Synthesis, antifungal activity, and molecular docking study of some novel highly substituted 3-indolylthiophene derivatives

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Background and objectives
The currently available antifungal drugs have the limitations of toxicity, potential drug interaction with other drugs, insufficient pharmacokinetics properties, and development of resistance. Thus, development of new antifungal agents with less toxicity is urgently required. The present work aimed to synthesize new 3-indolylthiophene derivatives and evaluate their antifungal activity by studying their molecular docking.

Materials and methods
New series of thiadiazoles **4a-c**, morpholinyl-acetamides **6a-c**, 4-methylpiperazinylacetamides **7a-c**, thiazolidines **10a-c**, azetidines **12a-c**–**13a-c**, sulfonamides **14a-c**–**15a-c**, benzamides **16a-c**, pyrrolidines **17a-c**, succinamic acids **18a-c**, acetamides **19a-c**, thieno(2,3-*c*)pyridines **20a-c**, thieno(2,3-*e*)-1,2,4-triazolo(1,5-*c*)pyrimidines **23a-c**, thieno(2,3-*d*) pyrimidines **24a-c**–**26a-c**, and thieno(2,3-*b*)pyridines **27a-c** derivatives incorporated into *N*-substituted 3-indolylthiophenes were prepared by an initial reaction of 2-amino-4-(*N*-substituted-1*H*-indol-3-yl)thiophene-3-carbonitriles **1a-c** with different reagents. The antifungal activity of the newly synthesized compounds was evaluated against two strains of fungi, namely, *Candida albicans* (ATCC-10231) and *Aspergillus niger* (ATCC-10535). However, the mode of action of the most promising antifungal compounds was assessed by docking with cytochrome P450 14 α-sterol demethylase (CYP51) (PDB ID: 1EA1).

Results and conclusion
Compound **4a** showed good inhibitory activity against both *C. albicans* (ATCC-10231) and *A. niger* **(**ATCC-10535), with minimum inhibitory concentrations values of 9 and 36 μg/disk, respectively, compared with fluconazole, with minimum inhibitory concentrations values of 8 and 34 μg/disk. Docking results showed that compound **4a** had the highest docking score, with a binding energy of –30.25 kJ/mol, which is in agreement with the experimental activity value.

Keywords:
2-aminothiophene-3-carbonitriles, antifungal activity, heterocycles, indole, molecular docking

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Introduction

Thiophene derivatives are important heterocyclics found in various biologically active and natural compounds [1–5]. Especially, 2-aminothiophene has a broad spectrum, and is used in pharmaceuticals [6,7] and as starting materials for of the construction of fused heterocycle systems [8–11]. Also, indole nucleus and its derivatives have unique biological activities such as anti-inflammatory [12,13], anticancer [13,14], and antimicrobial activities [15,16]. On the basis of these findings and in view of our continuous work on the synthesis of new indole heterocycle derivatives with biological activities [12–14,16], here, we report on the synthesis of a novel series of thiophene derivatives starting from and 2-amino-4-(*N*-substituted-1*H*-indol-3-yl)thiophene-3-carbonitriles **1a-c** [17] and evaluate their antifungal activity. In addition, a molecular docking study of the most biologically active compounds was carried out using the Molecular Operating Environment (MOE) program and cytochrome P450 14 α-sterol demethylase (CYP51) (PDB ID: 1EA1) for a better understanding of the drug–receptor interaction.

Materials and methods

Chemistry

The chemicals and solvents were of commercial grade and used without further purification. Melting points were determined in open capillary tubes on an Electrothermal 9100 digital melting point apparatus (serial no. 8694; Electrothermal Engineering Ltd, Rochford, UK) and were uncorrected. Elemental analyses were carried out on a Perkin-Elmer 2400 analyzer (Perkin-Elmer, Waltham, Massachusetts, USA) and were found to be within ±0.4% of the theoretical values. Infrared (IR) spectra were recorded on a Perkin-Elmer 1600 FTIR spectrophotometer (Perkin-Elmer). The nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance digital spectrometer at 500 MHz for ^{1}H and at 125 MHz for ^{13}C (Bruker BioSpin GmbH, Rheinstetten, Germany) in DMSO-d_6; chemical shifts (δ) are reported in ppm units relative to the internal tetramethylsilane standard. Mass spectra (EI) were recorded using a Jeol-JMS-AX500 mass spectrometer (Jeol Ltd, Tokyo, Japan) at 70 eV.

*Ethyl-2-(3-cyano-4-(N-substituted-1**H**-indol-3-yl) thiophen-2-ylamino)acetate **2a-c***

A mixture of compound **1a**, **1b**, or **1c** (0.35 mol), ethyl chloroacetate (42.87 g, 0.35 mol), and potassium carbonate (6.21 g, 0.045 mol) in absolute methanol (300 ml) was kept overnight at room temperature. The reaction mixture was heated at reflux on a steam bath for 1–3 h. After cooling, the reaction mixture was poured onto cold water (20 ml) and the solid formed was filtered off, air-dried, and crystallized from absolute ethanol.

*2-(2-(3-Cyano-4-(N-substituted-1H-indol-3-yl)thiophen-2-ylamino)acetyl)hydrazine carbothioamides **3a-c***

A mixture of compound **2a**, **2b**, or **2c** (0.17 mol) and thiosemicarbazide (15.49 g, 0.17 mol) in absolute methanol (250 ml) was heated at reflux on a steam bath for 10–13 h. The reaction mixture was filtered while hot. After cooling, the filtrate was poured onto cold water (20 ml). The solid formed was filtered off, air-dried, and crystallized from absolute ethanol.

*2-(5-Amino-1,3,4-thiadiazol-2-yl-methylamino)-4-(N-substituted-1H-indol-3-yl) thiophene-3-carbonitriles **4a-c***

A mixture of compound **3a**, **3b**, or **3c** (0.15 mol) and concentrated sulfuric acid (6 ml) in absolute methanol (50 ml) was kept overnight at room temperature. The reaction mixture was heated under reflux on a steam bath for 7–8 h. After cooling, the reaction mixture was neutralized with an ammonia solution (25%) and the solid formed was filtered off, washed with water, air-dried, and crystallized from absolute ethanol.

*N-(3-Cyano-4-(N-substituted-1H-indol-3-yl) thiophen-2-yl)-2-morpholinyl-acetamides **6a-c** and N-(3-cyano-4-(N-substituted-1H-indol-3-yl) thiophen-2-yl)-2- (4-methylpiperazinyl)acetamides **7a-c***

A mixture of compound **5a**, **5b**, or **5c** (0.006 mol) and morpholine or *N*-methylpiperazine (0.006 mol) in acetone (50 ml) containing potassium carbonate (1.65 g, 0.012 mol) was heated at reflux for 15–20 h. The solvent was evaporated under vacuo to half its volume and the solid formed was filtered off, washed with a 5% sodium bicarbonate solution and then with water, air-dried, and crystallized from absolute ethanol.

*N-(3-Cyano-4-(N-substituted-1H-indol-3-yl)thiophen-2-yl)-2-hydrazinyl acetamides **8a-c***

A mixture of compound **5a**, **5b**, or **5c** (0.02 mol) and hydrazine hydrate (1 ml, 0.02 mol, 80%) in absolute methanol (30 ml) was heated at reflux for 9–10 h. After cooling, the solid formed was filtered off, air-dried, and crystallized from chloroform.

*2-(N-Benzylidenehydrazinyl)-N-(3-cyano-4-(N-substituted-1H-indol-3-yl) thiophen-2-yl)acetamides **9a-c***

A mixture of compound **8a**, **8b**, or **8c** (0.01 mol) and benzaldehyde (1.06 g, 0.01 mol) in absolute methanol containing a few drops of glacial acetic acid was heated at reflux for 4–6 h. After cooling, the reaction mixture was concentrated to half its volume and the solid formed was filtered off, air-dried, and crystallized from chloroform.

*N-(3-Cyano-4-(N-substituted-1H-indol-3-yl)thiophen-2-yl)-2-(4-oxo-2-phenylthiazolidin-3-ylamino)acetamides **10a-c***

To a stirred solution of compound **9a**, **9b**, or **9c** (0.01 mol) in absolute methanol (25 ml), thioglycolic acid (1.39 g, 0.015 mol) was added. The reaction mixture was stirred for 4 h; then, anhydrous sodium sulfate (30 g) was added and heated at reflux for a further 6 h. The reaction mixture was filtered while hot. After cooling, the solid formed was filtered off, air-dried, and crystallized from chloroform.

*2-(3-Chloro-2-(4-nitrophenyl)-4-oxo-azetidin-1-yl)-4-(N-substituted-1H-indol-3-yl)thiophene-3-carbonitriles **12a-c** and 4-(N-substituted-1H-indol-3-yl)-2-(2-(4-nitrophenyl)-4-oxo-3-phenylazetidin-1-yl)thiophene-3-carbonitriles **13a-c***

To a solution of compound **11a**, **11b**, or **11c** (0.01 mol) in dry dioxane (5 ml), a solution of chloroacetyl chloride or phenacyl bromide (0.01 mol) in dry dioxane and triethylamine (0.59 ml, 0.01 mol) was added. The reaction mixture was heated at reflux for 15–16 h. The reaction mixture was filtered off while hot and the solvent was removed under vacuum. The residue that formed was collected, washed with water, air-dried, and crystallized from absolute ethanol.

*N-(3-Cyano-4-(N-substituted-1H-indol-3-yl) thiophen-2-yl)benzene-sulfonamides **14a-c** and 4-bromo-N-(3-cyano-4-(N-substituted-1H-indol-3-yl)thiophen-2-yl) benzenesulfonamides **15a-c***

A mixture of compound **1a**, **1b**, or **1c** (0.01 mol) and benzenesulfonyl chloride or 4-bromobenzenesulfonyl chloride (0.01 mol) in dry dioxane (30 ml) containing a few drops of triethylamine was heated at reflux for 8 h. After cooling, the reaction mixture was poured onto cold water (20 ml). The solid formed was filtered off, air-dried, and crystallized from dioxane.

*N-(3-Cyano-4-(substituted-1H-indol-3-yl)thiophen-2-yl) benzamides **16a-c***

A mixture of compound **1a**, **1b**, or **1c** (0.01 mol) and benzoyl chloride (0.01 mol) in dry dioxane (30 ml) containing a few drops of triethylamine was heated at reflux for 8 h. After cooling, the reaction mixture was poured onto cold water (20 ml). The solid

formed was filtered off, air-dried, and crystallized from dioxane.

2-(2,5-Dioxopyrrolidin-1-yl)-4-(N-substituted-1H-indol-3-yl)thiophene-3-carbonitriles ***17a-c***

A mixture of compound **1a**, **1b**, or **1c** (0.01 mol) and succinic anhydride (1 g, 0.01 mol) was heated at 180°C in a test tube on a sand bath for 4 h. After cooling, the product was solidified by the addition of absolute ethanol (50 ml). The solid formed was filtered off, washed with water, air-dried, and crystallized from dimethylformamide.

N-(3-Cyano-4-(N-substituted-1H-indol-3-yl)thiophen-2-yl) succinamic acids ***18a-c***

A mixture of compound **1a**, **1b**, or **1c** (0.01 mol) and succinic anhydride (1 g, 0.01 mol) in absolute ethanol (10 ml) was heated at reflux for 10 h. After cooling, the reaction mixture was poured onto cold water (20 ml). The solid formed was filtered off, air-dried, and crystallized from dimethylformamide–water.

N-(3-Cyano-4-(N-substituted-1H-indol-3-yl)thiophen-2-yl) acetamides ***19a-c***

A solution of compound **1a**, **1b**, or **1c** (0.01 mol) in a mixture of (10 ml) acetic anhydride and glacial acetic acid (2 : 1) was heated at reflux for 8–10 h. After cooling, the reaction mixture was poured onto ice-water (20 ml) and the solid formed was filtered off, air-dried, and crystallized from absolute ethanol.

