European Journal of Medicinal Chemistry 46 (2011) 1926–1934

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Synthesis, spectral, crystal structure and in vitro antimicrobial evaluation of imidazole/benzotriazole substituted piperidin-4-one derivatives

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ARTICLE INFO

Article history:
Received 22 October 2010
Received in revised form
13 February 2011
Accepted 15 February 2011
Available online 23 February 2011

Keywords:
2,6-Diarylpiperidin-4-ones
Imidazoles
Benzotriazole
Antimicrobial activity

ABSTRACT

Imidazole/benzotriazole analogues substituted piperidin-4-one derivatives **(17–26)** have been synthesized. Their chemical structures were characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectral analysis. In addition, single crystal X-ray diffraction has also been recorded for compounds **21** and **23**. The synthesized compounds were subjected to their *in vitro* antibacterial and antifungal activities against pathogenic microbial strains. The results pointed out that compounds **19** & **24** against *B. subtilis* and **20** & **24** against *E. coli* were explored superior inhibition activity.

1. Introduction

Imidazole and benzotriazole derivatives are of synthetically important analogues and are associated with several biological and pharmacological properties [1,2]. It is known that clinically useful drugs such as miconazole, econazole and oxiconazole (Fig. 1) containing imidazole moiety exhibit strong antifungal activity. Similarly, benzotriazole derivatives are of wide interest because of their diverse biological activity and potential clinical applications [3,4]. The 1H-benzotriazol compounds regulate important pharmacological activities such as anti-inflammatory, antiviral, antifungal, antineoplastic and antidepressant activities [5,6]. 1- and 2-[3-(1-piperazinyl)propyl]-benzotriazoles exhibit remarkable antiserotonergic, antiadrenergic and antihistaminic activities, as well as *in vivo* analgesic action [5]. Besides, recent studies have shown that several benzotriazole compounds act antiinflammation, antivirus, antifungal, and antitumor agents, and as selective inhibitors of PTP1B and antidepressants, resulting from the potent bioactivity of benzotriazole [7–11].

Recently our team have reported some heterocyclic compounds with significant biological activity viz. 2-[(2,4-diaryl-3-azabicyclo[3.3.1]nonan-9-ylidene)hydrazono]-1,3-thiazolidin-4-ones [12], 2,4-[diaryl-3-azabicyclo[3.3.1]nonan-9-yl]-5-spiro-4-acetyl-2-(acetylamino)-Δ^2-1,3,4-thiadiazoline [13], 2,6-diarylpiperidin-4-one O-benzyloxime ethers [14], 2,4-diaryl-3-azabicyclo[3.3.1]nonan-9-ones and their oxime ethers/ [15]. In connection to these, we report some imidazole and benzotriazole analogues substituted piperidin-4-one derivatives and their *in vitro* antibacterial and antifungal activities.

2. Chemistry

The synthetic pathway that leads to the formation of title compounds (**17–26**) are sketched in Scheme 1. A well numbered target compound structure was given in Fig. 2 for structural and biological analysis. By adopting the literature precedent [16], 2,6-diarylpiperidin-4-ones were prepared using one pot multicomponent Mannich reaction by condensing suitable aromatic aldehydes, ketones and ammonium acetate in 1:2:1 ratio. This upon nucleophilic substitution reaction with chloroacetyl chloride in the presence of base catalyst (triethylamine) afforded *N*-chloroacetyl-2,6-diarylpiperidin-4-ones **(9–16)** [17]. Further, the imidazole/benzotriazole undergoes nucleophilic substitution with *N*-chloroacetyl-2,6-diarylpiperidin-4-ones under reflux condition afforded the title compounds (**17–26**). All the newly synthesized compounds were fully characterized on the basis of spectroscopic techniques (IR, ^{1}H NMR, ^{13}C NMR and mass). In addition, for compounds **21** and **23,** single crystal X-ray diffraction was also recorded.

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doi:10.1016/j.ejmech.2011.02.036

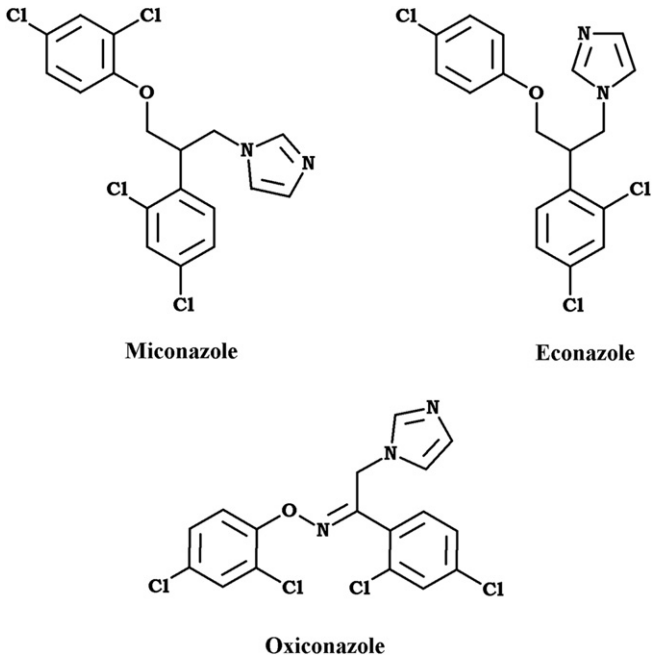


Fig. 1. Structures of the active antifungal agents.

3. Results and discussion

3.1. Spectral characterization

For both imidazole and benzotriazole substituted compounds, an intense band was observed around 1715 cm^{-1} corresponds to ring carbonyl stretching frequency (C=O at C-4). Similarly, a sharp band appeared around 1650 cm^{-1} which is due to amide carbonyl stretching frequency. A bunch of medium intense bands observed in the region 3079–2809 cm^{-1} is due to aromatic and aliphatic C–H stretching vibrations. The obtained molecular mass (m/z) of 21 and 23 are in good agreement with the proposed molecular formula.

