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REVIEW ARTICLE

Synthesis, NMR spectral studies and antimicrobial evaluation of some 2-(benzothiazol-2-yl)-1-(alkyl-2*r*,6*c*-diarylpiperidin-4-ylidine)hydrazine derivatives

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Abstract Substituted 2-(benzothiazol-2-yl)-1-(alkyl-2,6-diarylpiperidin-4-ylidine)hydrazines **10–17** were synthesized by the condensation of different 2*r*,6*c*-diarylpiperidin-4-ones **1–8** with 2-hydrazinobenzothiazole **9**. All the synthesized compounds were investigated in solution and in the solid state by IR, ^{1}H, ^{13}C and 2D NMR spectral techniques. The structure–activity relationships were studied by the screening of the antimicrobial activity over a representative panel of bacterial and fungal strains using two-fold serial dilution method.

Keywords 2*r*,6*c*-Diarylpiperidin-4-one · 2-Mercaptobenzothiazole · Hydrazine hydrate · NMR techniques · Antibacterial activity · Antifungal activity

Introduction

Thiazoles are the important group of heterocyclic compounds due to their drug utility (Valverde and Torroba, 2005), and thiazolidinones exhibited good antimicrobial activities (Barreca *et al.*, 2001; Kucukguzel *et al.*, 2006; Verma and Saraf, 2008; Aridoss *et al.*, 2009). 2,3-Disubstituted analogues of thiazolidinones proved to be predominantly effective in non-nucleoside HIV reverse transcriptase inhibitors and it was found in the drug development programme for the treatment of inflammation (Sharma *et al.*, 1998) and HIV (Bell *et al.*, 1995). 2-Mercaptobenzothiazole derivatives played a vital role in antitubercular (Mistry and Desai, 2006), antimicrobial (Pattan *et al.*, 2006, Guru *et al.*, 2006), anti-inflammatory (Srivastava *et al.*, 2002), antiviral (Rani *et al.*, 1990) and anticancer (Chande *et al.*, 1995) activities. Many hydrazine derivatives showed whole panoply of chemotherapeutic properties (Beraldo, 2004).

Substituted 2,6-diarylpiperidin-4-ones (Geneste *et al.*, 1976) were subjected to quite a large number of synthetic (Noller and Balliah, 1948) and physico-chemical studies (Pandiarajan *et al.*, 1987, Sivasubramanian *et al.*, 1981). Owing to the importance of the title compound, eight piperidone derivatives of hydrazines have been synthesized and the structures have been confirmed by spectral studies and biological activities have also been estimated for the synthesized compounds.

Materials and methods

Physical measurements

The melting points were recorded in an open capillary tube and were uncorrected. IR spectra were recorded in AVATAR-330 FT-IR spectrometer (Thermo Nicolet). ^{1}H, ^{13}C and 2D NMR (^{1}H–^{1}H and ^{1}H–^{13}C COSY) spectra were recorded at 500 MHz, on a BRUKER AMX 500 MHz spectrometer using DMSO as the solvent and TMS as the internal standard. All the spectra of **10–17** were measured at room temperature (298 K).

Experimental section

Synthesis of substituted 2*r*,6*c*-diarylpiperidin-4-ones **1–8**

2*r*,6*c*-Diarylpiperidin-4-ones were prepared using one spot multicomponent Mannich reaction by condensing suitable aromatic aldehydes, ketones and ammonium acetate in

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1:2:1 ratio using ethanol as the solvent. The mixture was heated to boiling and allowed to stand at room temperature overnight. 50 mL concentrated HCl was added and the obtained precipitate was washed with ethanol–ether (1:5) mixture. The hydrochloride salt in acetone was treated with strong ammonia solution and the free base was obtained by pouring water. The product was recrystallised from ethanol (Noller and Balliah, 1948).