3-(N-Substituted-1H-indol-3-yl)-6-methyl-5H-thieno(2,3-c) pyridin-4-ones ***20a-c***

A solution of compound **1a**, **1b**, or **1c** (0.01 mol) in a mixture of concentrated hydrochloric acid and glacial acetic acid (20 ml, 3 : 1) was heated at reflux for 6 h. After cooling, the reaction mixture was poured onto ice-water (20 ml) and the solid formed was filtered off, air-dried, and crystallized from dioxane.

Ethyl N-3-cyano-4-(N-substituted-1H-indol-3-yl)thiophen-2-yl formamidates ***21a-c***

A solution of compound **1a**, **1b**, or **1c** (0.01 mol) in triethylorthoformate (12 ml) was heated at reflux for 18 h. Excess triethylorthoformate was removed under vacuo and the residue was triturated with ethanol (10 ml). The solid separated was filtered, air-dried, and crystallized from absolute ethanol.

4-Imino-5-(N-substituted-1H-indol-3-yl)-4H-thieno(2,3-d) pyrimidin-3-yl-amines ***22a-c***

A mixture of compound **21a**, **21b**, or **21c** (0.01 mol) and hydrazine hydrate (10 ml, 0.2 mol, 80%) was stirred in absolute ethanol (20 ml) for 2 h at room temperature. The reaction mixture was poured onto cold water (10 ml) and the solid formed was filtered off, air-dried, and crystallized with dioxane.

9-(N-Substituted-1H-indol-3-yl)thieno(2,3-e)[1,2,4] triazolo(1,5-c)pyrimidines ***23a-c***

A mixture of compound **22a**, **22b**, or **22c** (0.05 mol) and triethylorthoformate (0.6 ml) in dimethylformamide (2 ml) was heated at reflux on a water bath for 2–3 h. After cooling, the reaction mixture was poured onto cold water (10 ml) and the solid formed was filtered off, air-dried, and crystallized from absolute ethanol–chloroform.

5-(N-Substituted-1H-indol-3-yl)-1,2,3,4-tetrahydrothieno(2,3-d)pyramidi-ne-2,4-dithiones ***24a-c***

A mixture of compound **1a**, **1b**, or **1c** (0.01 mol) and excess of carbon disulfide (10 ml) in absolute ethanolic potassium hydroxide solution [1.12 g, 0.02 mol of KOH in absolute ethanol (20 ml)] was heated at reflux for 12 h. The excess carbon disulfide was evaporated under vacuo and the residue obtained was dissolved in water (20 ml). The reaction mixture was filtered off and the filtrate was acidified with diluted hydrochloric acid (1 : 1). The solid that formed was filtered off, washed with water, air-dried, and crystallized from dimethylformamide — water.

4-Amino-5-(N-substituted-1H-indol-3-yl)-1H-thieno(2,3-d) pyrimidin-2-ones ***25a-c*** *and 4-amino-5-(N-substituted-1H-indol-3-yl)-1H-thieno(3,2-d)pyrimidine-2-thiones* ***26a-c***

A mixture of compound **1a**, **1b**, or **1c** (0.01 mol), urea, and/or thiourea (0.01 mol) was heated at 180°C in a test tube on a sand bath for 4 h. After cooling, the reaction mixture was solidified with the addition of absolute ethanol (50 ml). The solid formed was filtered off, washed with water, air-dried, and recrystallized from dimethylformamide.

5-Amino-6-hydroxy-3-(N-substituted-1H-indol-3-yl) thieno(2,3-b)pyridine-6-carbonitriles ***27a-c***

A mixture of compound **1a**, **1b**, or **1c** (0.01 mol) and ethylcyanoacetate (1.13 g, 0.01 mol) in dry dioxane (30 ml) containing a few drops of piperidine was heated at reflux for 8–10 h. After cooling, the reaction mixture was poured onto cold water (20 ml). The solid formed was filtered off, air-dried, and crystallized from dioxane.

Biological assay

Cells

Fungal strains, namely, *Candida albicans* (ATCC-10231) and *Aspergillus niger* (ATCC-10535), were

supplied from the American Type Culture Collection (Biomerieux Inc., Durham, North Carolina, USA).

Antifungal assay

Antifungal activity of new synthesized compounds was determined *in vitro* using the disc diffusion method [18] against *C. albicans* (ATCC-10231) and *A. niger* (ATCC-10535). The antifungal activities of the tested compounds were estimated by placing presterilized filter paper discs (6 mm in diameter) impregnated with tested compounds at 200 μg/disc on nutrient and Sabouraud dextrose agar. Dimethylformamide was used as a solvent for impregnation. Inhibition zones of the test compounds were measured after 5 days of incubation at 28°C. Fluconazole (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) was used as the reference drug. Minimum inhibitory concentration (MIC) was read as the lowest concentration of the test compounds at which the test strain dose shows no visible growth. MIC was determined twice in duplicate experiments.

Molecular docking study

Docking studies of the most active compounds were carried out using the MOE 2008.10 release (Chemical Computing Group, Montreal, Quebec, Canada; *http://www.chemcomp.com*). The program operated on an Intel(R) core(TM) i5-3210M CPU@2.50 GHz, 2.50 GHz processor, 4.00 GB memory, with a Windows 7 Ultimate operating system. The protein crystal structure of cytochrome P450 14 α-sterol demethylase (CYP51) (PDB ID: 1EA1) in complex with 2-(2,4-difluorophenyl)-1,3-di(1*H*-1,2,4-triazol-1-yl)propan-2-ol (fluconazole) was downloaded from *http://www.rcsb.org/-pdb* (ID: 1EA1) [19], refinement of the crude PDB structure was performed, and then saved as MOE file to be used for docking simulation.

The active pocket was considered to be the site where fluconazole complexes with cytochrome P450 14 α-sterol demethylase (CYP51) (PDB ID: 1EA1). The active pocket consisted of 23 amino acid residues such as Ala256, Gln72, Phe255, Met79, Leu100, Arg96, Tyr76, Arg326, His392, Leu324, Gly388, Leu321, Cys394, Pro386, Phe78, Thr264, Ala400, Ser261, Phe399, Gly396, and Thr260. The structures of ligands were drawn in ChemDraw Ultra 7.0 (Cheminformatics Software company based in Cambridge, Massachusetts, USA) and saved as mol. The two-dimensional structure of the selected compounds was converted into their three-dimensional form and energy minimized using the MMFF94x force field until a root-mean-square deviation of atomic position gradient of 0.01 Kcal/mol/Å was reached [20]. The docking scores were expressed in negative energy terms; the lower the binding free energy, the better the binding affinity [21].

Results and discussion

Chemistry

The reaction route for the synthesis of the target compounds is outlined in Schemes 1–3. The reaction of 2-amino-4-(*N*-substituted-1*H*-indol-3-yl)thiophene-3-carbonitriles **1a-c** [17] with chloroethyl acetate in absolute methanol in the presence of potassium carbonate as a base yielded ethyl-2-(3-cyano-4-(*N*-substituted-1*H*-indol-3-yl)thiophen-2-ylamino) acetate **2a-c** (Scheme 1). Treatment of compounds **2a-c** with thiosemicarbazide yielded hydrazine carbothioamide derivatives **3a-c**. Cyclization of the latter compounds by their reaction with equimolar amounts of sulfuric acid in absolute methanol led to the formation of 2-(5-amino-1,3,4-thiadiazol-2-ylmethylamino)-4-(*N*-substituted-1*H*-indol-3-yl) thiophene-3-carbonitriles **4a-c** (Scheme 1).

The reaction of compounds **1a-c** with chloroacetyl chloride in dry benzene led to the formation of *N*-chloroacetamido derivatives **5a-c** (Scheme 1) [17]. The reaction of **5a-c** with morpholine or *N*-methylpiperazine in acetone and in the presence of potassium carbonate yielded *N*-(3-cyano-4-(*N*-substituted-1*H*-indol-3-yl)thiophen-2-yl)-2-(morpholinyl)acetamides **6a-c** and *N*-(3-cyano-4-(*N*-substituted-1*H*-indol-3-yl) thiophen-2-yl)-2-(4-methylpiperazinyl)acetamides **7a-c**, respectively (Scheme 1).

Amination of chloroacetamido compounds **5a-c** with hydrazine hydrate in absolute methanol yielded the corresponding hydrazinylacetamide derivatives **8a-c** (Scheme 1). Acid-catalyzed reaction of the latter compounds with benzaldehyde under reflux in absolute methanol yielded the corresponding Schiff's bases, namely, *N*-(3-cyano-4-(*N*-substituted-1*H*-indol-3-yl)thiophen-2-yl)-2-(*N*-benzylidenehydrazino) acetamides **9a-c** (Scheme 1). Cyclization of the latter Schiff's bases by their reaction with thioglycolic acid in dry dioxane in the presence of anhydrous sodium sulfate led to the formation of *N*-(3-cyano-4-(*N*-substituted-1*H*-indol-3-yl)thiophen-2-yl)-2-(4-oxo-2-phenyl thiazolidin-3-yl)amino)acetamides **10a-c** (Scheme 1).

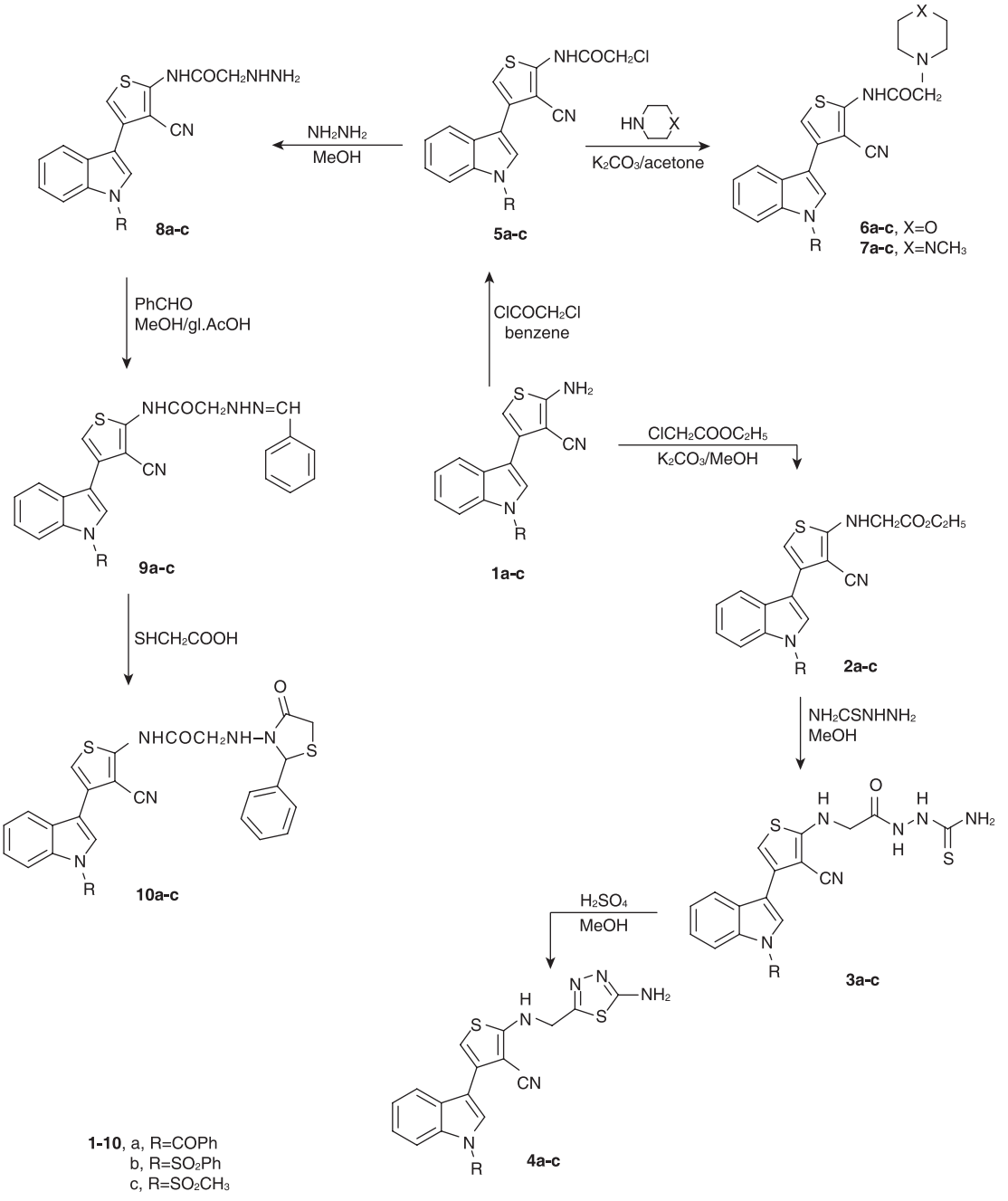
However, acid-catalyzed reaction of compounds **1a-c** with 4-nitrobenzaldehyde yielded the corresponding Schiff's bases **11a-c** [17] (Scheme 2). The reaction of **11a-c** with chloroacetyl chloride and/or phenacyl bromide in dry dioxane in the presence of triethylamine

as a base led to the formation of 2-(3-chloro-2-(4-nitrophenyl)-4-oxo-cyclobutyl)-4-(*N*-substituted-1*H*-indol-3-yl)thiophene-3-carbonitriles **12a-c** and 4-(*N*-substituted-1*H*-indol-3-yl)-2-(2-(4-nitrophenyl)-4-oxo-3-phenylcyclobutyl)thiophene-3-carbonitriles **13a-c**, respectively (Scheme 2).