In order to assign the ^{1}H and ^{13}C NMR signals, we have chosen **19** and **20** as the representative compounds. The aryl proton signals for compounds **19** and **20** are observed as multiplet in the region 6.70–7.40 ppm. The acetyl methylene protons of compound **20** appeared as a singlet at 4.55 ppm whereas the same protons in compound **19** appeared as two double doublet at 4.60 ppm ($J = 16.38$ Hz) and 4.39 ppm ($J = 18.01$ Hz). It is mainly due to the loss of symmetry in the molecule (**19**) [18–20]. For compound **20**, there is a broad singlet observed at 5.41 ppm. As the singlet corresponds to two protons integral, it is ascribed to benzylic protons (H-2 and H-6) whereas for **19**, the same protons signal appeared as two broad singlets at 6.43 and 5.23 ppm. Here, the each signal represents one proton integral value. By considering the substituent effect at C-3 position, the deshielded signal is assigned to H-2 proton whereas shielded one is assigned to H-6 proton. For compound **20**, the two methyl groups at C-3/C-5 is observed as doublet at 1.07 ppm ($J = 6.90$ Hz) with six proton integration whereas for **19**, the isopropyl methyl group signal observed as two separate doublet at 1.08 ppm ($J = 1.14$ Hz) and 1.10 ppm ($J = 1.32$ Hz). In addition, one more signal observed for the same compound (**19**) at 2.07 is due to methine proton in isopropyl analogue. There is a well resolved multiplet centered at 3.18 ppm for compound **20**, which corresponds to two protons and may be due to H-3 and H-5 protons. For compound **19**, H-3 and H-5 protons observed as multiplet and double doublet at 2.82–2.92 and 2.65 ppm, respectively.

^{13}C NMR spectra of compounds **19** and **20** show a pair of less intense signals in the higher frequency region at 208.22/169.03 and 210.384/169.32 ppm. These two set of signals in each compound represent carbonyl and amide carbonyl carbons of **19** and **20**, respectively. Benzylic carbon signals of both compounds are observed at 57.26 ppm (C-2)/56.93 ppm(C-6) (**19**) and 61.44 ppm (C-2 and C-6) (**20**). The C-3/C-5 carbon signals are observed at 54.68/45.39 and 45.46 ppm respectively for **19** and **20**. The acetyl methylene carbon signal for **19** and **20** are observed at 48.93 and 49.03 ppm, respectively. Two methyl carbon signals at C-3 and C-5 of **20** are observed at 14.20 ppm whereas the signal of methyl group in isopropyl analogue of **19** is observed at 20.89 and 20.43 ppm. The other compounds signal assignments were made based on the assignment of compounds **19** and **20**.

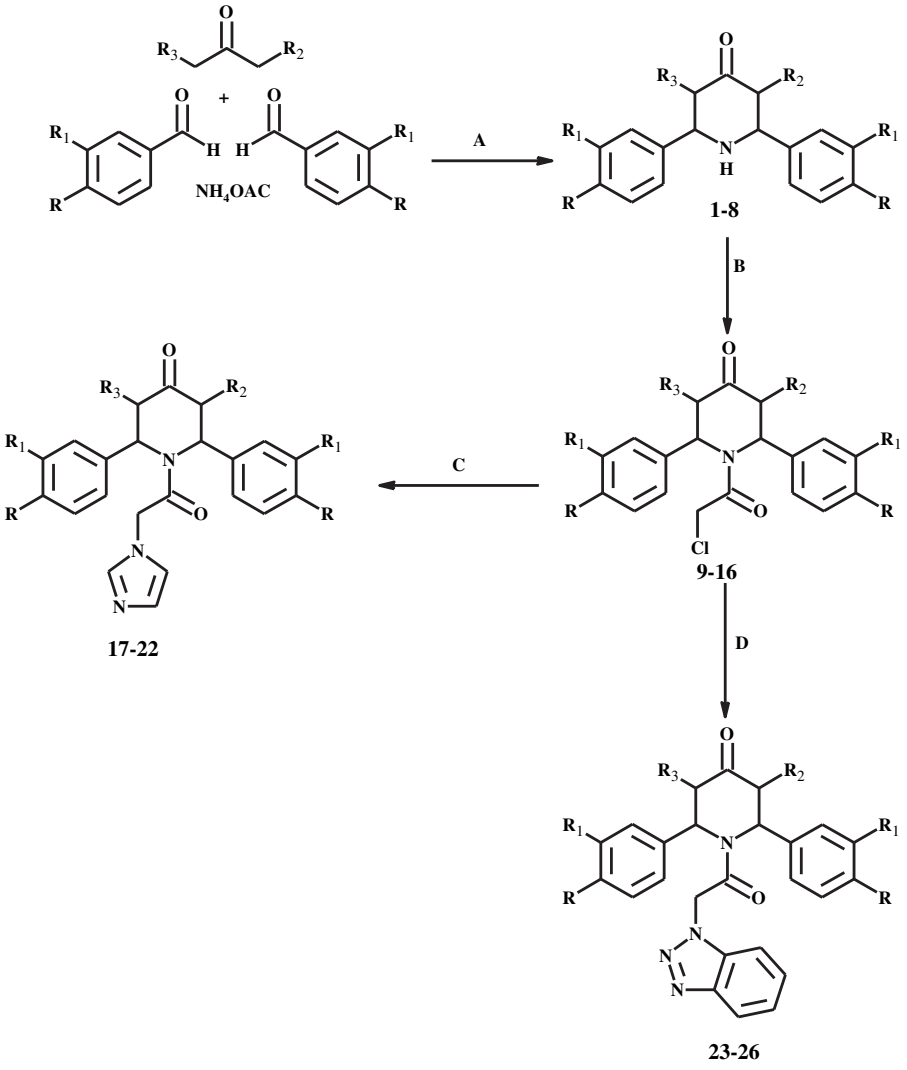
3.2. Crystal structure analysis of *21* and *23*

Good quality crystals were grown by the slow evaporation technique using absolute ethanol as solvent. Table 1 gives crystal data, data collection, and refinement parameters of **21** and **23** whereas Tables 2 and 3 show the selected bond lengths and angles of non hydrogen atoms and Figs. 3 and 4 shows the ORTEP diagram of the compounds **21** and **23** respectively with 30% probability. All the hydrogen atoms are placed at chemically acceptable positions. The bond distances and bond angles of compounds **21** and **23** are in good agreement with the standard values. The observed bond length N1–C6 (1.376 (3) Å) **(21)** and (N1–C18/N5–C45 1.353(5) Å/1.359(5) Å) **(23)** are shorter than reported values. This indicates the effective conjugation between lone pair of nitrogen in piperidine ring and amide carbonyl group. The N–$COCH_2$ group is coplanar as confirmed by the torsion angles C5–N1–C6–O2 & C1–N1–C6–C7 of −8.44° and 2.83° **(21)** and C1–N1–C18–O2 and C5–N1–C18–C19 of 6.3° and −11.1° **(23)**.