Synthesis of 2-hydrazinobenzothiazole **9**

2-Hydrazinobenzothiazole was prepared by refluxing an equimolar solution of 2-mercaptobenzothiazole (0.2 mol) and hydrazine hydrate (0.2 mol) in methanol (150 mL) on a steam bath for 10 h. It was cooled, filtered and washed with ice water. The product was dried and recrystallised from ethanol to yield the pure compound **9** (Dua *et al.*, 2010) (yield 61 %; m.p. 202 °C).

Synthesis of 2-(benzothiazol-2-yl)-1-(alkyl-2*r*,6*c*-diarylpiperidin-4-ylidine)hydrazines **10–17**

To a boiling solution of 2*r*,6c-diarylpiperidin-4-ones (0.1 mol) in methanol, 2-hydrazinobenzothiazole (0.1 mol) was added with constant stirring and the reaction mixture was refluxed for 2–3 h on a water bath. After cooling, the product was filtered and then washed with water. The products, **10–17**, were purified by column chromatography. All the synthesized compounds were obtained in good yield and their analytical and spectral data are given below.

2-(Benzothiazol-2-yl)-1-(2,6-diphenylpiperidin-4-ylidene)hydrazine ***10***

Yield 74 %, m.p. 161 °C; IR (KBr, cm^{-1}): *v* 1601 (benzothiazole C=N), 1556 (C=N), 3425 (NH), 2853–3069 (aromatic); ^{1}H NMR (DMSO, 500 MHz, ppm): δ 2.04 (t, 1H, H-5a), 2.43 (m, 1H, H-3e), 2.52 (d, 1H, H-3e), 2.84 (s, 1H, NH), 3.44 (1H, H-5e), 3.86 (m, 1H, H-2a), 3.94 (d, 1H, H-6a), 7.04–7.68 (aromatic), 11.42 (s, 1H, N–NH); ^{13}C NMR (DMSO, 500 MHz, ppm): δ 37.1 (C-5), 43.7 (C-3), 60.6 (C-6), 61.6 (C-2), 121.5–128.8 (aromatic), 157.2 (C-4), 167.0 (thiazole C=N).

2-(Benzothiazol-2-yl)-1-(3-ethyl-2,6-diphenylpiperidin-4-ylidene)hydrazine ***11***

Yield 69 %, m.p. 191 °C; IR (KBr, cm^{-1}): *v* 1630 (benzothiazole C=N), 1597 (C=N), 3331, 3439 (NH), 2855–3222 (aromatic); ^{1}H NMR (DMSO, 500 MHz, ppm): δ 0.87 (t, 3H, CH_3), 1.17 (s, 1H, H-7a), 1.63 (s, 1H, H-7b), 2.06 (t, 1H, H-5a), 2.39 (m, 1H, H-3a), 2.85 (s, 1H, NH), 3.46 (1H, H-5e), 3.61 (d, 1H, H-2a), 3.83 (d, 1H, H-6a), 7.05–7.80 (aromatic), 11.51 (s, 1H, N–NH); ^{13}C NMR (DMSO, 500 MHz, ppm): δ 12.4 (CH_3), 19.3 (CH_2), 37.7 (C-5), 51.3 (C-3), 59.8 (C-6), 66.6 (C-2), 120.4–143.9 (aromatic and *ipso*), 162.3 (C-4), 169.7 (thiazole C=N).

2-(Benzothiazol-2-yl)-1-(3-methyl-2,6-diphenylpiperidin-4-ylidene)hydrazine ***12***

Yield 64 %, m.p. 173 °C; IR (KBr, cm^{-1}): *v* 1663 (benzothiazole C=N), 1601 (C=N), 3358, 3455 (NH), 2924–3210 (aromatic); ^{1}H NMR (DMSO, 500 MHz, ppm): δ 0.87 (d, 3H, CH_3), 2.12 (t, 1H, H-5a), 2.56 (m, 1H, H-3a), 3.51 (d, 1H, H-2a, H-5e), 3.86 (d, 1H, H-6a), 7.04–7.70 (aromatic), 11.46 (s, 1H, N–NH); ^{13}C NMR (DMSO, 500 MHz, ppm): δ 12.8 (CH_3), 37.4 (C-5), 44.7 (C-3), 60.5 (C-6), 69.2 (C-2), 117.6–144.6 (aromatic and *ipso*), 160.4 (C-4), 168.6 (thiazole C=N).

