Furthermore, the in vitro cytotoxicity study and in vivo acute oral toxicity study revealed that compounds 7j and 7k are non toxic in nature.

References

Albataineh MT, Sutton DA, Fothergill AW, Wiederhold NP (2016) Update from the laboratory clinical identification and susceptibility testing of fungi and trends in antifungal resistance. Infect Dis Clin North Am 30:13–35

Article PubMed Google Scholar

Arthington-Skaggs BA, Warnock DW, Morrison CJ (2000) Quantitation of *Candida albicans* ergosterol content improves the correlation between *in vitro* antifungal susceptibility test results and *in vivo* outcome after fluconazole treatment in murine model of invasive *candidiasis*. Antimicrob Agents Chemother 44:2081–2085

Article CAS PubMed PubMed Central Google Scholar

Brown GD, Denning DW, Gow NAR, Levitz SM, Netea MG, White TC (2012) Hidden killers: human fungal infections. SciTransl Med 4:165–13

Google Scholar

Denning DW, Bromley MJ (2015) How to bolster the antifungal pipeline. Science 347:1414–1416

Article CAS PubMed Google Scholar

Franchetti P, Cappellacci L, Pasqualini M, Petrelli R, Jayaprakasan V, Jayaram HN, Boyd DB, Jain MD, Grifantini M (2005) Synthesis, conformational analysis, and biological activity of new analogues of thiazole-4-carboxamide adenine dinucleotide (TAD) as IMP dehydrogenase inhibitors. Bioorg Med Chem 13:2045–2053

Article CAS PubMed Google Scholar

Gu XH, Wan XZ, Jiang B (1999) Syntheses and biological activities of bis(3-indolyl)thiazoles, analogues of marine bis(indole) alkaloid nor topsentins. Bioorg Med Chem Lett 9:569–572

Article CAS PubMed Google Scholar

Kumar PSV, Suresh L, Chandramouli GVP (2015) Ionic liquid catalyzed multi-component synthesis, antifungal activity, docking studies and *in-silico* ADMET properties of novel fused chromeno-pyrazolo-phthalazine derivatives. J Saudi Chem Soc https://doi.org/10.1016/

j.jscs.2015.08.001

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

Article CAS PubMed Google Scholar

Lamberth C, Dumeunier R, Trah S, Wendeborn S, Godwin J, Schneiter P, Corran A (2013) Synthesis and fungicidal activity of tubulin polymerisation promoters. Part 3: Imidazoles. Bioorg Med Chem 21:127–134

Article CAS PubMed Google Scholar

Lima IO, De Medeiros Nobrega F, De Oliveira WA, Lima EO, Menezes EA, Cunha F, MeloDiniz AMFF (2012) Anti-candida albicans effectiveness of citral and investigation of mode of action. Pharma Biol 50:1536–1541

Article CAS Google Scholar

Medime E, Capan G (1994) Synthesis and anticonvulsant activity of new 4-thiazolidone and 4-thiazoline derivatives. II Farmaco 49:449–451

Google Scholar

Meunier B (2008) Hybrid molecules with a dual mode of action: dream or reality? Acc Chem Res 41:69–77

Article CAS PubMed Google Scholar

Nikalje AG, Ghodke MS, Kalam Khan FA, Sangshetti JN (2015) CAN catalyzed one-pot synthesis and docking study of some novel substituted imidazole coupled 1, 2, 4-triazole-5-carboxylic acids as antifungal agents. Chin Chem Lett 26:108–112

Article CAS Google Scholar

National Committee for Clinical Laboratory Standards (2002) Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts Approved Standard. Document M27-A2. National Committee for Clinical Laboratory Standards, Wayne, PA

Google Scholar

Pianalto KM, Alspaugh JA (2016) New horizons in antifungal therapy. J Fungi 2:1–24

Article Google Scholar

Roemer T, Krysan DJ (2014) Antifungal drug development: challenges, unmet clinical needs, and new approaches. Cold Spring Harb Perspect Med 4:a019703

Article PubMed PubMed Central Google Scholar

Sangshetti JN, Lokwani DK, Sarkate AP, Shinde DB (2011) Synthesis, antifungal activity, and docking study of some new 1,2,4-triazole analogs. Chem Biol Drug Des 78:800–809

Article CAS PubMed Google Scholar

Strushkevich N, Usanov SA, Park H (2010) Structural basis of human CYP51 inhibition by antifungal azoles. J Mol Biol 397:1067–1078