3.3. Antimicrobial studies

All the newly synthesized compounds **17**–**26** were screened for their ***in vitro*** antibacterial activity against *Staphylococcus aureus* (ATCC-25930), *Bacillus subtilis* (ATCC-530), *Salmonella typhi* (ATCC-25021), *Escherichia coli* (ATCC-26032) and *Klebsiella pneumoniae* (ATCC-16425) and antifungal activity against *Candida albicans* (ATCC-3430), *Cryptococcus neoformans* (ATCC-3235), *Rhizopus species* (ATCC-2842), *Aspergillus niger* (ATCC-635) and *Aspergillus flavus* (ATCC-525) and their MIC values were determined by serial dilution method. DMSO is used as a control while Streptomycin and Amphotericin B are used as standard drugs respectively for bacterial and fungal strains.

The antibacterial activity of MIC values are given in Table 4. Among the compounds reported in this series, **18** and **21** against *B. subtilis*, **20** against *S. aureus*, **19** and **24** against *K. pneumonia* and **26** against *E. coli* did not show any inhibitory activity even at maximum concentration (200 μg/mL). However, bulkier isopropyl group at C-3 position in the piperidine ring (**19**) against *B. subtilis* and methyl group at C-3 and C-5 in piperidine ring (**20**) against *E. coli* explored good inhibitory activity (12.5 and 6.25 μg/mL respectively). Compounds containing *para* fluorophenyl substituent at C-2 & C-6 in the piperidine ring (**24**) increase the growth inhibition activity against *E. coli* (12.5 μg/mL). Surprisingly, replacement of methyl group by ethyl at C-3 and hydrogen at C-5 in compound **24** (Compound **26**) shows superior inhibition activity



Scheme 1. Schematic diagram showing the synthesis of compounds (**17**–**26**).

against *B. subtilis* (6.25 μg/mL). The other compounds exhibit minimum to moderate activity (25–200 μg/mL).

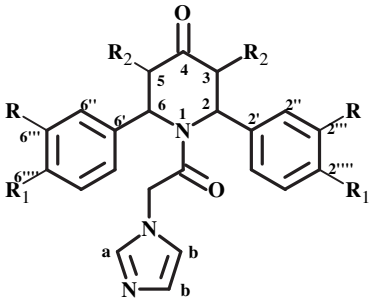
The antifungal activity results (Table 5) show that alkyl group substitution at the C-3/C-5 of piperidine ring exhibit moderate activity (25–200 μg/ml). But compound **19** against *A. niger* shows better activity at 12.5 μg/mL. It is due to the replacement of ethyl group by bulkier isopropyl function at C-3 in piperidine ring. Compound **20** (having methyl group at C-3 & C-5 and unsubstituted phenyl group at C-2 & C-6) shows minimum to poor activity (100–200 μg/mL). But, introduction of fluoro function at meta position of the two phenyl groups **(21)** exhibit maximum inhibition at 6.25 μg/mL against *C. neoformans* and moderate activity (25 μg/mL) against *Rhizopus sp.* Similarly, compound **20** against *C. albicans* was inactive but introduction of electron donating group (OCH_3) (**22**) shows superior activity but modification of imidazole group by benzotriazole analogue shows poor inhibition activity. Modification of fluoro substitution and imidazole analogue in **22** by methoxy function and benzotriazole analogue respectively, shows significant activity against *Rhizopus sp* and *A. niger.* Rest of the compounds exhibit minimum to poor activity (25–200 μg/ml). However, compounds **18** and **22** against *A. niger,* **20** and **23** against *C. albicans*, **21** and **24** against *A. flavus* and **25** against *Rhizopus sp.* did not show any growth inhibition activity even at the maximum concentration (200 μg/mL).

4. Conclusion

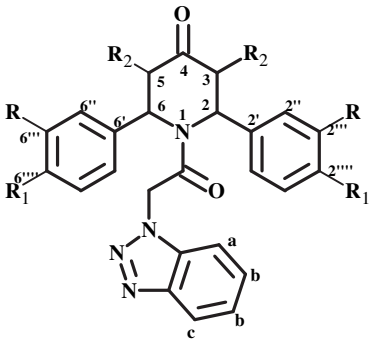
In summary, a close examination of *in vitro* antibacterial and antifungal activities of variously substituted 1-[2-(1H-imidazol/benzotriazole-1-yl)acetyl]-2,6-diarylpiperidin-4-ones against the tested bacterial and fungal strains provide a better structure–activity correlation. Of the tested compounds, those with methyl group at C-3 and C-5 along with *m*-fluorophenyl and *p*-methoxyphenyl at C-2 and C-6 exerted highest level of antibacterial and antifungal activity. Thus, in future, this class of imidazole and benzotriazole derivatives may be used as templates to generate better drugs to fight bacterial and fungal infections.

5. Experimental

All the reagents and solvents used were of high grade and purchased from Alfa Aesar and were distilled prior to use unless otherwise stated. The melting points were recorded in open capillaries and are uncorrected. IR spectra were recorded using AVATAR-330 FT-IR spectrophotometer (Thermo Nicolet) ^{1}H-NMR spectra were recorded at 400 and 500 MHz on BRUKER AMX 400 and 500 MHz spectrophotometer using $CDCl_3$ and DMSO-d_6 as solvent and TMS as an internal standard. ^{13}C-NMR spectra were



17-22



23-26

Compound	R	R_1	R_2	R_3
17	H	H	CH_3	H
18	H	H	CH_2CH_3	H
19	H	H	$CH(CH_3)_2$	H
20	H	H	CH_3	CH_3
21	F	H	CH_3	CH_3
22	H	OCH_3	CH_3	CH_3
23	F	H	CH_3	CH_3
24	H	F	CH_3	CH_3
25	H	OCH_3	CH_3	CH_3
26	H	F	CH_2CH_3	H

Fig. 2. A well numbered target molecules (**17**–**26**).

recorded at 100 MHz on BRUKER AMX 400 MHz spectrometer in $CDCl_3$ and DMSO-d_6 and tetramethylsilane (TMS) as internal standard. Results are presented in the following format; chemical shift δ in ppm, multiplicity, J values in Hertz (Hz), number of protons, proton's position. Multiplicities are shown as the following abbreviations: s (singlet), bs (broad singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet) and m (multiplet). Mass spectra were recorded on JEOL JMS-700 spectrophotometer.

Table 1
Crystal data and structure refinement parameters of compounds **21** and **23**.