1-(2,6-Bis(4-bromophenyl)-3-methylpiperidin-4-ylidene)-2-(benzothiazol-2-yl)hydrazine ***13***

Yield 67 %, m.p. 174 °C; IR (KBr, cm^{-1}): *v* 1600 (benzothiazole C=N), 1554 (C=N), 3413 (NH), 2853–3183 (aromatic); ^{1}H NMR (DMSO, 500 MHz, ppm): δ 0.85 (d, 3H, CH_3), 2.05 (t, 1H, H-5a), 2.51 (m, 1H, H-3a), 2.91 (s, 1H, NH), 3.49 (m, 1H, H-2a), 3.84 (d, 1H, H-6a), 11.49 (s, 1H, N–NH), 7.05–7.68 (aromatic), 11.49 (s, 1H, N–NH); ^{13}C NMR (DMSO, 500 MHz, ppm): δ 12.6 (CH_3), 37.3 (C-5), 44.6 (C-3), 59.7 (C-6), 68.2 (C-2), 121.0–148.0 (aromatic and *ipso*), 160.8 (C-4), 168.1 (thiazole C=N).

1-(2,6-Bis(4-fluorophenyl)-3-methylpiperidin-4-ylidene)-2-(benzothiazol-2-yl)hydrazine ***14***

Yield 72 %, m.p. 188 °C; IR (KBr, cm^{-1}): *v* 1649 (benzothiazole C=N), 1602 (C=N), 3319, 3418 (NH), 2852–3068 (aromatic); ^{1}H NMR (DMSO, 500 MHz, ppm): δ 0.85 (d, 3H, CH_3), 1.06 (t, 1H, H-5a), 2.08 (m, 1H, H-3a), 2.82 (s, 1H, NH), 3.45 (1H, H-5e), 3.52 (m, 1H, H-2a), 3.86 (d, 1H, H-6a), 7.05–7.70 (aromatic); ^{13}C NMR (DMSO, 500 MHz, ppm): δ 12.7 (CH_3), 19.0 (C-5), 44.6 (C-3), 59.7 (C-6), 68.2 (C-2), 115.2–152.4 (aromatic and *ipso*), 160.9 (C-4), 171.4 (thiazole C=N).

1-(2,6-Bis(4-chlorophenyl)-3-methylpiperidin-4-ylidene)-2-(benzothiazol-2-yl)hydrazine ***15***

Yield 68 %, m.p. 188 °C; IR (KBr, cm^{-1}): *v* 1643 (benzothiazole C=N), 1603 (C=N), 3418 (NH), 2853–2924 (aromatic); ^{1}H NMR (DMSO, 500 MHz, ppm): δ 0.85 (d, 3H, CH_3), 2.06 (t, 1H, H-5a), 2.49 (m, 1H, H-3a), 2.86 (s, 1H, NH), 3.48 (m, 1H, H-2a, H-5e), 3.85 (d, 1H, H-6a), 11.48 (s, 1H, N–NH), 7.03–7.69 (aromatic), 11.48 (s, 1H,

N–NH); ^{13}C NMR (DMSO, 500 MHz, ppm): δ 12.6 (CH_3), 37.3 (C-5), 44.6 (C-3), 59.7 (C-6), 68.2 (C-2), 122.0–152.9 (aromatic and *ipso*), 168.9 (C-4), 174.3 (thiazole C=N). Anal. found (Cal.) for $C_{24}H_{22}Cl_2N_4S$ (%): C, 57.46 (57.48); H, 4.39 (4.42); N, 11.15 (11.18).