Article CAS PubMed Google Scholar

Shiradkar MR, Murahari KK, Reddy H, Tatikonda S, Chakravarthy AK, Panchal D, Kaur R, Burange P, Ghogare J, Mokalec V, Raut M (2007) Synthesis of new S-derivatives of clubbed triazolylthiazole as anti-Mycobacterium tuberculosis agents. Bioorg Med Chem 15:3997–4008

Article CAS PubMed Google Scholar

Tiwari SV, Seijas JA, Vazquez-Tato MP, Sarkate AP, Karnik KS, Nikalje AP (2017a) Facile synthesis of novel coumarin derivatives, antimicrobial analysis, enzyme assay, docking study, ADMET prediction and toxicity study. Molecules 22:1172–1180

Article Google Scholar

Tiwari SV, Nikalje APG, Lokwani DK, Sarkate AP, Jamir K (2017b) Synthesis, biological evaluation, molecular docking study and acute oral toxicity study of coupled Imidazolyl-Pyrimidine derivatives. Lett Drug Des Discov 14: https://doi.org/10.2174/1570180814666170704101817

Turan-Zitouni G, Kaplancikli ZA, Yildiz MT, Chevallet P, Kaya D (2005) Synthesis and antimicrobial activity of 4-phenyl/cyclohexyl-5-(1-phenoxyethyl)-3-[N-(2-thiazolyl)acetamido]thio-4H-1,2,4-triazole derivatives. Eur J Med Chem 40:607–613

Article CAS PubMed Google Scholar

Tsuruoka A, Kaku Y, Kakinuma H, Tsukada I, Yanagisawa M, Nara K, Naito T (1998) Synthesis and antifungal activity of novel thiazole-containing triazole antifungals. II. optically active ER-30346 and its derivatives. Chem Pharm Bull 46:623–630

Article CAS PubMed Google Scholar

Tsuruoka A, Kaku Y, Kakinuma H, Tsukada I, Yanagisawa M, Naito T (1997) Synthesis and antifungal activity of novel thiazole-containing triazole antifungals. Chem Pharm Bull 45:1169–1176

Article CAS PubMed Google Scholar

Nimbalkar UD, Tupe SG, Seijas Vazquez JA, Khan FAK, Sangshetti JN, Nikalje APG (2016) Ultrasound and molecular sieves-assisted synthesis, molecular docking and antifungal evaluation of 5-(4-(benzyloxy)-substituted phenyl)-3-((phenylamino)methyl)-1,3,4-oxadiazole-2(3H)-thiones. Molecules 21:484–497

Article Google Scholar

Vichai V, Kirtikara K (2006) Sulforhodamine B colorimetric assay for cytotoxicity screening. Nat Prot 1:1112–1116

Article CAS Google Scholar

Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD (2002) Molecular properties that influence the oral bioavailability of drug candidates. J Med Chem 45:2615–2623

Article CAS PubMed Google Scholar

Vandenberg JI, Walker BD, Campbell TJ (2001) HERG K^+ channels: friend and foe. Trends PharmacolSci 22:240–246

Article CAS Google Scholar

Yasnitskii BG, Dolberg EB (1971) Mechanism of the formation of 2-aminothiazole in the reaction of chloroacetaldehyde with thiourea. Chem Heterocycl Comp 7:866–868

Article Google Scholar

Zhai B, Lin XR (2011) Recent progress on antifungal drug development. Curr Pharm Biotechnol 12:1255–1262

Article CAS PubMed Google Scholar

Zhou CH, Mi JL (2009) Preparation of Fluotrimazole ether derivatives as antimicrobial agents. CN Patent, CN101391986 (A).

Zhao B, Zhou YC, Fan MJ, Li ZY, Wang LY, Deng QG (2013) Synthesis, fluorescence properties and selective Cr(III) recognition of tetraaryl imidazole derivatives bearing thiazole group. Chinese Chem Lett 24:257–259

Article CAS Google Scholar

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Author information

Authors and Affiliations

Y. B. Chavan College of Pharmacy, Dr. Rafiq Zakaria Campus, Rauza Bagh, Aurangabad, Maharashtra, 431001, India
Anna Pratima G. Nikalje & Shailee V. Tiwari

Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra, 431004, India
Aniket P. Sarkate & Kshipra S. Karnik

Corresponding author

Correspondence to Anna Pratima G. Nikalje.

Ethics declarations

Conflict of interest

The authors declare that they have no competing interests.

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