The reaction of compounds **1a-c** with benzenesulfonyl chloride, 4-bromobenzenesulfonyl chloride and/or benzoyl chloride under reflux in dry dioxane and in the presence of triethylamine yielded the corresponding *N*-(3-cyano-4-(*N*-substituted-1*H*-indol-3-yl) thiophen-2-yl)benzene sulfonamides **14a-c**, 4-bromo-*N*-(3-cyano-4-(*N*-substituted-1*H*-indol-3-yl) thiophen-2-yl)benzene sulfonamides **15a-c** and *N*-(3-cyano-4-(*N*-substituted-1*H*-indol-3-yl)thiophen-2-yl)benzamides **16a-c**, respectively (Scheme 2).

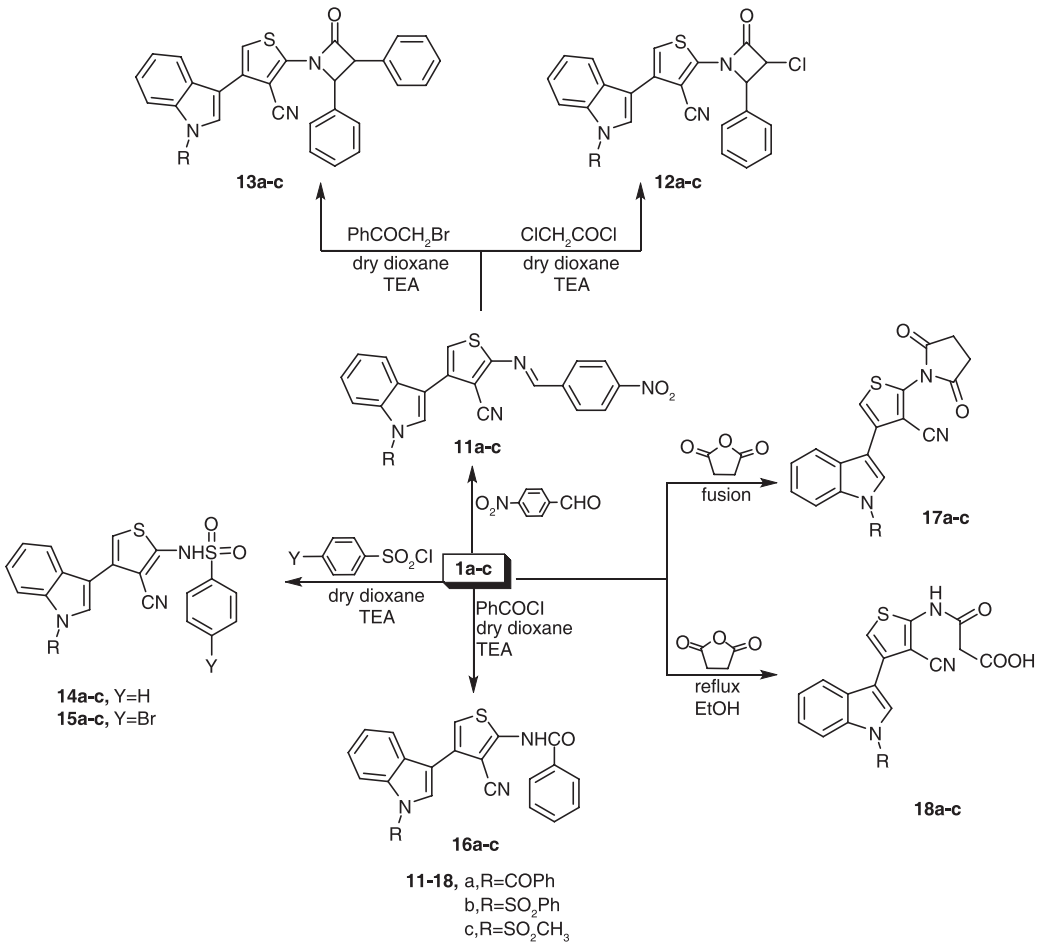
However, fusion of compounds **1a-c** with succinic anhydride yielded 2-(2,5-dioxopyrrolidin-1-yl)-4-(*N*-substituted-1*H*-indol-3-yl)thiophene-3-carbonitriles **17a-c** (Scheme 2), whereas heating of compounds **1a-c** with succinic anhydride in absolute ethanol yielded *N*-(3-cyano-4-(*N*-substituted-1*H*-

Scheme 1



Synthesis of compounds **1a-c**–**10a-c**.

Scheme 2



Synthesis of compounds **11a-c**–**18a-c**.

indol-3-yl)thiophen-2-yl)succinamic acids **18a-c** (Scheme 2).

Moreover, acetylation of compounds **1a-c** under reflux in acetic anhydride and glacial acetic acid (2 : 1) yielded the corresponding *N*-(3-cyano-4-(*N*-substituted-1*H*-indol-3-yl)thiophen-2-yl)acetamides **19a-c** (Scheme 3), whereas heating of compounds **1a-c** in a mixture of concentrated hydrochloric acid and glacial acetic acid (3 : 1) yielded the fused 5-(*N*-substituted-1*H*-indol-3-yl)-2-methyl-3*H*-thieno(2,3-*d*) pyrimidin-4-ones **20a-c** (Scheme 3).

Condensation of compounds **1a-c** with excess triethylorthoformate at reflux yielded ethyl *N*-3-cyano-4-(*N*-substituted-1*H*-indol-3-yl)thiophen-2-ylformamidates **21a-c** (Scheme 2). Cyclization of the latter compounds upon treatment with hydrazine hydrate in absolute ethanol under reflux yielded the fused thieno(2,3-*d*)pyrimidine derivatives **22a-c**, which, under further cyclization, upon heating with triethylorthoformate in dimethylformamide, led to the formation of fused thieno(2,3-*e*)-1,2,4-triazolo(1,5-*c*) pyrimidine derivatives **23a-c** (Scheme 3).

The reaction of compounds **1a-c** with excess carbon disulfide in absolute ethanolic potassium hydroxide solution yielded the fused 1,2,3,4-tetrahydrothieno(2,3-*d*) pyrimidine-2,4-dithione derivatives **24a-c** (Scheme 3).

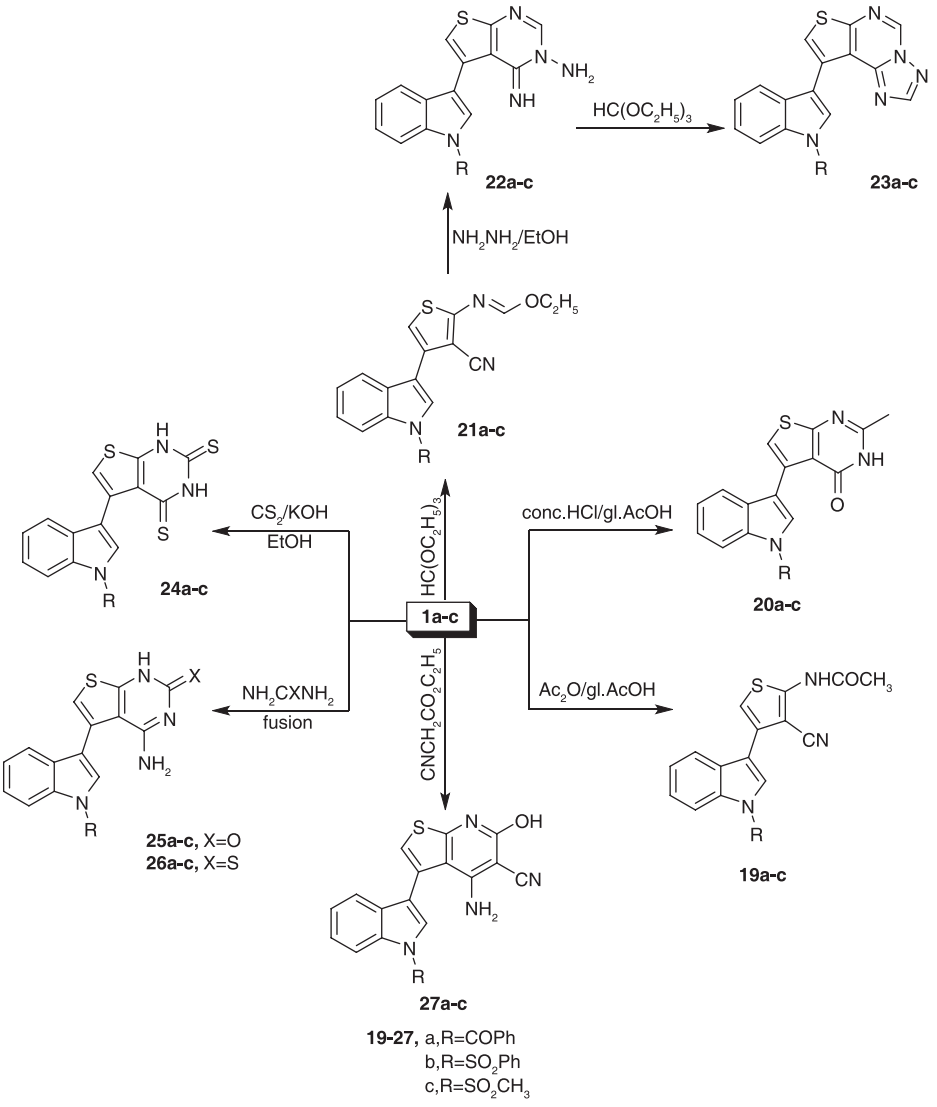
However, fusion of compounds **1a-c** with urea and/or thiourea yielded the fused thieno(2,3-*d*)pyrimidine derivatives **25a-c** and thieno(2,3-*d*)pyrimidine-2-thione derivatives **26a-c**, respectively (Scheme 3), whereas the reaction of compounds **1a-c** with ethyl cyanoacetate under reflux in dry dioxane and in the presence of piperidine as a catalyst yielded thieno(2,3-*b*) pyridine-5-carbonitrile derivatives **27a-c** (Scheme 3).

The structures of the newly synthesized compounds were confirmed on the basis of elemental analyses (Table 1) as well as IR, NMR, and mass spectral data (Table 2).

Antifungal activity

The in-vitro antifungal activity of the newly prepared compounds was evaluated against *C. albicans* (ATCC-10231) and *A. niger* **(**ATCC-10535) using fluconazole

Scheme 3



Synthesis of compounds **19a-c**–**27a-c**.