1-(2,6-Bis(2-chlorophenyl)-3-methylpiperidin-4-ylidene)-2-(benzothiazol-2-yl)hydrazine ***16***

Yield 69 %, m.p. 171 °C; IR (KBr, cm^{-1}): *v* 1645 (benzothiazole C=N), 1601 (C=N), 3317 (NH), 2856–2924 (aromatic); ^{1}H NMR (DMSO, 500 MHz, ppm): δ 0.93 (d, 3H, CH_3), 2.06 (t, 1H, H-5a), 2.51 (m, 1H, H-3a), 2.94 (s, 1H, NH), 4.13 (m, 1H, H-2a, H-5e), 4.23 (d, 1H, H-6a), 6.97–7.88 (aromatic), 11.48 (s, 1H, N–NH); ^{13}C NMR (DMSO, 500 MHz, ppm): δ 12.1 (CH_3), 37.2 (C-5), 45.2 (C-3), 56.9 (C-6), 63.5 (C-2), 120.7–130.8 (aromatic), 168.8 (C-4), 174.3 (thiazole C=N).

1-(2,6-Bis(4-methoxyphenyl)-3-methylpiperidin-4-ylidene)-2-(benzothiazol-2-yl)hydrazine ***17***

Yield 66 %, m.p. 177 °C; IR (KBr, cm^{-1}): *v* 1643 (benzothiazole C=N), 1605 (C=N), 3424 (NH), 2800–2950 (aromatic); ^{1}H NMR (DMSO, 500 MHz, ppm): δ 0.86 (d, 3H, CH_3), 2.08 (t, 1H, H-5a), 2.51 (m, 1H, H-3a), 3.18 (s, 1H, NH), 3.43 (d, 2H, H-2a, H-5e), 3.79 (d, 1H, H-6a), 6.90–7.68 (aromatic), 11.43 (s, 1H, N–NH); ^{13}C NMR (DMSO, 500 MHz, ppm): δ 12.8 (CH_3), 37.6 (C-5), 44.9 (C-3), 55.4, 55.5 (O–CH_3), 59.9 (C-6), 68.6 (C-2), 113.9–158.9 (aromatic and *ipso*), 162.7 (C-4), 169.0 (thiazole C=N).

Biological activities

In vitro evaluation of antimicrobial activities

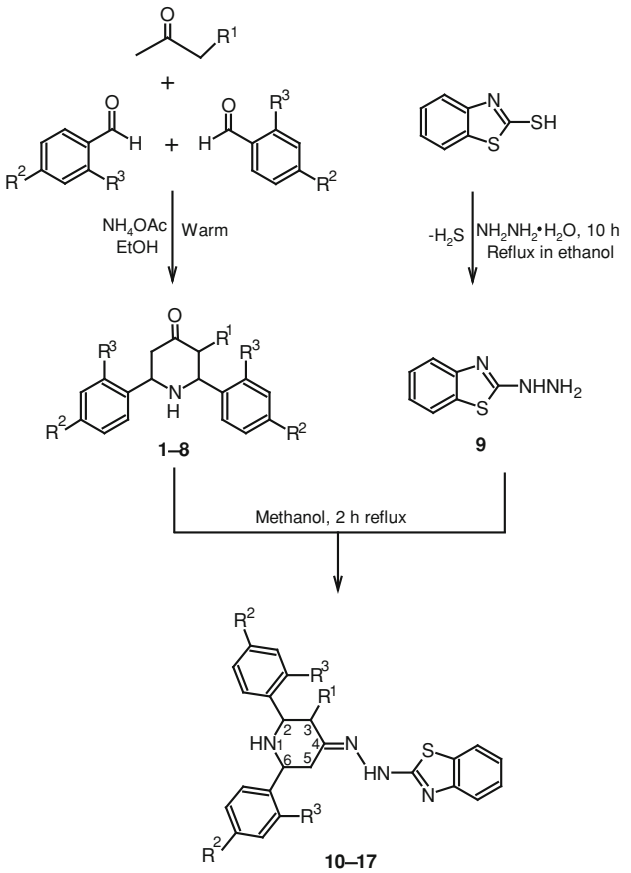
All the bacterial strains viz., *Klebsiella pneumoniae* (MTCC 2272), *Escherichia coli* (MTCC 443), *Bacillus subtilis* (MTCC 121), *Pseudomonas aeruginosa* (MTCC 741), *Staphylococcus aureus* (MTCC 96) and the fungal strains viz., *Candida albicans*, *Fusarium oxysporum*, *Aspergillus flavus*, *Aspergillus niger* and *Cryptococcus neoformans* were obtained from the Faculty of Medicine, Annamalai University, India.