Empirical formula	$C_{24}\ H_{22}\ F_2\ N_3\ O_2$	$C_{54}\ H_{48}\ F_4\ N_8\ O_{4.50}$
Formula weight	422.45	478
Wavelength	0.71073 Å	0.71073 Å
Crystal system, space group	Orthorhombic , P2(1)2(1)2(1)	Monoclinic, P21/n
Cell dimensions	$a = 8.8467(5)$ Å; $b = 9.1233(5)$ Å; $c = 26.3523(12)$ Å ; $\alpha = \beta = \gamma = 90^\circ$	$a = 18.6505(7)$ Å, $b = 13.9451(5)$ Å, $c = 19.0945(6)$ Å; $\beta = 105.343(2)$
Z, Density (calculated)	4, 1.319 mg m^{-3}	4, 1.327
Absorption coefficient	0.097 mm^{-1}	0.097 mm^{-1}
Volume	2126.92(19) $Å^3$	4789.2(3) $Å^3$
θ range for data collection	1.55–28.28°	1.36–22.71°
F(000)	884	2000
Crystal size	0.40 × 0.35 × 0.32 mm	0.30 × 0.20 × 0.20 mm
Index range	$-11 \le h \le 11$, $-5 \le k \le 12$, $-31 \le l \le 23$	$-20 \le h \le 20$, $-15 \le k \le 12$, $-20 \le l \le 20$
Reflections collected/unique	9324/4944 [R(int) = 0.0222]	39136/6421 [R(int) = 0.0368]
No. of parameters:	305	686
Goodness-of-fit	0.838	1.099
Largest diff. peak and hole	00.242 and −0.226 e $Å^{-3}$	0.837 and −0.407 e $Å^{-3}$
Data/restraints/parameters	4944/1/305	6421/4/686
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
CCDC	704386	705115

Table 2
Geometric parameters of compound **21**.

Atom	Bond length (Å)	Atom	Bond length (Å)
C(1)–N(1)	1.482(3)	C(6)–N(1)	1.376(3)
C(1)–C(11)	1.525(3)	C(10)–N(2)	1.356(4)
C(3)–O(1)	1.211(3)	C(13)–F(1)	1.308(4)
C(4)–C(5)	1.547(3)	C(15)–F(1A)	1.112(6)
C(5)–N(1)	1.488(3)	C(23)–F(2)	1.352(4)
Atom	**Bond angle (°)**	**Atom**	**Bond angle (°)**
N(1)–C(1)–C(11)	111.82(18)	F(1)–C(13)–C(12)	121.3(3)
C(3)–C(2)–C(17)	108.8(3)	C(24)–C(19)–C(5)	120.6(2)
C(3)–C(2)–C(1)	112.4(2)	F(2)–C(23)–C(24)	118.0(3)
C(2)–C(3)–C(4)	117.2(2)	F(2)–C(23)–C(22)	119.2(3)
N(1)–C(5)–C(4)	111.36(18)	C(6)–N(1)–C(1)	117.26(19)
C(19)–C(5)–C(4)	108.14(17)	C(6)–N(1)–C(5)	122.03(18)
N(1)–C(6)–C(7)	117.07(19)	C(10)–N(2)–C(7)	128.2(2)
N(2)–C(7)–C(6)	112.0(2)	C(8)–N(3)–C(9)	104.3(3)
C(10)–C(9)–N(3)	110.5(3)	C(5)–N(1)–C(6)–O(2)	−8.44 (5)
C(9)–C(10)–N(2)	106.8(3)	C(1)–N(1)–C(6)–C(7)	2.83 (5)

5.1. General procedure for the preparation of 1-[2-(1H-imidazo-1-yl)acetyl]-2,6-diarylpiperidin-4-ones and 1-[2-(1H-benzotriazol-1-yl)acetyl]-2,6-diarylpiperidin-4-ones ***(17–26)***

A mixture of *N*-chloroacetyl-2,6-diarylpiperidin-4-one (1 mmol), triethylamine (3 mmol) and imidazole/1,2,3-benzotriazole (1.2 mmol) in toluene or benzene was refluxed for about 5–6 h. The reaction was monitored by TLC. After the completion of reaction, excess of solvent was removed under reduced pressure. The final mass was poured into 3% sodium bicarbonate solution to remove the formed quaternary ammonium salt. This was then extracted with ether and dried over anhydrous sodium sulphate. The residue thus obtained was purified by column chromatography.

The above general method was adopted for the synthesis of following compounds.

*5.1.1. 1-[2-(1H-imidazol-1-yl)acetyl]-3-methyl-2,6-diphenylpiperidin-4-one (**17**)*

A white solid; yield (71%); mp 123 °C; IR (cm^{-1}): 3049, 2972, 2927, 2852, (C–H stretching); 1717 (C=O stretching); 1654 (N–C=O stretching); 1509, 1454, 1395, 1274, 1233, 1080, 1031, 918, 777, 743, 702, 667, 616, 465. ^{13}C NMR (δ ppm): 62.467 (C-2); 54.259 (C-6); 46.263 (C-3); 43.042 (C-5); 207.888 (C=O at C-4); 169.035 (N–C=O); 140.779 (C-2′ ipso); 141.056 (C-6′ *ipso*) 128.532, 127.404, 126.626 (other aryl carbons); 49.144 (N–COCH_2); 137.998 (C-a Carbon of imidazole ring); 120. 204 (C-b Carbon of imidazole ring); 129.625, 129.318, 129.191, 128.829, 128.368, 128.296, 127.494, 126.761, 125.412 (other aryl carbons); 13.378 (CH_3 at C-3).

Table 3
Geometric parameters of compound **23**.