Table 1 Physical and analytical data of the synthesized compounds

Compound numbers	Molecular formula (molecular weight)	Melting point (°C)	Yield (%)	Analysis calculated %/found		
				C	H	N
2a	$C_{24}H_{19}N_3O_3S$ (429.49)	146–148	70	67.12/67.00	4.46/4.31	9.78/9.60
2b	$C_{23}H_{19}N_3O_4S_2$ (465.54)	121–123	69	59.34/59.20	4.11/4.00	9.03/9.00
2c	$C_{18}H_{17}N_3O_4S_2$ (403.48)	139–141	60	53.58/53.65	4.25/4.05	10.41/10.28
3a	$C_{23}H_{18}N_6O_2S_2$ (474.56)	169–171	80	58.21/58.02	3.82/4.00	17.71/17.56
3b	$C_{22}H_{18}N_6O_3S_3$ (510.61)	277–279	70	51.75/51.52	3.55/3.65	16.46/16.50
3c	$C_{17}H_{16}N_6O_3S_3$ (448.54)	232–234	65	45.52/45.45	3.60/3.46	18.74/18.58
4a	$C_{23}H_{16}N_6OS_2$ (456.54)	197–199	60	60.51/60.38	3.53/3.60	18.41/18.25
4b	$C_{22}H_{16}N_6O_2S_3$ (492.60)	265–267	55	53.64/53.51	3.27/3.10	17.06/17.00
4c	$C_{17}H_{14}N_6O_2S_3$ (430.53)	166–168	46	47.43/47.26	3.28/3.15	19.52/19.34
6a	$C_{26}H_{22}N_4O_3S$ (470.54)	132–134	70	66.37/66.20	4.71/4.59	11.91/11.82
6b	$C_{25}H_{22}N_4O_4S_2$ (506.60)	221–223	61	59.27/59.04	4.38/4.20	11.06/11.00
6c	$C_{20}H_{20}N_4O_4S_2$ (444.53)	59–61	60	54.04/54.22	4.53/4.45	12.60/12.54
7a	$C_{27}H_{25}N_5O_2S$ (483.58)	182–184	55	67.06/67.11	5.21/5.00	14.48/14.30
7b	$C_{26}H_{25}N_5O_3S_2$ (519.64)	93–95	50	60.10/60.21	4.85/5.00	13.48/13.51
7c	$C_{21}H_{23}N_5O_3S_2$ (457.57)	130–132	41	55.12/55.00	5.07/5.00	15.31/15.24
8a	$C_{22}H_{17}N_5O_2S$ (415.47)	132–134	60	63.60/63.43	4.12/4.00	16.86/16.71
8b	$C_{21}H_{17}N_5O_3S_2$ (451.52)	116–118	55	55.86/56.00	3.79/3.61	15.51/15.36
8c	$C_{16}H_{15}N_5O_3S_2$ (389.45)	104 decomposition	49	49.34/49.18	3.88/4.00	17.98/17.80

(*Continued*)

Table 1 Continued

Compound numbers	Molecular formula (molecular weight)	Melting point (°C)	Yield (%)	Analysis calculated %/found		
				C	H	N
9a	$C_{29}H_{21}N_5O_2S$ (503.57)	198–200	60	69.17/69.00	4.20/4.03	13.91/14.00
9b	$C_{28}H_{21}N_5O_3S_2$ (539.63)	71–73	56	62.32/62.12	3.92/4.00	12.98/13.00
9c	$C_{23}H_{19}N_5O_3S_2$ (477.56)	170–172	52	57.85/58.00	4.01/4.22	14.66/14.46
10a	$C_{31}H_{23}N_5O_3S_2$ (577.68)	100–102	45	64.45/64.57	4.01/3.91	12.12/12.00
10b	$C_{30}H_{23}N_5O_4S_3$ (613.73)	101–102	40	58.71/58.54	3.78/3.61	11.41/11.30
10c	$C_{25}H_{21}N_5O_4S_3$ (551.66)	120–122	32	54.43/54.31	3.84/3.70	12.70/12.54
12a	$C_{29}H_{17}ClN_4O_4S$ (552.99)	85–87	50	62.99/63.03	3.10/3.21	10.13/10.02
12b	$C_{28}H_{17}ClN_4O_5S_2$ (589.04)	344 dec.	45	57.09/57.22	2.91/3.04	9.51/9.40
12c	$C_{23}H_{15}ClN_4O_5S_2$ (526.97)	216–218	32	52.42/52.26	2.87/3.00	10.63/10.51
13a	$C_{35}H_{22}N_4O_4S$ (594.64)	99–101	49	70.69/70.56	3.73/3.61	9.42/9.26
13b	$C_{34}H_{22}N_4O_5S_2$ (630.69)	219–221	45	64.75/64.59	3.52/3.43	8.88/9.00
13c	$C_{29}H_{20}N_4O_5S_2$ (568.09)	75–77	32	61.26/61.08	3.55/3.41	9.85/10.01
14a	$C_{26}H_{17}N_3O_3S_2$ (483.56)	55–57	70	64.58/64.40	3.54/3.32	8.69/8.51
14b	$C_{25}H_{17}N_3O_4S_3$ (519.62)	70–72	66	57.79/57.61	3.30/3.48	8.09/8.00
14c	$C_{20}H_{15}N_3O_4S_3$ (457.55)	80–82	56	52.50/52.36	3.30/3.21	9.18/9.05
15a	$C_{26}H_{16}BrN_3O_3S_2$ (562.46)	61–63	66	55.52/55.38	2.87/3.00	7.47/7.31
15b	$C_{25}H_{16}BrN_3O_4S_3$ (598.51)	69–71	65	50.17/50.02	2.69/2.71	7.02/7.12
15c	$C_{20}H_{14}BrN_3O_4S_3$ (536.44)	73–75	50	44.78/44.59	2.63/2.50	7.83/7.68
16a	$C_{27}H_{17}N_3O_2S$ (447.51)	123–125	56	72.47/72.31	3.83/3.99	9.39/9.21
16b	$C_{26}H_{17}N_3O_3S_2$ (483.56)	73–75	50	64.58/64.65	3.54/3.42	8.69/8.51
16c	$C_{21}H_{15}N_3O_3S_2$ (421.06)	79–81	49	59.84/60.01	3.59/3.68	9.97/10.00
17a	$C_{24}H_{15}N_3O_3S$ (425.46)	372–374	50	67.75/67.59	3.55/3.37	9.88/9.65
17b	$C_{23}H_{15}N_3O_4S_2$ (461.51)	395–397	46	59.86/60.01	3.28/3.15	9.10/9.30
17c	$C_{18}H_{13}N_3O_4S_2$ (399.44)	342–346	36	54.12/54.04	3.28/3.09	10.52/10.35
18a	$C_{24}H_{17}N_3O_4S$ (443.47)	140–142	61	65.00/64.93	3.86/3.70	9.48/9.61
18b	$C_{23}H_{17}N_3O_5S_2$ (479.53)	119–121	56	57.61/57.46	3.57/3.63	8.76/8.57
18c	$C_{18}H_{15}N_3O_5S_2$ (417.46)	165–167	45	51.79/51.82	3.62/3.42	10.07/10.14
19a	$C_{22}H_{15}N_3O_2S$ (385.44)	168–170	60	68.55/68.61	3.92/3.85	10.90/11.00
19b	$C_{21}H_{15}N_3O_3S_2$ (421.49)	127–129	59	59.84/59.66	3.59/3.63	9.97/9.84
19c	$C_{16}H_{13}N_3O_3S_2$ (359.42)	95–97	55	53.47/53.30	3.65/3.45	11.69/11.51
20a	$C_{22}H_{15}N_3O_2S$ (385.44)	221–223	67	68.55/68.38	3.92/4.00	10.90/10.81
20b	$C_{21}H_{15}N_3O_3S_2$ (421.49)	152–154	60	59.84/59.73	3.59/3.42	9.97/10.01
20c	$C_{16}H_{13}N_3O_3S_2$ (359.42)	177–179	55	53.47/53.31	3.65/3.49	11.69/11.55
21a	$C_{23}H_{17}N_3O_2S$ (399.46)	53–55	45	69.15/69.00	4.29/4.16	10.52/10.38
21b	$C_{22}H_{17}N_3O_3S_2$ (435.52)	120–122	30	60.67/60.48	3.93/4.00	9.65/9.52
21c	$C_{17}H_{15}N_3O_3S_2$ (373.45)	105–107	39	54.67/54.50	4.05/4.22	11.25/11.14
22a	$C_{21}H_{15}N_5OS$ (385.44)	134–136	50	65.44/65.25	3.92/3.80	18.17/18.02
22b	$C_{20}H_{15}N_5O_2S_2$ (421.50)	164–166	40	56.99/57.11	3.59/3.41	16.62/16.51
22c	$C_{15}H_{13}N_5O_2S_2$ (359.43)	228–230	36	50.12/50.00	3.65/3.55	19.48/19.57
23a	$C_{22}H_{13}N_5OS$ (395.44)	189–191	40	66.82/66.70	3.31/3.51	17.71/17.60
23b	$C_{21}H_{13}N_5O_2S_2$ (431.49)	197–199	30	58.45/58.51	3.04/3.23	16.23/16.08
23c	$C_{16}H_{11}N_5O_2S_2$ (369.42)	184–186	15	52.02/52.12	3.00/3.10	18.96/18.82
24a	$C_{21}H_{13}N_3OS_3$ (419.54)	199–201	60	60.12/60.03	3.12/3.24	10.02/10.11
24b	$C_{20}H_{13}N_3O_2S_4$ (455.60)	90–92	59	52.73/52.60	2.88/3.05	9.22/9.03
24c	$C_{15}H_{11}N_3O_2S_4$ (393.53)	103–105	49	45.78/45.62	2.82/2.70	10.68/10.52
25a	$C_{21}H_{14}N_4O_2S$ (386.43)	168–170	70	65.27/65.31	3.65/3.51	14.50/14.38
25b	$C_{20}H_{14}N_4O_3S_2$ (422.48)	152 dec.	66	56.86/57.00	3.34/3.20	13.26/13.10
25c	$C_{15}H_{12}N_4O_3S_2$ (360.41)	213–215	59	49.99/50.01	3.36/3.41	15.55/15.63
26a	$C_{21}H_{14}N_4OS_2$ (402.49)	262–264	72	62.67/62.49	3.51/3.43	13.92/14.01
26b	$C_{20}H_{14}N_4O_2S_3$ (438.55)	244–246	65	54.78/54.63	3.22/3.10	12.78/12.60
26c	$C_{15}H_{12}N_4O_2S_3$ (376.48)	265 dec.	52	47.85/47.72	3.21/3.31	14.88/14.72
27a	$C_{23}H_{14}N_4O_2S$ (410.45)	140–142	71	67.30/67.18	3.44/3.51	13.65/13.51
27b	$C_{22}H_{14}N_4O_3S_2$ (446.50)	170–172	69	59.18/59.04	3.16/3.01	12.55/12.63
27c	$C_{17}H_{12}N_4O_3S_2$ (384.43)	141–143	65	53.11/53.02	3.15/3.05	14.57/14.40

as a reference drug. The preliminary antifungal screening for all the synthesized compounds at a concentration of 200 μg/disk showed that compounds **4a,b**, **6a,b**, **7a,b**, **10a**, **12a**, **13a**, **15a,c**, **16a-c**, **17a,b**, **18b**, **20a,b**, **23a,b**, **25a,b**, and **27a,c** were the most active ones. The MICs results of the active compounds were estimated and compared