In vitro antimicrobial activities of the compounds were tested in Sabouraud's dextrose broth (SDB, Hi-Media, Mumbai) for fungi and in nutrient broth (NB, Hi-Media, Mumbai) for bacteria by the two-fold serial dilution method (Dhar *et al.*, 1968). The test compounds were dissolved in dimethylsulphoxide (DMSO) to obtain 1 mg/mL stock solutions. The seeded broth (broth containing microbial spores) was prepared in nutrient broth from 24-h-old bacterial cultures on nutrient agar (Hi-Media, Mumbai) at 37 ± 1 °C, while fungal spores from 24 h to 7-days-old Sabouraud's agar slant cultures were suspended in SDB. The colony-forming units (cfu) of the seeded broth were determined by the plate count technique and were adjusted with the help of McFarland standards in the range of 10^4–10^5 cfu/mL. The final inoculums size was 10^5 cfu/mL for antibacterial assay, and 1.1–1.5 to 10^2 cfu/mL for the antifungal assay. The testing was performed at pH 6.5 ± 0.2 for bacteria, and pH 5.6 ± 0.2 for fungal studies. Exactly 0.2 mL solution of the test compound was added to 1.8 mL of the seeded broth to form the first dilution. One millilitre of this was diluted with a further 1 mL of the seeded broth to give the second dilution and so on, till six such dilutions were obtained. A set of assay tubes containing only the seeded broth was kept as control. Simillarly the solvent controls were also run simultaneously. The tubes were incubated in biochemical oxygen demand (BOD) incubators at 37 ± 1 °C for bacteria, and 28 ± 1 °C for fungal studies. The minimum inhibitory concentrations (MIC) 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 studies, respectively.

Results and discussion

The reported new hydrazine derivatives **10**–**17** were synthesized as shown in Scheme 1 and their analytical data and the nature of the substituents are given in Table 1. In the IR spectra, the presence of C=N stretching frequency around 1,646 and 1,603 cm^{-1} confirms the formation of the new hydrazine derivatives. However, the absence of C=O stretching frequency, around 1,720 cm^{-1} also confirms the formation of the new compound. A collection of bands observed in the region of 3,418–3,309 cm^{-1} are due to the N–H stretching frequency. The absorption bands observed in the region 3,057–2,853 cm^{-1} are ascribed due to the aromatic and aliphatic C–H vibrations. The numbering of target compounds are shown in Fig. 1. Based on the previous studies, it has been concluded that piperidin-4-ones **1**–**8** exist in chair conformation (Pandiarajan *et al.*, 1991; Krishnapillay and Fazal Mohamed, 1997). In this conformation, the aryl groups are present in the equatorial orientation and the alkyl group at C-3 also occupies the equatorial orientation in 3-alkyl substituted compounds. The observed vicinal coupling constants suggest that the synthesized hydrazines **10**–**17** also exist as a chair conformation.

The ^{1}H NMR spectral analysis of the representative compound **15** is discussed as follows. In compound **15**, H-5e is deshielded by 1.42 ppm than the H-5a proton.

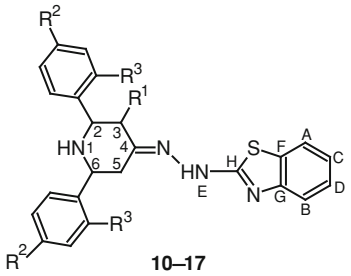


	R^1	R^2	R^3
10;	H	H	H
11;	CH_2CH_3	H	H
12;	CH_3	H	H
13;	CH_3	Br	H
14;	CH_3	F	H
15;	CH_3	Cl	H
16;	CH_3	H	Cl
17;	CH_3	OCH_3	H