Atom	Bond length (Å)	Atom	Bond length (Å)
C(1)–N(1)	1.488(5)	C(5)–N(1)	1.487(5)
C(1)–C(2)	1.522(6)	C(5)–C(6)	1.514(5)
C(2)–C(3)	1.495(6)	C(18)–O(2)	1.211(4)
C(1)–C(12)	1.511(6)	C(18)–N(1)	1.353(5)
C(2)–C(26)	1.526(6)	C(18)–C(19)	1.510(5)
C(3)–O(1)	1.206(5)	C(18)–O(2)	1.211(4)
C(3)–C(4)	1.506(6)	C(18)–N(1)	1.353(5)
C(4)–C(27)	1.519(5)	C(18)–C(19)	1.510(5)
C(4)–C(5)	1.542(5)	C(19)–N(2)	1.447(5)
Atom	**Bond angle (°)**	**Atom**	**Bond angle (°)**
N(1)–C(1)–C(12)	112.1(3)	C(2)–C(3)–C(4)	115.8(4)
N(1)–C(1)–C(2)	109.6(3)	C(3)–C(4)–C(5)	108.6(3)
N(1)–C(5)–C(6)	114.0(3)	C(4)–C(5)–C(27)	112.9(3)
N(1)–C(5)–C(4)	110.3(3)	C(6)–C(5)–C(4)	109.7(3)
O(2)–C(18)–N(1)	122.9(4)	C(13)–C(12)–C(1)	118.0(4)
C(12)–C(1)–C(2)	117.6(3)	C(17)–C(12)–C(1)	124.2(4)
C(1)–C(2)–C(3)	112.4(3)	C(1)–N(1)–C(18)–O(2)	6.3(2)
C(1)–C(2)–C(26)	111.6(4)	C(5)–N(1)–C(18)–C(19)	−11.1(2)

*5.1.2. 1-[2-(1H-imidazol-1-yl)acetyl]-3-ethyl-2,6-diphenylpiperidin-4-one (**18**)*

A white solid; yield (68%); mp 147 °C; IR (cm^{-1}): 3065, 2964, 2930, 2869 (C–H stretching); 1714 (C=O stretching); 1663 (N–C=O stretching); 1505, 1453, 1386, 1291, 1234, 1080, 1031, 769, 743, 702, 664, 465 (other stretching frequency). ^{1}H NMR (δ ppm): 6.08 (bs, 1H, H-2a); 5.41 (bs, 1H, H-6a); 4.59, 4.45 (2d, 2H, J = 16.36 and 14.87, NCOCH_2); 2.94–3.08 (m, 2H, H-5a and H-3a); 2.67 (dd, 1H, $^2J_{5a,5e}$ = 17.75 Hz and $^3J_{5a,6a}$ = 4 Hz, H-5e); 1.52–1.72 (m, 2H, CH_2–CH_3); 1.05 (t, 3H, J = 7.45 Hz, CH_3 at C-3); 7.05 (s, 1H, H-a proton of Imidazole); 6.77–7.35 (m, 12H, aryl and H-b-Imidazole protons). ^{13}C NMR (δ ppm): 56.72 (C-2 & C-6); 51.92 (C-3); 45.03 (C-5); 208.23 (C=O at C-4); 169.22 (N-C=O); 140.63 (C-2′ *ipso*); 141.33 (C-6′ *ipso*); 137.93 (C-a Carbon of imidazole ring); 120.0 (C-b Carbon of imidazole ring); 129.58, 129.07, 128.82, 128.40, 128.20, 127.47, 126.10, 125.35 (other aryl carbons); 48.92 (N-COCH_2); 23.01 (CH_2 at CH_2 CH_3); 11.76 (CH_3 at CH_2 CH_3).

*5.1.3. 1-[2-(1H-imidazol-1-yl)acetyl]-3-isopropyl-2,6-diphenylpiperidin-4-one (**19**)*

A white solid; yield (72%); mp 63 °C; IR (cm^{-1}): 3061, 2963, 2929, 2874 (C–H stretching); 1714 (C=O stretching); 1658 (N–C=O stretching); 1507, 1450, 1400, 1274, 1226, 1080, 1032, 917, 812, 762, 700, 664, 623, 519, 468 (other stretching frequency). ^{1}H NMR (δ ppm): 6.43 (bs, 1H, H-2a); 5.23 (bs, 1H, H-6a); 4.64 (d, 1H, J = 16.38, NCOCHH); 4.39 (d, 1H, J=18.01, NCOCHH); 2.82–2.92 (m, 2H, H-3a and H-5a protons are merged together); 2.65 (dd, 1H, $^2J_{5a,5e}$ = 19.38 Hz and $^3J_{5a,6a}$ = 4 Hz, H_{5e}); 2.07 [m, 1H, (CH $(CH_3)_2$) at C-3]; 1.10 (d, 3H, J = 1.32 Hz, [CH(CH_3') (CH_3'')]); 1.08 (d, 3H, J = 1.14 Hz, [CH(CH_3') (CH_3'')]); 6.78–7.38 (m, 13H, aryl and H-a & H-b-Imidazole protons). ^{13}C NMR (δ ppm): 57.261 (C-2); 56.934 (C-6); 54.685 (C-3); 45.399 (C-5); 208.229 (C=O at C-4); 169.039 (N–C=O); 140.921 (C-2′ *ipso*); 141.214 (C-6′ *ipso*) 128.532, 127.404, 126.626 (other aryl carbons); 48.936 (N–COCH_2); 137.966 (C-a Carbon of imidazole ring); 120.278 (C-b Carbon of imidazole ring); 129.414, 128.819, 128.630, 128.164, 127.903, 127.421, 125.910, 125.599, (other aryl carbons); 28.872 [CH–(CH_3') (CH_3'')]; 20.890 [CH–(CH_3') (CH_3'')]; 20.436 [CH–(CH_3') (CH_3'')].

*5.1.4. 1-[2-(1H-imidazol-1-yl)acetyl]-3,5-dimethyl-2,6-diphenylpiperidin-4-one (**20**)*

A pale yellow solid; yield (75%); mp 163 °C; IR (cm^{-1}): 3061, 2978, 2936, 2876 (C–H stretching); 1716 (C=O stretching); 1644 (N–C=O stretching); 1502, 1453, 1385, 1285, 1233, 1203, 1080, 1031, 916, 817, 762, 703, 665, 628, 530, 421. ^{1}H NMR (δ ppm): 5.41 (bs, 2H, H-2a and H-6a); 4.55 (s, 2H, NCOCH_2); 3.18 (m, 2H, H-3a and H-5a); 7.05 (s, 1H, H-a proton of imidazole); 6.73–7.36 (12H, aryl and H-b proton of imidazole); 1.07 (d, 6H, J = 6.90 Hz, CH_3 at C-3 and C-5). ^{13}C NMR (δ ppm): 61.447 (C-2 and C-6); 45.466 (C-3 and C-5); 210.384 (C=O at C-4); 169.327 (N–C=O); 140.776 (C-2′ and C-6′ *ipso*); 137.952 (C-a Carbon of imidazole ring); 120.291 (C-b Carbon of imidazole ring); 129.223, 128.562, 128.412, 128.128, 127.613, 127.084, (other aryl carbons); 49.038 (N-COCH_2); 14.205 (CH_3 at C-3 and C-5).