Table 2 Spectral characterization of the prepared compounds

Compound numbers	IR (γ_{max}/cm)	^{1}H NMR (δ, ppm)	Mass (*m/z*, %)
2a	3200 (NH), 2215 (CN), 1733 (C = O), 1655 (C = C), 1084 (C-O-C)	–	429 (M^+, 22), 144 (100)
2b	3127 (NH), 2208 (CN), 1740 (C = O), 1652 (C = C), 1386 and 1149 (SO_2-N)	^{1}H NMR: 11.91 (1H, s, NH), 8.55 (1H, s, H-2 ind.), 8.30 (1H, s, H-5 thiophene), 8.17–7.18 (9H, m, Ar-H), 4.57 (2H, s, CH_2), 2.70 (2H, q, CH_2), 1.25 (3H, t, CH_3)^{13}C NMR: 192.5 (C = O), 136.5–111.9 (Ar-C), 116.1 (CN), 41.4 (CH_2), 27.6 (CH_2), 27.1 (CH_3)	–
2c	3122 (NH), 2211 (CN), 1727 (C = O), 1612 (C = C), 1372 and 1175 (SO_2-N)	^{1}H NMR: 10.22 (1H, s, NH), 8.32 (1H, s, H-2 ind.), 8.19 (1H, s, H-5 thiophene), 7.95–7.13 (4H, m, Ar-H), 4.32 (2H, s, CH_2), 3.83 (3H, s, CH_3-SO_2), 2.82 (2H, q, CH_2), 1.56 (3H, t, CH_3)	403 (M^+, 10), 77 (100)
3a	3360 (NH_2), 3160 and 3112 (NH), 2209 (CN), 1735 (C = O), 1571 (C = C), 1245 (C = S)	^{1}H NMR: 10.31 (1H, s, NH), 8.28 (1H, s, H-2 ind.), 8.15 (1H, s, H-5 thiophene), 7.70–7.01 (9H, m, Ar-H), 6.44 (1H, s, NH), 5.03 (2H, s, CH_2), 2.46 (2H, s, NH_2), 1.84 (1H, s, NH)^{13}C NMR: 193.1 (C = O), 136.9–112.2 (Ar-C), 117.0 (CN), 42.1 (CH_2)	474 (M^+, 11), 144 (100)
3b	3416 (NH_2), 3240 and 3162 (NH), 2195 (CN), 1705 (C = O), 1520 (C = C), 1251 (C = S), 1372 and 1175 (SO_2-N)	^{1}H NMR: 11.22 (2H, s, NH_2), 8.76 (1H, s, NH), 8.22 (1H, s, H-2 ind.), 8.11 (1H, s, H-5 thiophene), 7.77–7.03 (9H, m, Ar-H), 6.11 (1H, s, NH), 5.20 (2H, s, CH_2), 2.21 (1H, s, NH)	–
3c	3399 (NH_2), 3205 (br. NH) 2225 (CN), 1654 (C = O), 1509 (C = C), 1245 (C = S), 1362 and 1148 (SO_2-N)	–	448 (M^+, 0.13), 65 (100)
4a	3414 (NH_2), 3208 (NH), 2202 (CN), 1741 (C = O), 1631 (C = N), 1530 (C = C)	^{1}H NMR: 8.21 (1H, s, H-2 ind.), 8.11 (1H, s, H-5 thiophene), 7.98–7.03 (9H, m, Ar-H), 6.76 (2H, s, NH_2), 5.64 (2H, s, CH_2), 2.21 (1H, s, NH)^{13}C NMR: 141.1–111.1 (Ar-C), 116.8 (CN), 42.6 (CH_2)	–
4b	3368 (NH_2), 3112 (NH), 2217 (CN), 1618 (C = N), 1523 (C = C), 1384 and 1135 (SO_2-N)	–	492 (M^+, 2.31), 144 (100)
4c	3365 (NH_2), 3174 (NH), 2222 (CN), 1614 (C = N), 1527 (C = C), 1373 and 1132 (SO_2-N)	^{1}H NMR: 8.71 (2H, s, NH_2), 8.24 (1H, s, H-2 ind.), 8.15 (1H, s, H-5 thiophene), 7.88–7.01 (4H, m, Ar-H), 5.55 (2H, s, CH_2), 3.01 (3H, s, CH_3-SO_2), 2.64 (1H, s, NH)^{13}C NMR: 136.3–111.5 (Ar-C), 116.2 (CN), 41.5 (CH_2), 27.6 and 27.3 (CH_3)	430 (M^+, 13), 85 (100)
6a	3158 (NH), 2217 (CN), 1705 (C = O), 1571 (C = C), 1148 (C-O-C)	^{1}H NMR: 8.53 (1H, s, H-2 ind.), 8.33 (1H, s, H-5 thiophene), 8.01–7.24 (9H, m, Ar-H), 4.03 (2H, s, CH_2), 3.84–2.37 (8H, m, CH_2), 2.45 (1H, s, NH)	470 (M^+, 23), 144 (100)
6b	3162 (NH), 2224 (CN), 1720 (C = O), 1573 (C = C), 1386 and 1169 (SO_2-N), 1096 (C-O-C)	–	506 (M^+, 0.54), 77 (100)
6c	3300 (NH), 2197 (CN), 1675 (C = O), 1608 (C = C), 1386 and 1161 (SO_2-N), 1099 (C-O-C)	^{1}H NMR: 8.60 (1H, s, H-2 ind.) 8.33 (1H, s, H-5 thiophene), 8.02–7.22 (4H, m, Ar-H), 4.13 (2H, s, CH_2), 3.73–2.11 (8H, m, CH_2), 3.06 (3H, s, CH_3), 1.75 (1H, s, NH)	–
7a	3187 (NH), 2195 (CN), 1659 (C = O), 1517 (C = C)	^{1}H NMR: 9.32 (1H, s, NH), 8.32 (1H, s, H-2 ind.), 8.15 (1H, s, H-5 thiophene), 7.82–7.10 (9H, m, Ar-H), 4.15 (2H, s, CH_2), 3.94–2.29 (8H, m, CH_2), 1.68 (3H, s, CH_3)	–
7b	3240 (NH), 2205 (CN), 1670 (C = O), 1521 (C = C), 1389 and 1151 (SO_2-N)	^{1}H NMR: 10.00 (1H, s, NH), 8.56 (1H, s, H-2 ind.), 8.30 (1H, s, H-5 thiophene), 8.12–7.01 (9H, m, Ar-H), 4.23 (2H, s, CH_2), 3.84–2.37 (8H, m, CH_2), 1.49 (3H, s, CH_3)	519 (M^+, 21), 144 (100)
7c	3160 (NH), 2215 (CN), 1739 (C = O), 1568 (C = C), 1385 and 1148 (SO_2-N)	–	457 (M^+, 0.62), 199 (100)
8a	3330 (NH_2), 3187 and 3174 (NH), 2210 (CN), 1708 (C = O), 1578 (C = C)	^{1}H NMR: 10.12 (2H, s, NH_2), 8.25 (1H, s, H-2 ind.), 8.05 (1H, s, H-5 thiophene), 7.84–7.10 (9H, m, Ar-H), 6.11 (1H, s, NH), 5.04 (2H, s, CH_2), 1.85 (1H, s, NH)^{13}C NMR: 192.2 (C = O), 135.8–112.5 (Ar-C), 116.4 (CN), 41.6 (CH_2)	415 (M^+, 5.4) 144 (100)
8b	3420 (NH_2), 3240 and 3160 (NH), 2222 (CN), 1687 (C = O), 1517 (C = C), 1365 and 1140 (SO_2-N)	–	451 (M^+, 7.2), 224 (100)
8c	3361 (NH_2), 3232 (NH), 2227 (CN), 1720 (C = O), 1557 (C = C), 1384 and 1153 (SO_2-N)	^{1}H NMR: 9.50 (1H, s, NH), 8.51 (1H, s, H-2 ind.), 8.24 (1H, s, H-5 thiophene), 8.10–7.10 (4H, m, Ar-H), 6.76 (2H, s, NH_2), 5.24 (2H, s, CH_2), 3.41 (3H, s, CH_3-SO_2), 2.01 (1H, s, NH)	–
9a	3223 and 3157 (NH), 2240 (CN), 1660 (C = O), 1614 (C = N), 1576 (C = C)	^{1}H NMR: 11.86 (1H, s, NH), 9.95 (1H, s, NH), 8.95 (1H, s, CH = N), 8.43 (1H, s, H-2 ind.), 8.26 (1H, s, H-5 thiophene), 8.16–7.00 (14H, m, Ph-H), 5.31 (2H, s, CH_2)^{13}C NMR: 189.9 (C = O), 136.1–112.1 (Ar-C), 116.5 (CN), 41.6 (CH_2)	503 (M^+, 0.27), 144 (100)