Scheme 1 2-(Benzothiazol-2-yl)-1-(alkyl-2*r*,6*c*-diarylpiperidin-4-ylidine)hydrazine derivatives

Table 1 Analytical data for compounds **10–17**

Compounds	R^1	R^2	R^3	Yield (%)	m.p. (°C)
10	H	H	H	74	161
11	CH_2CH_3	H	H	69	191
12	CH_3	H	H	64	173
13	CH_3	Br	H	67	174
14	CH_3	F	H	72	188
15	CH_3	Cl	H	68	182
16	CH_3	H	Cl	69	171
17	CH_3	OCH_3	H	66	177



	R^1	R^2	R^3
10;	H	H	H
11;	CH_2CH_3	H	H
12;	CH_3	H	H
13;	CH_3	Br	H
14;	CH_3	F	H
15;	CH_3	Cl	H
16;	CH_3	H	Cl
17;	CH_3	OCH_3	H

Fig. 1 Numbering of compounds **10–17**

Further, the C-5 carbon is shielded by 7.30 ppm than the C-3 carbon. This indicates the ***E***-configuration of the C=N bond. The doublet appearing at 3.85 ppm with a coupling constant value of $J_{aa} = 11.0$ Hz is assigned to the H-6a proton of the piperidone ring system. Further, H-6a has correlation with 3.48/2.06 ppm signals of the methylene protons of C-5. The unresolved signal at 3.48 ppm has almost two protons integral value. This may be due to the overlapping of H-2a and H-5e protons. The shoulder at 3.49 ppm and the signal at 3.48 ppm are assigned to H-2a and H-5e protons. The signal at 2.06 ppm is due to H-5a proton. The deshielding effect of the H-5e proton is pronounced due to the interaction between H-5e proton and the proton of the nitrogen bearing benzothiazole moiety. Furher, H-5a proton is highly shielded due to the transmittance of negative charge from C-5 to H-5a. From these observations, it is concluded that the higher frequency signal appeared at 3.48 ppm and the lower frequency signal observed at 0.85 ppm are assigned to the H-5e proton and the methyl protons present at the C-3 carbon.

The signal which appeared at 2.49 ppm with one proton integral value is assigned to H-3a proton. The broad singlets observed at 2.86 and 11.48 ppm are assigned to the NH protons of piperidone and 2-hydrazinobenzothiazole moiety. Further, these NH signals are unambiguously identified by D_2O exchange. The signals which appeared in the region of 7.03–7.69 ppm with 12 protons integral value are assigned to aromatic protons. The proton signal positions, intensity and splitting patterns observed in the ^{1}H NMR spectra of all the other synthesized compounds **10–17** are almost same as in the compound **15**.

In compound **11**, three signals observed at 1.17, 1.63 and 0.87 ppm are due to the presence of ethyl group in the

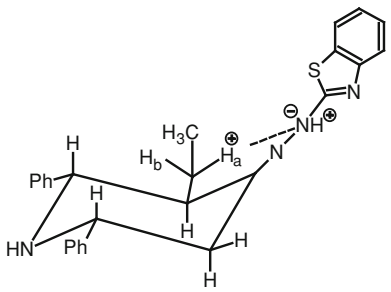


Fig. 2 Hydrogen bond interaction of **11**

equatorial position of C-3. The H-3a proton showed a signal at 2.39 ppm. Among the obtained chemical shift values, 0.87 ppm is assigned for CH_3 protons. The signals that appeared at 1.17 and 1.63 ppm are due to H_a and H_b (Fig. 2) protons of the ethyl group. These observations indicate that the higher frequency signal at 1.63 ppm is due to the interaction between H_b proton with the nitrogen atom present in the C-4 carbon. In compound **17**, a singlet appearing at 3.75 ppm with six protons integral value is assigned to the methoxy substitution at C-2 and C-6.