*5.1.5. 1-[2-(1H-imidazol-1-yl)acetyl]-3,5-dimethyl-2,6-bis(m-fluorophenyl)piperidin-4-one (**21**)*

A pale yellow solid; yield (67%); mp 127 °C; IR (cm^{-1}): 3073, 2969, 2931, 2871, 2853, 2809 (C–H stretching); 1710 (C=O stretching); 1666 (N–C=O stretching); 1591, 1513, 1488, 1450, 1386, 1354, 1277, 1240, 1192, 1079, 1038, 950, 884, 789, 747 (other stretching frequency). ^{1}H NMR (δ ppm): 5.49 (bs, 2H, H-2a and H-6a); 3.10 (m, 2H, H-3a and H-5a); 4.71 (s, 2H, NCOCH_2); 7.76 (s, 1H,

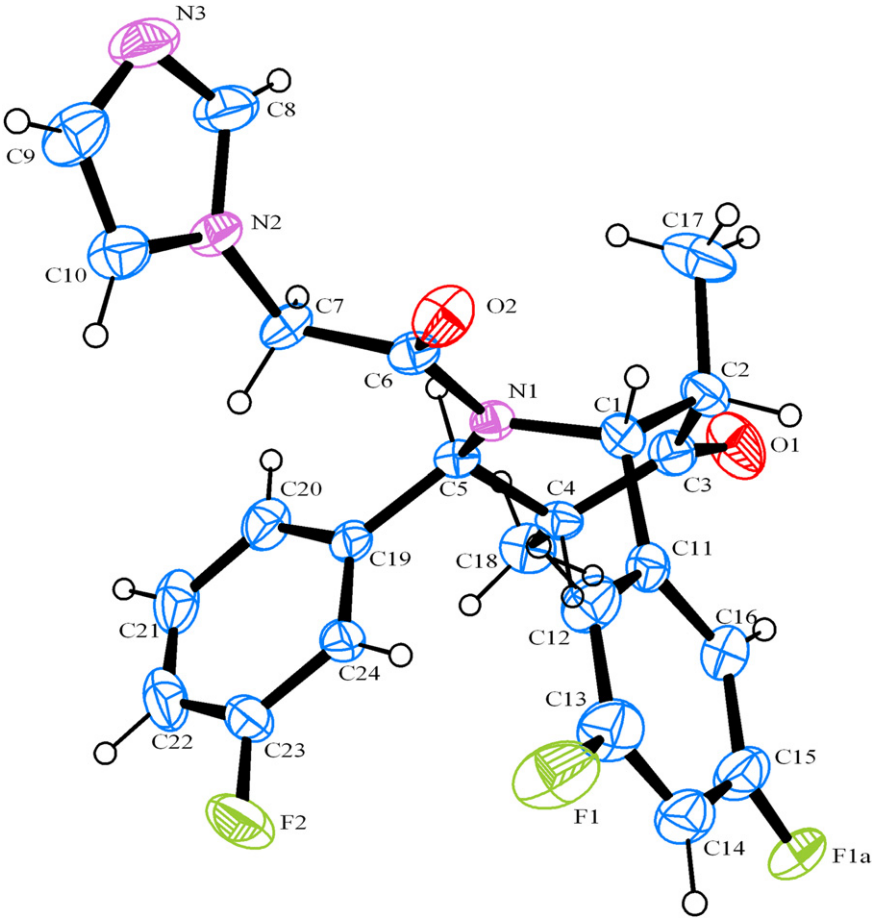


Fig. 3. ORTEP diagram of compound **21** with 30% probability.

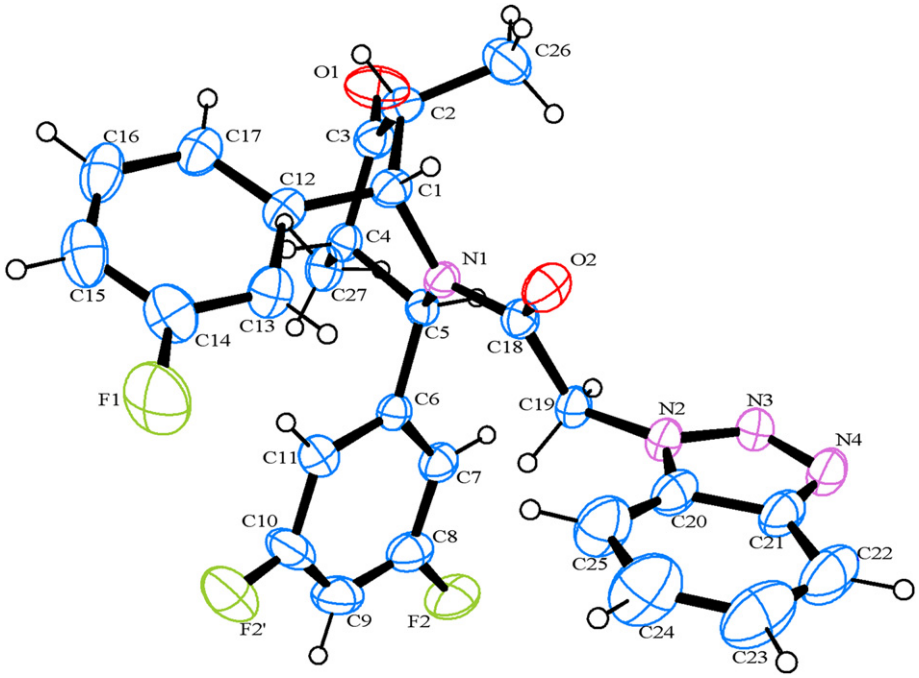


Fig. 4. ORTEP diagram of compound **23** with 30% probability.

Table 4
In vitro antibacterial activity of compounds **17**–**26** against selected bacterial strains.

Compounds	Minimum inhibitory concentration (MIC) in μg/ml				
	S. aureus (ATCC-25930)	*B. subtils* (ATCC-530)	*S. tyhpi* (ATCC-25021)	*E. coli* (ATCC-26032)	*K. pneumonia* (ATCC-16425)
17	100	25	200	100	50
18	200	–	200	50	100
19	100	12.5	25	100	–
20	–	100	100	6.25	100
21	50	–	50	200	200
22	100	50	100	25	200
23	50	25	25	50	25
24	100	50	50	12.5	–
25	25	200	100	50	100
26	50	6.25	50	–	100
Streptomycin	50	12.5	50	12.5	25

–, No inhibition even at maximum concentration.