Continued

Table 2 Continued

Compound numbers	IR (γ_{max}/cm)	^{1}H NMR (δ, ppm)	Mass (*m/z*, %)
9b	3150 and 3112 (NH), 2197 (CN), 1721 (C = O), 1617 (C = N), 1575 (C = C), 1364 and 1137 (SO_2-N)	^{1}H NMR: 10.55 (1H, s, NH), 9.12 (1H, s, CH = N), 8.21 (1H, s, H-2 ind.), 8.07 (1H, s, H-5 thiophene), 8.00–7.01 (14H, m, Ar-H), 5.25 (2H, s, CH_2), 1.91 (1H, s, NH)	539 (M^+, 33), 239 (100)
9c	3154 and 3128 (NH), 2195 (CN), 1697 (C = O), 1620 (C = N), 1575 (C = C), 1380 and 1135 (SO_2-N)	10.15 (1H, s, NH), 9.43 (1H, s, CH = N), 8.36 (1H, s, H-2 ind.), 8.24 (1H, s, H-5 thiophene), 7.97–7.01 (9H, m, Ar-H), 6.51 (1H, s, NH), 5.29 (2H, s, CH_2), 3.21 (3H, s, CH_3-SO_2)^{13}C NMR: 192.3 (C = O), 136.6–111.2 (Ar-C), 117.3 (CN), 41.8 (CH_2), 27.17 (CH_3)	–
10a	3193 and 3164 (NH), 2245 (CN), 1710 (C = O), 1552 (C = C)	^{1}H NMR: 10.55 (1H, s, NH), 8.21 (1H, s, H-2 ind.), 8.11 (1H, s, H-5 thiophene), 7.93–7.10 (14H, m, Ar-H), 5.35 (1H, s, H-2 thiazolidinone), 5.22 (2H, s, CH_2), 4.61 (2H, s, CH_2 thiazolidinone), 2.61 (1H, s, NH)^{13}C NMR: 192.1 (C = O), 138.2–112.5 (Ar-C), 116.3 (CN), 42.2 (CH_2)	577 (M^+, 12), 144 (100)
10b	3159 and 3120 (NH), 2224 (CN), 1705 (C = O), 1586 (C = C), 1372 and 1130 (SO_2-N)	^{1}H NMR: 11.00 (1H, s, NH), 8.36 (1H, s, H-2 ind.), 8.24 (1H, s, H-5 thiophene), 8.00–7.02 (14H, m, Ar-H), 5.33 (1H, s, H-2 thiazolidinone), 5.20 (2H, s, CH_2), 4.43 (2H, s, CH_2 thiazolidinone), 2.69 (1H, s, NH)	–
10c	3212 and 3175 (NH), 2210 (CN), 1678 (C = O), 1575 (C = C), 1366 and 1142 (SO_2-N)	^{1}H NMR: 8.72 (1H, s, H-2 ind.), 8.35 (1H, s, H-5 thiophene), 8.16–7.19 (9H, m, Ar-H), 6.01 (1H, s, NH), 5.41 (1H, s, H-2 thiazolidinone), 5.19 (2H, s, CH_2), 4.52 (2H, s, CH_2 thiazolidinone), 3.71 (3H, s, CH_3-SO_2), 1.85 (1H, s, NH)	551 (M^+, 24), 166 (100)
12a	2208 (CN), 1676 and 1692 (C = O), 1602 (C = C), 739 (C-Cl)	^{1}H NMR: 8.34 (1H, s, H-2 ind.), 8.15 (1H, s, H-5 thiophene), 8.04–7.00 (13H, m, Ar-H), 5.22 (1H, d, CH), 5.07 (1H, d, CH)^{13}C NMR: 192.5 (C = O), 136.5–111.9 (Ar-C), 116.6 (CN), 45.6 (CH), 27.1 (CH)	552/554 (M^+/M^++2, 11/3.61), 144 (100)
12b	2207 (CN), 1712 (C = O), 1596 (C = C), 1353 and 1164 (SO_2-N), 751 (C-Cl)	^{1}H NMR: 8.26 (1H, s, H-2 ind.), 8.15 (1H, s, H-5 thiophene), 7.78–7.13 (13H, m, Ar-H), 5.51 (1H, d, CH), 5.00 (1H, d, CH)^{13}C NMR: 192.2 (C = O), 136.2–112.0 (Ar-C), 116.7 (CN), 42.6 (CH), 27.5 (CH)	–
12c	2210 (CN), 1727 (C = O), 1607 (C = C), 1374 and 1133 (SO_2-N), 747 (C-Cl)	^{1}H NMR: 8.51 (1H, s, H-2 ind.), 8.30 (1H, s, H-5 thiophene), 8.17–7.13 (4H, m, Ar-H), 5.45 (1H, d, CH), 5.12 (1H, d, CH), 3.66 (3H, s, CH_3-SO_2)	526/528 (M^+/M^++2, 23/7.67), 290 (100)
13a	2215 (CN), 1707 (C = O), 1546 (C = C)	^{1}H NMR: 8.16 (1H, s, H-2 ind.), 7.90 (1H, s, H-5 thiophene), 7.86–7.01 (18H, m, Ar-H), 5.53 (1H, d, CH), 5.10 (1H, d, CH)	594 (M^+, 23), 144 (100)
13b	2220 (CN), 1709 (C = O), 1614 (C = C), 1386 and 1149 (SO_2-N)	^{1}H NMR: 8.63 (1H, s, H-2 ind.), 8.35 (1H, s, H-5 thiophene), 8.14–7.11 (18H, m, Ar-H), 5.65 (1H, d, CH), 5.11 (1H, d, CH)^{13}C NMR: 192.3 (C = O), 139.1–112.1 (Ar-C), 116.5 (CN), 41.0 (2CH)	630 (M^+, 16), 77 (100)
13c	2232 (CN), 1645 (C = O), 1545 (C = C), 1372 and 1175 (SO_2-N)	^{1}H NMR: 8.27 (1H, s, H-2 ind.), 7.99 (1H, s, H-5 thiophene), 7.78–7.05 (13H, m, Ar-H), 5.24 (1H, d, CH), 5.00 (1H, d, CH), 4.01 (3H, s, CH_3-SO_2)^{13}C NMR: 193.26 (C = O), 138.67–111.42 (Ar-C), 116.51 (CN), 42.34 (2CH), 27.13 (CH_3)	–
14a	3212 (NH), 2225 (CN), 1759 (C = O), 1578 (C = C), 1366 and 1142 (SO_2-N)	^{1}H NMR: 8.81 (1H, s, H-2 ind.), 8.15 (1H, s, H-5 thiophene), 7.95–7.64 (14H, m, Ar-H), 3.60 (1H, s, NH)	–
14b	3214 (NH), 2212 (CN), 1579 (C = C), 1395 and1134 (SO_2-N)	^{1}H NMR: 8.32 (1H, s, H-2 ind.), 8.03 (1H, s, H-5 thiophene), 7.73–7.13 (14H, m, Ar-H), 2.64 (1H, s, NH)^{13}C NMR: 135.1–113.1 (Ar-C), 116.2 (CN)	–
14c	3138 (NH), 2199 (CN), 1563 (C = C), 1395 and1153 (SO_2-N)	–	457 (M^+, 25), 77 (100)
15a	3157 (NH), 2204 (CN), 1710 (C = O), 1567 (C = C), 1377 and 1176 (SO_2), 747 (C-Br)	^{1}H NMR: 8.22 (1H, s, H-2 ind.), 8.01 (1H, s, H-5 thiophene), 7.79–7.06 (14H, Ar-H), 1.65 (1H, s, NH)	–
15b	3205 (NH), 2223 (CN), 1599 (C = C), 1387 and 1181 (SO_2), 741 (C-Br)	^{1}H NMR: 8.38 (1H, s, H-2 ind.), 8.17 (1H, s, H-5 thiophene), 7.87–7.11 (14H, Ar-H), 6.65 (1H, s, NH)	597/599 (M^+/M^++2, 2.43/2.41), 144 (100)
15c	3166 (NH), 2205 (CN), 1565 (C = C), 1366 and 1136 (SO_2), 749 (C-Br)	^{1}H NMR: 8.52 (1H, s, H-2 ind.), 8.30 (1H, s, H-5 thiophene), 8.17–7.16 (8H, m, Ar-H), 3.62 (3H, s, CH_3-SO_2), 2.90 (1H, s, NH)^{13}C NMR: 134.8–111.4 (Ar-C), 116.6 (CN), 22.8 (CH_3)	535/537 (M^+/M^++2, 15/15), 65 (100)
16a	3200 (NH), 2215 (CN), 1733 (C = O), 1655 (C = C)	^{1}H NMR: 8.51 (1H, s, H-2 ind.), 8.23 (1H, s, H-5 thiophene), 7.79–7.06 (14H, Ar-H), 6.46 (1H, s, NH)	447 (M^+, 5.62), 144 (100)

Continued

Table 2 Continued

Compound numbers	IR (γ_{max}/cm)	^{1}H NMR (δ, ppm)	Mass (*m/z*, %)
16b	3127 (NH), 2208 (CN), 1733 (C = O), 1652 (C = C), 1384 and 1135 (SO_2-N)	^{1}H NMR: 8.32 (1H, s, H-2 ind.), 8.12 (1H, s, H-5 thiophene), 7.79–7.06 (14H, Ar-H), 1.53 (1H, s, NH)^{13}C NMR: 192.1 (C = O), 136.3–111.1 (Ar-C), 116.7 (CN)	–
16c	3122 (NH), 2211 (CN), 1727 (C = O), 1612 (C = C), 1373 and 1132 (SO_2-N)	–	421 (M^+, 24), 85 (100)
17a	2214 (CN), 1710 and 1699 (C = O), 1552 (C = C)	^{1}H NMR: 8.21 (1H, s, H-2 ind.), 8.11 (1H, s, H-5 thiophene), 7.98–7.03 (9H, m, Ar-H), 3.32–3.20 (4H, m, CH_2-CH_2)	425 (M^+, 11), 144 (100)
17b	2198 (CN), 1722 and 1701 (C = O), 1628 (C = C), 1364 and 1134 (SO_2-N)	^{1}H NMR: 8.25 (1H, s, H-2 ind.), 8.15 (1H, s, H-5 thiophene), 7.98–7.04 (9H, m, Ar-H), 3.75–3.63 (4H, m, CH_2-CH_2)^{13}C NMR:192.4 (C = O), 138.2–111.1 (Ar-C), 117.1 (CN), 41.7 ($2CH_2$)	–
17c	2205 (CN), 1695 and 1678 (C = O), 1548 (C = C), 1344 and 1125 (SO_2-N)	^{1}H NMR: 8.24 (1H, s, H-2 ind.), 8.15 (1H, s, H-5 thiophene), 7.88–7.01 (4H, m, Ar-H), 3.75–3.63 (4H, m, CH_2-CH_2), 3.16 (3H, s, CH_3-SO_2)	399 (M^+, 5.23), 98 (100)
18a	3408 (OH), 3135 (NH), 2215 (CN), 1695 and 1639 (C = O), 1587 (C = C)	^{1}H NMR: 12.12 (1H, s, OH), 8.77 (1H, s, H-2 ind.), 8.22 (1H, s, H-5 thiophene), 8.08–7.24 (9H, m, Ar-H), 3.95–3.83 (4H, m, CH_2-CH_2), 2.69 (1H, s, NH)^{13}C NMR: 192.9 and 192.0 (C = O), 136.4–111.2 (Ar-C), 116.7 (CN), 42.1 ($2CH_2$)	–
18b	3368 (OH), 3206 (NH), 2202 (CN), 1731 and 1649 (C = O), 1585 (C = C), 1386 and 1161 (SO_2-N)	^{1}H NMR: 12.00 (1H, s, OH), 8.56 (1H, s, H-2 ind.), 8.21 (1H, s, H-5 thiophene), 7.98–7.11 (9H, m, Ar-H), 3.85–3.73 (4H, m, CH_2-CH_2), 2.21 (1H, s, NH)	479 (M^+, 7.12), 199 (100)
18c	3320 (OH), 3162 (NH), 2210 (CN), 1686 and 1617 (C = O), 1522 (C = C), 1384 and 1135 (SO_2-N)	^{1}H NMR: 11.92 (1H, s, OH), 9.31 (1H, s, NH), 8.28 (1H, s, H-2 ind.), 8.15 (1H, s, H-5 thiophene), 7.70–7.11 (4H, m, Ar-H), 4.05 and 3.80 (4H, m, CH_2-CH_2), 3.46 (3H, s, CH_3)^{13}C NMR: 192.5 (C = O), 136.6–112.0 (Ar-C), 116.8 (CN), 41.5 ($2CH_2$), 27.1 (CH_3)	417 (M^+, 1.43), 167 (100)
19a	3157 (NH), 2215 (CN), 1699 (C = O), 1571 (C = C)	^{1}H NMR: 8.71 (1H, s, H-2 ind.), 8.50 (1H, s, H-5 thiophene), 7.79–7.06 (9H, Ar-H), 1.95 (3H, s, CH_3), 1.23 (1H, s, NH)^{13}C NMR: 192.3 (C = O), 137.3–111.1 (Ar-C), 116.1 (CN), 24.2 (CH_3)	–
19b	3139 (NH), 2222 (CN), 1719 (C = O), 1563 (C = C), 1317 and 1150 (SO_2-N)	–	421 (M^+, 31), 283 (100)
19c	3127 (NH), 2235 (CN), 1698 (C = O), 1564 (C = C), 1384 and 1163 (SO_2-N)	^{1}H NMR: 8.32 (1H, s, H-2 ind.), 8.12 (1H, s, H-5 thiophene), 8.21–7.20 (4H, Ar-H), 3.63 (3H, s, SO_2-CH_3), 2.12 (3H, s, CH_3), 1.30 (1H, s, NH)	359 (M^+, 6.43), 197 (100)
20a	3158 (NH), 1705 (C = O), 1604 (C = N), 1571 (C = C)	^{1}H NMR: 10.87 (1H, s, NH), 8.68 (1H, s, H-5 thiophene), 8.29 (1H, s, H-2 ind.), 8.18 (1H, d, H-7 ind.), 7.81 (1H, d, H-4 ind.), 7.76–7.00 (7H, m, Ar-H), 3.82 (3H, s, CH_3)	385 (M^+, 7.21), 144 (100)
20b	3362 (NH), 1645 (C = O), 1606 (C = N), 1573 (C = C), 1386 and 1169 (SO_2-N),	^{1}H NMR: 8.41 (1H, s, H-5 thiophene), 8.12 (1H, s, H-2 ind.), 7.81–7.10 (9H, m, Ar-H), 6.26 (1H, s, NH), 3.55 (3H, s, CH_3)^{13}C NMR: 192.2 (C = O), 134.8–112.6 (Ar-C), 27.0 (CH_3)	421 (M^+, 0.45), 265 (100)
20c	3300 (NH), 1637 (C = O), 1608 (C = C), 1386 and 1161 (SO_2-N)	^{1}H NMR: 9.11 (1H, s, NH), 8.38 (1H, s, H-5 thiophene), 8.11 (1H, s, H-2 ind.), 7.97–7.00 (4H, m, Ar-H), 3.58 (3H, s, CH_3), 3.23 (3H, s, CH_3)	–
21a	2217 (CN), 1710 (C = O), 1628 (C = N), 1599 (C = C), 1099 (C-O-C)	^{1}H NMR: 8.51 (1H, s, H-2 ind.), 8.20 (1H, s, H-5 thiophene), 8.05–7.22 (9H, Ar-H), 6.62 (1H, s, CH = N), 4.12 (2H, q, CH_2), 1.34 (3H, t, CH_3)^{13}C NMR: 192.4 (C = O), 136.8–111.6 (Ar-C), 117.1 (CN), 62.6 (CH = N), 27.6 (CH_2), 14.6 (CH_3)	–
21b	2213 (CN), 1623 (C = N), 1516 (C = C), 1386 and 1143 (SO_2-N), 1096 (C-O-C)	^{1}H NMR: 8.81 (1H, s, H-2 ind.), 8.41 (1H, s, H-5 thiophene), 8.25–7.30 (9H, m, Ar-H), 6.60 (1H, s, CH = N), 3.75 (2H, q, CH_2), 1.20 (3H, t, CH_3)^{13}C NMR: 137.2–112.5 (Ar-C), 116.1 (CN), 61.5 (CH = N), 27.1 (CH_2), 14.7 (CH_3)	435 (M^+, 8.65), 77 (100)
21c	2202 (CN), 1617 (C = N), 1574 (C = C), 1381 and 1138 (SO_2-N), 1148 (C-O-C)	–	373 (M^+, 14), 65 (100)
22a	3407 (NH_2), 3201 (NH), 1721 (C = O), 1612 (C = N), 1526 (C = C)	^{1}H NMR: 10.32 (1H, s, NH), 8.58 (1H, s, H-2 pyrimidine), 8.35 (1H, s, H-5 thiophene), 8.11 (1H, s, H-2 ind.), 8.04–7.13 (9H, m, Ar-H), 2.64 (2H, s, NH_2)^{13}C NMR: 193.3 (C = O), 139.4–112.1 (Ar-C)	385 (M^+, 4.53), 144 (100)
22b	3424 (NH_2), 3126 (NH), 1619 (C = N), 1580 (C = C), 1366 and 1134 (SO_2-N)	^{1}H NMR: 9.72 (1H, s, NH), 8.51 (1H, s, H-2 pyrimidine), 8.35 (1H, s, H-5 thiophene), 7.91 (1H, s, H-2 ind.), 7.81–7.15 (9H, m, Ar-H), 2.69 (2H, s, NH_2)	–
22c	3381 and 3280 (NH_2), 3165 (NH), 1621 (C = N), 1528 (C = C), 1379 and 1176 (SO_2-N)	^{1}H NMR: 9.32 (1H, s, NH), 8.62 (1H, s, H-2 pyrimidine), 8.41 (1H, s, H-5 thiophene), 8.12 (1H, s, H-2 ind.), 7.94–7.13 (4H, m, Ar-H), 3.42 (3H, s, CH_3), 1.45 (2H, s, NH_2)	359 (M^+, 0.65), 172 (100)
23a	1734 (C = O), 1638 (C = N), 1572 (C = C)	^{1}H NMR: 9.33 (1H, s, CH triazolo), 8.92 (1H, s, H-2 pyrimidine), 8.51 (1H, s, H-5 thiophene), 8.25 (1H, s, H-2 ind.), 7.94–7.15 (9H, m, Ar-H)	395 (M^+, 0.19), 144 (100)