In the ^{13}C NMR spectrum of **15**, two weak intense signals observed at 174.3 and 168.9 ppm are due to the C=N carbon atom of benzothiazole and piperidine moieties. The signals at 143.5 and 142.6 ppm are due to the *ipso* carbon of the aryl ring at C-2′ and C-6′ carbon atom. Four intense signals appeared in the aliphatic region. The signals at 68.2 and 59.7 ppm are due to C-2 and C-6 carbons. Further, the signals which appeared at 44.6 and 37.3 ppm are assigned to the C-3 and C-5 carbon atoms of the piperidine ring system. For the compounds **11**–**17**, the alkyl carbon signals are observed around 12.0 ppm.

The ^1H NMR spectral assignments have been made based on the characteristic signals and 2D NMR (^1H–^1H and ^1H–^{13}C COSY) spectral data. The ^1H–^1H and ^1H–^{13}C cosy spectral correlations of **15** are given in Table 2. In ^1H–^1H COSY spectrum of **15**, the signal at 3.85 ppm as a doublet ($J_{aa} = 11.0$ Hz) with one proton integral value (H-6a) has correlation with signals at 3.48 and 2.06 ppm. This confirms that these signals are due to H-5e and H-5a protons. A signal appeared at 3.49 ppm with one proton integral value (H-2a) and its correlation with a signal at 2.49 ppm confirmed that the latter signal was due to the H-3a proton. In the ^1H–^{13}C COSY spectrum of **15**, the signals observed at 68.2 and 59.7 ppm have cross peaks with H-2a and H-6a protons. Hence, these signals are assigned to C-2 and C-6 carbons, respectively. The carbon signal at 37.3 ppm has ^1H–^{13}C COSY correlation with proton signals at 3.48 and 2.06 ppm confirms that the signal at 37.3 ppm is due to C-5 carbon. In the higher frequency region, a doublet appeared at 3.85 ppm ($J_{aa} = 11.0$ Hz) with one proton integral value (H-6a) which has correlation with a carbon signal at 59.7 ppm. Hence, the signal that appeared at 59.7 ppm is due to C-6 carbon atom. The signals that appeared in the range of 118.4–132.2 ppm show a cross peak with the aryl protons signal in ^1H–^{13}C COSY.

Table 2 Correlations in ^1H–^1H and ^1H–^{13}C COSY spectra (ppm) of **15**

Signal	^1H–^1H COSY spectrum	^1H–^{13}C COSY spectrum
3.49 (H-2a)	2.49	68.2
2.49 (H-3a)	3.49	44.6
2.06 (H-5a)	3.48	37.3
3.48 (H-5e)	2.06, 3.85	37.3
3.85 (H-6a)	3.48	59.7

Antibacterial activity

The synthesized compounds were screened for their in vitro antibacterial activity by disc diffusion method. MIC values were determined by two-fold serial dilution method. Streptomycin was used as a standard for the comparison of the antibacterial activity, and the MIC results are summarized in Table 3. Compounds **10** and **11** showed antibacterial activity against *K. pneumoniae*, *B. subtilis* and *P. aeruginosa*. However, the unsubstituted compound **12** showed a noticeable activity against *E. coli* and a good activity against *K. pneumoniae* and *B. subtilis*. The bromo substituted compound **13** had a good activity against *K. pneumoniae* and *B. subtilis*. The compound **14** exhibited a strong activity against *K. pneumoniae* at 25μg/mL. Introduction of a chlorine atom in the *para* position of the phenyl ring (compound **15)** displayed a strong activity against *K. pneumoniae*, *E. coli*, *B. subtilis* and *S. aureus*. The chlorine atom substituted in the *ortho* position of the phenyl ring (compound **16)** demonstrated good activities against *K. pneumoniae*, *B. subtilis* and *P. aeruginosa*. The methoxy substitution at the *para* position of the phenyl ring (compound **17**) revealed a good activity against *K. pneumoniae*. Among the synthesized compounds, none of the compounds exhibited antibacterial activity against *S. aureus*. The obtained antibacterial results revealed that the nature of the substituents and the substitution pattern on the aryl ring have considerable impact on the antibacterial activities of the target hydrazine. In this context, the *para* substituted compounds appeared to be more beneficial for antibacterial activity than the *ortho* substituted compounds.