H-a imidazole proton); 6.89–7.39 (m, 10H, aryl and H-b imidazole protons); 1.10 (d, 6H, $J = 6.92$ Hz, CH_3 at C-3 and C-5). ^{13}C NMR (δ ppm): 60.878 (C-2 and C-6); 45.368 (C-3 and C-5); 209.348 (C=O at C-4); 169.160 (N–C=O); 143.151 (C-2′ and C-6′ *ipso*); 137.702 (C-a Carbon of imidazole ring); 120.706 (C-b Carbon of imidazole ring); 143.165 and 143.102 (C-2′ and C-6′); 164.341 and 161.869 (C-2‴ and C-6‴); 130.966, 130.883, 127.408, 123.336, 123.308, 115.717, 115.509, 114.695, 114.472 (other aryl carbons); 49.223 (N–CO$\underline{C}H_2$); 14.181 (CH_3 at C-3 and C-5). HR-MS, (m/z) 423.10 (M^+).

*5.1.6. 1-[2-(1H-imidazol-1-yl)acetyl]-3,5-dimethyl-2,6-bis(p-methoxyphenyl)piperidin-4-one (**22**)*

A white solid; yield (77%); mp 139 °C; IR (cm^{-1}): 2997, 2935, 2839 (C–H stretching); 1715 (C=O stretching); 1645, 1610 (N–C=O and C=N stretching); 1513, 1548, 1396, 1286, 1180, 1112, 1108, 1080, 835, 663, 591 and 539 (other stretching frequency). ^{1}H NMR (δ ppm): 5.32 (bs, 2H, H-2a and H-6a); 4.51 (s, 2H, NCO$\underline{C}H_2$); 3.15 (m, 2H, H-3a and H-5a); 6.87 (d, 4H, aryl protons ortho to methoxy group); 7.08 (d, 4H, aryl protons meta to methoxy group); 7.22 (s, 1H H-a imidazole protons); 7.03 (2H, H-b proton of imidazole); 3.81 (s, 6H, OCH_3 at C-2‴′ and C-6‴′); 1.06 (d, 6H, $J = 6.90$ Hz, CH_3 at C-3 and C-5). ^{13}C NMR (δ ppm): 61.020 (C-2 and C-6); 45.696 (C-3 and C-5); 210.704 (C=O at C-4); 169.267 (N–C=O); 132.797 (C-2′ and C-6′ *ipso*); 159.469 (C-2‴′ and C-6‴′ *ipso*); 137.986 (C-a Carbon of imidazole ring); 120.100 (C-b Carbon of imidazole ring); 130.284, 129.343, 128.816 (C-2″ and C-6″) 114.219, 114.519 (C-2‴ and C-6‴); 48.970 (N–CO$\underline{C}H_2$); 55.386 (OCH_3 at C-2‴′ and C-6‴′), 14.241 (CH_3 at C-3 and C-5).

*5.1.7. 1-[2-(1H-benzotriazol-1-yl)acetyl]-3,5-dimethyl-2,6-bis(m-fluorophenyl)piperidin-4-one (**23**)*

A white yellow solid; yield (69%); mp 161 °C; IR (cm^{-1}): 3064, 2921, 2851 (C–H stretching); 1717 (C=O stretching); 1663 and 1613 (N–C=O and C=N stretching); 1588, 1490, 1454, 1386, 1319, 1274, 1240, 1133, 1098, 955, 878, 792, 746, 704 and 523 (other stretching frequency). ^{1}H NMR (δ ppm): 5.46 (bs, 2H, H-2a and H-6a); 5.27 (s, 2H, NCO$\underline{C}H_2$); 3.09 (m, 2H, H-3a and H-5a); 7.05–7.52 (m, 11H, aryl protons); 8.06 (d, 1H, H-c proton of benzotriazole). 1.00 (d, 6H, $J = 6.96$ Hz, CH_3 at C-3 and C-5). ^{13}C NMR (δ ppm): 50.892 (C-2 and C-6); 45.306 (C-3 and C-5); 209.179 (C=O at C-4); 167.938 (N–C=O); 164.325 and 161.851 (C-2‴′ and C-6‴′ *ipso*); 142.983, 142.923 (C-2′ and C-6′ *ipso* carbons); 133.603, 130.931, 128.320, 125.057, 123.357, 119.623, 115.691, 115.479, 114.720, 114.492, 109.984 (other aryl carbons); 60.971 (N–CO$\underline{C}H_2$); 14.037 (CH_3 at C-3 and C-5). HR-MS (m/z): 473.86 (M + 1).

*5.1.8. 1-[2-(1H-benzotriazol-1-yl)acetyl]-3,5-dimethyl-2,6-bis(p-methoxyphenyl)piperidine-4-one (**24**)*

A white solid; yield (70%); mp 183 °C; IR (cm^{-1}): 3079, 2969, 2922, 2851 (C–H stretching); 1715 (C=O stretching); 1643 and 1611 (N–C=O and C=N stretching); 1512, 1458, 1429, 1395, 1285, 1252, 1177, 1093, 1034, 840, 746, 666, 594, 541 (other stretching frequency). ^{1}H NMR (δ ppm): 5.38 (bs, 2H, H-2a and H-6a); 5.18 (s, 2H, NCO$\underline{C}H_2$); 3.08 (m, 2H, H-3a and H-5a); 3.75 (s, 6H, OCH_3 at C-2‴″ and C-6‴″); 0.96 (d, 6H, $J = 6.80$ Hz, CH_3 at C-3 and C-5); 6.82 (d, 4H, aryl protons ortho to the substituent); 7.08 (d, 4H, aryl protons meta to the substituent) 7.96 (d, 1H, H-c proton of benzotriazole); 7.24–7.40 (m, 3H, H-b proton in benzotriazole). ^{13}C NMR (δ ppm): 50.907 (C-2 and C-6); 45.610 (C-3 and C-5); 210.697 (C=O at C-4); 167.907 (N–C=O); 132.601 (C-2′ and C-6′ *ipso* carbon); 159.346 (C-2‴″ and C-4‴″); 128.836, 128.002 124.480, 119.683, 114.432, 109.954 (other aryl carbons); 55.340 (OCH_3 at C-2‴″ and C-6‴″); 14.083 (CH_3 at C-3 and C-5).

*5.1.9. 1-[2-(1H-benzotriazol-1-yl)acetyl]-3-ethyl-2,6-bis(p-fluorophenyl)piperidin-4-one (**25**)*

A pale yellow solid; yield (64%); mp 154 °C; IR (cm^{-1}): 3072, 2969, 2934, 2871 (C–H stretching); 1719 (C=O stretching); 1653

Table 5
In vitro antifungal activity of compounds **17**–**26** against selected fungal strains.