Continued

Table 2 Continued

Compound numbers	IR (γ_{max}/cm)	^{1}H NMR (δ, ppm)	Mass (*m/z*, %)
23b	1637 (C = N), 1606 (C = C), 1389 and 1151 (SO_2-N)	^{1}H NMR: 9.47 (1H, s, CH triazolo), 8.83 (1H, s, H-2 pyrimidine), 8.46 (1H, s, H-5 thiophene), 8.13 (1H, s, H-2 ind.), 7.90–7.09 (9H, m, Ar-H)^{13}C NMR: 160.6 (C = N), 139.4–112.1 (Ar-C)	431 (M^+, 4.12), 291 (100)
23c	1634 (C = N), 1571 (C = C), 1385 and 1148 (SO_2-N)	^{1}H NMR: 9.51 (1H, s, CH triazolo), 8.86 (1H, s, H-2 pyrimidine), 8.50 (1H, s, H-5 thiophene), 8.22 (1H, s, H-2 ind.), 8.04–7.16 (4H, m, Ar-H), 3.61 (3H, s, CH_3)	–
24a	3254 and 3135 (NH), 1692 (C = O), 1563 (C = C), 1238 (C = S)	^{1}H NMR: 10.21 (1H, s, NH), 8.23 (1H, s, H-5 thiophene), 8.13 (1H, s, H-2 ind.), 7.74–7.15 (9H, Ar-H), 2.93 (1H, s, NH)^{13}C NMR: 192.6 (C = O), 182.5 (C = S), 136.2–112.0 (Ar-C)	–
24b	3306 and 3167 (NH), 1585 (C = C), 1386 and 1161 (SO_2-N), 1242 (C = S)	–	455 (M^+, 31), 251 (100)
24c	3324 and 3162 (NH), 1615 (C = C), 1386 and 1169 (SO_2-N), 1248 (C = S)	^{1}H NMR: 8.92 (1H, s, NH), 8.30 (1H, s, H-5 thiophene), 8.17 (1H, s, H-2 ind.), 7.47–7.15 (4H, m, Ar-H), 3.51 (3H, s, CH_3), 1.93 (1H, s, NH)^{13}C NMR: 184.3 (C = S), 136.7–111.1 (Ar-C), 22.5 (CH_3)	333 (M^+, 12), 166 (100)
25a	3435 (br., NH_2, and NH), 1694 (C = O), 1614 (C = N), 1571 (C = C)	^{1}H NMR: 11.71 (1H, s, NH), 11.59 (2H, s, NH_2), 8.52 (1H, s, H-5 thiophene), 8.32 (1H, s, H-2 ind.), 8.20–7.00 (9H, m, Ar-H)^{13}C NMR: 193.7 (C = O), 136.4–111.7 (Ar-C)	386 (M^+, 4.81), 144 (100)
25b	3423 (NH_2), 3162 (NH), 1725 (C = O), 1615 (C = N), 1573 (C = C), 1386 and 1169 (SO_2-N)	^{1}H NMR: 10.54 (2H, s, NH_2), 8.31 (1H, s, H-5 thiophene), 8.15 (1H, s, H-2 ind.), 7.74–7.21 (9H, m, Ar-H), 6.02 (1H, s, NH)^{13}C NMR: 191.2 (C = O), 137.1–111.5 (Ar-C)	–
25c	3421 (NH_2), 3200 (NH), 1665 (C = O), 1637 (C = N), 1608 (C = C), 1386 and 1161 (SO_2-N)	–	360 (M^+, 0.43), 266 (100)
26a	3418 (NH_2), 3158 (NH), 1705 (C = O), 1614 (C = N), 1571 (C = C), 1245 (C = S)	^{1}H NMR: 11.71 (1H, s, NH), 8.40 (1H, s, H-5 thiophene), 8.13 (1H, s, H-2 ind.), 8.00–7.22 (9H, m, Ar-H), 6.91 (2H, s, NH_2)^{13}C NMR: 192.1 (C = O), 182.7 (C = S), 136.4–111.1 (Ar-C)	402 (M^+, 1.54), 144 (100)
26b	3363 (NH_2), 3162 (NH), 1615 (C = N), 1573 (C = C), 1386 and 1169 (SO_2-N), 1248 (C = S)	–	–
26c	3405 (NH_2), 3215 (NH), 1637 (C = N), 1608 (C = C), 1386 and 1161 (SO_2-N), 1250 (C = S)	^{1}H NMR: 10.46 (1H, s, NH), 8.52 (1H, s, H-5 thiophene), 8.02 (1H, s, H-2 ind.), 7.83–7.11 (4H, m, Ar-H), 6.91 (2H, s, NH_2), 4.01 (3H, s, CH_3)^{13}C NMR: 182.7 (C = S), 134.2–111.6 (Ar-C), 24.8 (CH_3)	376 (M^+, 3.21), 282 (100)
27a	3430 (OH), 3345 (NH_2), 2224 (CN), 1725 (C = O), 1619 (C = N), 1526 (C = C)	^{1}H NMR: 12.68 (1H, s, OH), 8.50 (1H, s, H-5 thiophene), 8.21 (1H, s, H-2 ind.), 8.13–7.12 (9H, Ar-H), 6.45 (2H, s, NH_2)^{13}C NMR: 192.6 (C = O), 138.1–111.5 (Ar-C), 116.1 (CN)	410 (M^+, 61), 144 (100)
27b	3491 (OH), 3315 (NH_2), 2208 (CN), 1628 (C = N), 1596 (C = C), 1342 and 1173 (SO_2-N)	–	446 (M^+, 43), 306 (100)
27c	3427 (OH), 3266 (NH_2), 2190 (CN), 1629 (C = N), 1605 (C = C), 1342 and 1175 (SO_2-N)	^{1}H NMR: 11.76 (1H, s, OH), 8.32 (1H, s, H-5 thiophene), 8.12 (1H, s, H-2 ind.), 7.74–7.23 (4H, Ar-H), 2.96 (2H, s, NH_2), 3.75 (3H, s, CH_3)^{13}C NMR: 137.3–111.1 (Ar-C), 116.2 (CN), 24.8 (CH_3)	–

IR, infrared; NMR, nuclear magnetic resonance.

with fluconazole (Table 3). From the data obtained, compound **4a**, in which the 5-amino-1,3,4-thiadiazol moiety incorporated into *N*-benzoylindole through the thiophene bridge, was found to be highly active toward both *C. albicans* (ATCC-10231) and *A. niger* (ATCC-10535), with MICs of 9 and 36 μg/disk, respectively, compared with fluconazole of MICs of 8 and 32 μg/disk.

Molecular docking study

The result of the antifungal activity of the test compounds led us to carry out molecular docking studies to understand the ligand–protein interactions in detail. Compounds **4a**, **4b**, **6a**, **7a**, **7b**, **10a**, **12a**, **13a**, **16a-c**, **18b**, and **23a** were docked using the MOE 2008.10 program and cytochrome P450 14 α-sterol demethylase (CYP51) (PDB ID: 1EA1). From the data obtained (Table 4 and Figs 1–5), it was found that most of the docked compounds showed a high docking score, with minimum binding energy ranging from −30.25 to −20.02 kJ/mol, in comparison with the cocrystallized ligand, namely, 2-(2,4-difluorophenyl)-1,3-di(1*H*-1,2,4-triazol-1-yl)propan-2-ol(fluconazole), which had a binding energy of −18.46 kJ/mol, an root-mean-square deviation value of 4.48, and formed only an arene–cation bond between the benzyl ring and Arg96 (Table 4 and Fig. 1a and b).

Table 3 In-vitro antifungal activity of the most active compounds in comparison with fluconazole

Compound numbers	Tested fungi (MICs in μg/disk)	
	Candida albicans (ATCC-10231)	*Aspergillus niger* (ATCC-10535)
4a	9	36
4b	16	64
6a	16	64
6b	32	>128
7a	16	64
7b	16	64
10a	16	64
12a	16	64
13a	16	64
15a	32	128
15c	32	128
16a	16	64
16b	16	64
16c	16	64
17a	32	128
17b	32	128
18b	16	64
20a	32	128
20b	32	128
23a	16	64
23b	32	128
25a	32	128
25b	32	128
27a	32	128
27b	32	128
Fluconazole	8	32

MIC, minimum inhibitory concentration.

Compound **4a** had highest docked score, with a binding energy of −30.25 kJ/mol, and showed good fitting inside the pocket of the protein residue through two H-bonds formed between (a) NH of His259 and C = N of the thiadiazole ring and (b) C = O of Met433 and NH of the thiadiazole ring at a good distance for interaction, 2.93 and 2.87 Å, respectively (Fig. 2a and b).

We can conclude that biological results were supported by docking results, which suggested that compound **4a** is a promising agent as an antifungal with drug likeness approach that has 1EA1 inhibitory activity.