Antifungal activity

All the synthesized compounds were screened for in vitro antifungal activity. The antifungal activities were evaluated

Table 3 In vitro antibacterial activities of **10–17**

Compounds	Entry			Minimum inhibitory concentrations (μg/mL)				
	R^1	R^2	R^3	*K. pneumoniae*	*E. coli*	*B. subtilis*	*P. aeruginosa*	*S. aureus*
10	H	H	H	200	200	100	200	–
11	CH_2CH_3	H	H	50	–	200	100	–
12	CH_3	H	H	100	25	100	200	200
13	CH_3	Br	H	100	200	200	200	–
14	CH_3	F	H	25	–	100	200	–
15	CH_3	Cl	H	50	25	50	100	–
16	CH_3	H	Cl	100	200	100	100	–
17	CH_3	OCH_3	H	50	100	200	–	100
Streptomycin				20	50	12.5	50	50

Table 4 In vitro antifungal activities of **10–17**

Compounds	Entry			Minimum inhibitory concentrations (μg/mL)				
	R^1	R^2	R^3	*C. albicans*	*F. oxysporum*	*A. flavus*	*A. niger*	*C. neoformans*
10	H	H	H	100	200	200	200	–
11	CH_2CH_3	H	H	200	100	100	–	200
12	CH_3	H	H	100	200	–	100	200
13	CH_3	Br	H	200	50	200	200	100
14	CH_3	F	H	100	100	100	100	200
15	CH_3	Cl	H	–	200	50	100	200
16	CH_3	H	Cl	200	100	200	50	100
17	CH_3	OCH_3	H	100	–	100	200	–
Amphotericin B				25	25	50	50	25

against different fungal strains, such as *C. albicans*, *F. oxysporum*, *A. flavus*, *A. niger* and *C. neoformans*. MIC values were determined by two-fold serial dilution method (Ruiz *et al.*, 2002). Amphotericin B was used as a standard for the comparison of antifungal activity. DMSO was used as solvent control. The MIC values of the tested compounds are presented in Table 4. Generally all the synthesized compounds exerted a wide range of modest in vitro antifungal activity against all the tested organisms. The compound **10** without any substituent at the *para* position of the aryl groups at C-2 and C-6 positions of the six membered heterocyclic moiety showed a good activity against *C. albicans.* The antifungal activity of compounds **11** and **12** was considerably enhanced by the presence of the alkyl group at C-3. Compound **12** exhibited a good activity against *F. oxysporum* and *A. flavus* whereas the compound **13** revealed a good activity against *C. albicans* and *A. niger*. The antifungal activity of compounds **13–16** against the tested fungal strains was significantly increased due to the introduction of halo functions at the *para*/*ortho* positions of aryl groups. Particularly compound **13** against *F. oxysporum*, compound **15** against *A. flavus* and compound **16** against *A. niger* showed significant antifungal activity that was compared with the standard Amphotericin B. The replacement of halo moieties at the *para* positions of the aryl groups at C-2 and C-6 positions in **17** by methoxy group caused a significant reduction in activity against *F. oxysporum*, *A. niger* and *C. neoformans*.

Conclusion

Novel 2-(benzothiazole-2-yl)-1-(alkyl-2*r*,6*c*-diarylpiperidin-4-ylidine)hydrazine derivatives **10–17** were synthesized and characterized by IR and NMR spectra. The chemical shift and the coupling constant values indicated that all the synthesized hydrazine derivatives adopt a chair conformation with equatorial orientation of alkyl and aryl substituents in the piperidone ring. A close examination of in vitro antibacterial and antifungal profile of various substituted hydrazine derivatives against the tested bacterial and fungal strains provide a better structure–activity correlations. The presence of chloro and methoxy functions at the *para* positions of the phenyl ring in the C-2 and C-6

position of piperidine moiety play an important role in eliciting inhibition of all the bacteria and fungi assayed. The Antimicrobial activity studies also revealed that electron withdrawing substituents present in the aryl moiety enhance the antimicrobial activities than the electron donating substituents.

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