Compounds	Minimum inhibitory concentration (MIC) in μg/ml				
	C. neoformans (ATCC-3235)	*C. albicans* (ATCC-3430)	*Rhizopus* sp. (ATCC-2842)	*A. niger* (ATCC-635)	*A. flavus*(ATCC-525)
17	100	100	100	200	100
18	50	200	100	–	200
19	25	100	50	12.5	100
20	100	–	200	100	100
21	6.25	50	25	100	–
22	50	6.25	100	–	100
23	100	–	100	200	200
24	200	50	6.25	12.5	–
25	100	200	–	50	50
26	50	100	25	100	50
Amphotericin-B	25	25	25	50	50

—, no inhibition even at maximum concentration.

and 1605 (N–C=O and C=N stretching); 1509, 1456, 1392, 1228, 1266, 1163, 1093, 1011, 955, 834, 749, 668, 597 and 520 (other stretching frequency). ^{1}H NMR (δ ppm): 6.02 (bs, 1H, H-2a); 5.14 (bs, 1H, H-6a); 5.50–5.68 (m, 2H, NCOCH$_2$); 3.03 (dd, $^2J_{5a5e} = 17.00$ Hz), $^3J_{5a6a} = 8.00$ Hz, 1H, H-5a; 2.85–2.97 (m, 1H, H-3a); 2.68 (m, 1H, H-5e); 1.50–1.58 (m, 2H, CH$_2$ CH$_3$); 0.88, t, 2H ($J = 7.25$ Hz, CH$_2$ CH$_3$); 6.89–7.53 (m, 11H, aryl protons); 8.08 (d, 1H, $J = 8.3$ Hz, H-c proton of benzotriazole). ^{13}C NMR (δ ppm): 55.763 (C-2); 54.759 (C-6); 52.553 (C-3); 44.032 (C-5); 207.516 (C=O at C-4); 167.742 (N–C=O); 163.510 & 161.061 (C-2′′′′ and C-6′′′′); (146.185 (C-2′ and C-6′ *ipso*)); 136.185, 133.546, 130.733, 129.328, 128.289, 128.070, 127.572, 124.281, 120.282, 116.409, 116.200, 115.952, 115.737, 115.422, 109.805 (other aryl carbons); 56.736 (N–COCH$_2$); 23.222 (CH$_2$ at CH$_2$ CH$_3$); 11.476 (CH$_3$ at CH$_2$ CH$_3$).

5.1.10. 1-[2-(1H-benzotriazol-1-yl)acetyl]-3,5-dimethyl-2,6-bis(p-fluorophenyl)piperidin-4-one (**26**)

A pale yellow solid; yield (68%); mp 208 °C; IR (cm^{-1}): 3090, 2969, 2923, 2853 (C–H stretching); 1716 (C=O stretching); 1647, 1604 (N-C=O and C=N stretching); 1510, 1457, 1396, 1321, 1285, 1231, 1162, 1095, 1019, 839, 750, 671, 588, 534 (other stretching frequency). ^{1}H NMR (δ ppm): 5.40 (bs, 2H, H-2a and H-6a); 3.02 (m, 2H, H-3a and H-5a); 5.22 (s, 2H, NCOCH$_2$); 0.94 (d, 6H, $J = 6.80$ Hz, CH$_3$ at C-3 and C-5); 7.98 (d, 2H, H-a proton of benzotriazole); 6.81–7.42 (aryl protons). ^{13}C NMR (δ ppm): 50.40 (C-2 and C-6); 45.53 (C-3 and C-5); 60.713, NCOCH$_2$; 209.63 (C=O at C-4); 167.99 (N–C=O); 145.62 (C-2′ and C-6′ *ipso* carbon); 161.58 & 161.10 (C-2′′′′ and C-4′′′′); 136.33, 136.30, 133.542, 130.74, 129.39, 124.38, 116.31, 116.10, 109.75 (other aryl carbons); 14.053 (CH$_3$ at C-3 and C-5).

5.2. Recording of single crystal XRD

Suitable crystals of the thiadiazoles **4a** and **5c** were grown from ethanol at room temperature. The data collection was carried out on Bruker APEXII CCD area detector diffractometer [21]. Graphite monochromatic Mo Kα radiation ($\lambda = 0.71073$ Å) was used throughout. The structures were solved by direct methods (SHELXS97) [22], which revealed the position of all non-hydrogen atoms and refined by full-matrix least-squares method (SHELXL97). Hydrogen atoms were geometrically fixed and were given riding model refinement. Molecular graphics and geometric calculations were obtained from ORTEP-3 [23].

5.3. Pharmacology

The bacterial strains viz., *S. aureus* (ATCC-25930), *B. subtilis* (ATCC-530), *S. typhi* (ATCC-25021), *E. coli* (ATCC-26032) and *K. pneumonia* (ATCC-16425) and fungal strains viz., *C. albicans* (ATCC-3430), *Crytococcus neoformans* (ATCC-3235), *R. species* (ATCC-2842), *A. niger* (ATCC-635) and *A. flavus* (ATCC-525) are procured from National Chemical Laboratory, Pune, India.

5.3.1. In vitro antibacterial and antifungal activity

In vitro activities of the compounds were tested in Sabourauds dextrose broth (SDB) for fungi and in Nutrient broth (NB) for bacteria by the two-fold serial dilution method [24]. Seeded broth (broth containing microbial spores) was prepared in NB from 24 h old bacterial cultures on nutrient agar (Hi-media, India) at 37 ± 1 °C while fungal spores from 24 h to 7 days old Sabourauds agar slant cultures were suspended in SDB. The bacterial suspension was adjusted with sterile saline to a concentration of 1×10^4–10^5 CFU. The tested compounds and reference drugs were prepared by two-fold serial dilution to obtain the required concentrations of 200, 100, 50 25, 12.5 and 6.25 μg/mL. The tubes were incubated in BOD incubators at 37 ± 1 °C for bacteria and 28 ± 1 °C for fungi. The minimum inhibitory concentrations (MICs) were recorded by visual observations after 24 h (for bacteria) and 72–96 h (for fungi except *C. albicans*) of incubation. Streptomycin and Amphotericin B were used as standards for bacterial and fungal study, respectively.

Acknowledgements

We thank NMR Research Centre, Indian Institute of Science Bangalore, for providing NMR facilities to record NMR spectra and Department of Chemistry & SAIF, Indian Institute of Technology – Madras for recording X-ray Data collection.

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