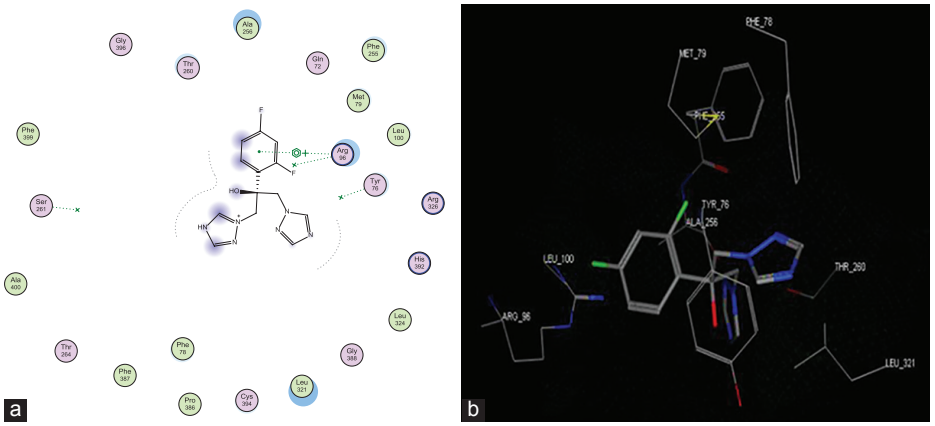
Conclusion

New series of thiadiazoles **4a-c**, morpholinyl-acetamides **6a-c**, 4-methylpiperazinylacetamides **7a-c**, thiazolidines **10a-c**, azetidines **12a-c–13a-c**, sulfonamides **14a-c–15a-c**, benzamides **16a-c**, pyrrolidines **17a-c**, succinamic acids **18a-c**, acetamides **19a-c**, thieno(2,3-*c*)pyridines **20a-c**, thieno(2,3-*e*)-1,2,4-triazolo(1,5-*c*) pyrimidines **23a-c**, thieno(2,3-*d*)pyrimidines **24a-c–26a-c**, and thieno(2,3-*b*)pyridines **27a-c** derivatives incorporated into *N*-substituted 3-indolylthiophenes were prepared. The antifungal activity of the newly synthesized compounds was tested against *C. albicans* (ATCC-10231) and *A. niger* (ATCC-10535). Compound **4a**, in which the

Table 4 Docking results of the most active compounds that docked with cytochrome P450 14 α-sterol demethylase (CYP51) (PDB ID: 1EA1)

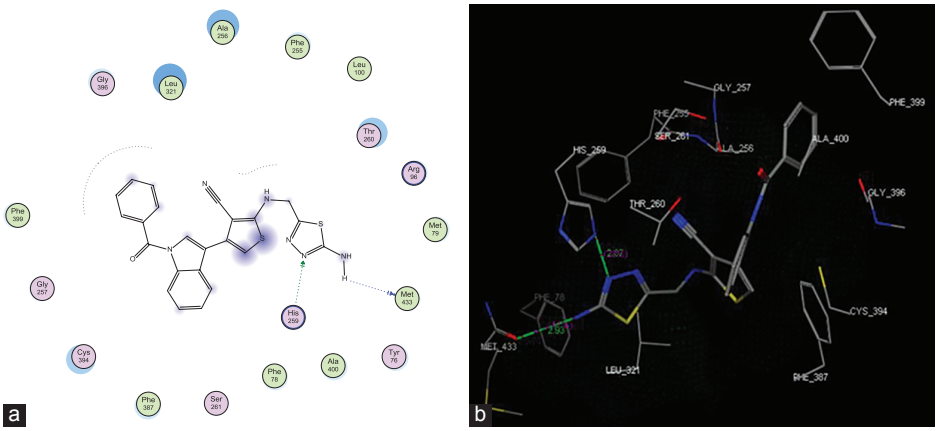
Compound numbers	Binding energy (kJ/mol)	Main atoms from the compounds	Main residue from 1EA1	Distance (Å)
Cocrystallized ligand	−18.46	Benzoyl ring	Arg96	Arene–cation
4a	−30.25	NH of thiadiazole ring	C = O of Met433	2.93
		C = N of thiadiazole ring	NH of His259	2.87
6a	−25.56	C = O	NH of Arg96	2.45
7a	−23.12	N-CH_3 of piperazine ring	NH of Arg96	2.73
			NH of Arg96	3.25
7b	−22.52	S = O	NH of Arg96	2.44
10a	−23.73	C = O of benzoyl ring	OH of Ser261	2.89
		C = O of thiazolidinone	OH of Tyr76	2.38
		C = O of urea group	NH of Arg96	2.85
		C = O of urea group	NH of Arg96	2.87
12a	−26.71	4-Nitro phenyl	Arg96	Arene–cation
13a	−27.00	C = O of benzoyl	OH of Ser261	2.80
		4-Nitro phenyl	Arg96	Arene–cation
		Phenyl ring	Arg96	Arene–cation
16a	−22.84	C = O of benzoyl ring	NH of Arg96	2.46
16b	−24.72	S = O	NH of Arg96	2.41
		Benzoyl ring	Tyr76	Arene–cation
16c	−20.02	S = O	NH of Arg96	2.44
		Indole ring	Arg96	Arene–cation
18b	−24.72	S = O	NH of Arg96	2.41
		Benzoyl ring	Tyr76	Arene–cation
23a	−23.61	Phenyl ring	Arg96	Arene–cation

Figure 1



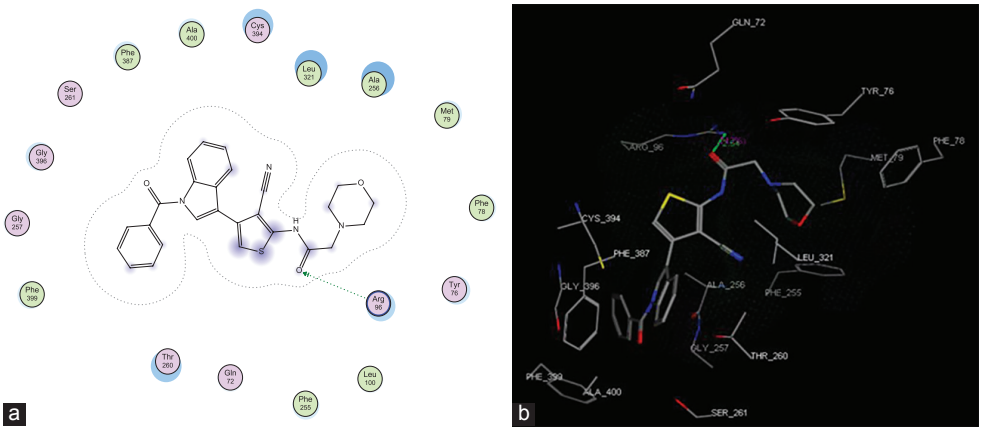
(a) Docked conformation alignment of cocrystallized ligand (fluconazole) in the cytochrome P450 14 α-sterol demethylase (CYP51) (PDB: 1EA1) binding site. (b) Simplified structure showing the interaction between fluconazole and the amino acid residues in the cytochrome P450 14 α-sterol demethylase (CYP51) (PDB: 1EA1) binding site.

Figure 2



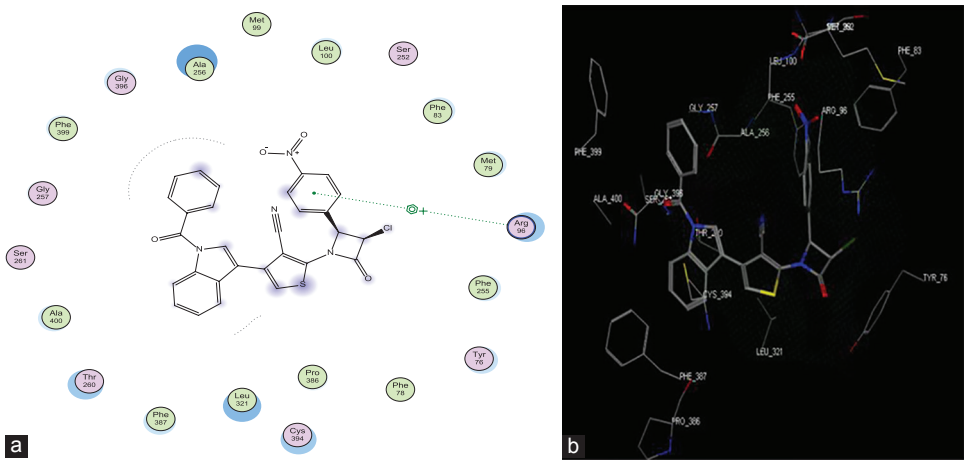
(a) Docked conformation alignment of **4a** and its original cocrystallized ligand in the cytochrome P450 14 α-sterol demethylase (CYP51) (PDB ID: 1EA1) binding site. (b) Simplified structure showing the interaction between **4a** and the amino acid residues in the cytochrome P450 14 α-sterol demethylase (CYP51) (PDB ID: 1EA1) binding site.

Figure 3



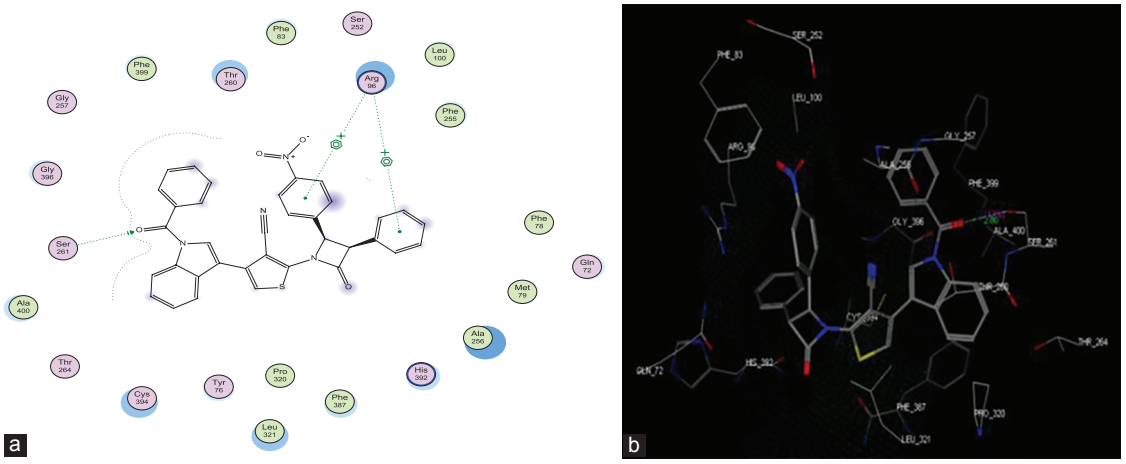
(a) Docked conformation alignment of **6a** and its original cocrystallized ligand in the cytochrome P450 14 α-sterol demethylase (CYP51) (PDB ID: 1EA1) binding site. (b) Simplified structure showing the interaction between **6a** and the amino acid residues in the cytochrome P450 14 α-sterol demethylase (CYP51) (PDB ID: 1EA1) binding site.

Figure 4



(a) Docked conformation alignment of **12a** and its original cocrystallized ligand in the cytochrome P450 14 α-sterol demethylase (CYP51) (PDB ID: 1EA1) binding site. (b) Simplified structure showing the interaction between **12a** and the amino acid residues in the cytochrome P450 14 α-sterol demethylase (CYP51) (PDB ID: 1EA1) binding site.

Figure 5



(a) Docked conformation alignment of **13a** and its original cocrystallized ligand in the cytochrome P450 14 α-sterol demethylase (CYP51) (PDB ID: 1EA1) binding site. (b) Simplified structure showing the interaction between **13a** and the amino acid residues in the cytochrome P450 14 α-sterol demethylase (CYP51) (PDB ID: 1EA1) binding site.

5-amino-1,3,4-thiadiazol moiety was incorporated into *N*-benzoylindole through the thiophene bridge, showed good inhibitory activity against both *C. albicans* (ATCC-10231) and *A. niger* (ATCC-10535), with MICs values of 9 and 36 µg/disk, respectively, compared with fluconazole, with MICs values of 8 and 34 µg/disk. The mode of action of the most promising antifungal compounds was assessed by docking with cytochrome P450 14 α-sterol demethylase (CYP51) (PDB ID: 1EA1) and the result showed that compound **4a** had the highest docking score, with a binding energy of −30.25 kJ/mol; thus, it may interact at the active site of cytochrome P450 14 α-sterol demethylase (CYP51), which is in agreement with the experimental activity value.

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Conflicts of interest

There are no conflicts of interest.

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