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Original Article

Synthesis, characterization, antibacterial and antifungal evaluation of novel cyclohexanone benzoylhydrazones

Gökçe Cihan-Üstündağ[1], Emel Mataracı-Kara[2], Gültaze Çapan[1]
[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Istanbul University, Istanbul, Turkey
[2]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Istanbul University, Istanbul, Turkey

ORCID IDs of the authors: G.C.Ü. 0000-0003-0516-6010; E.M.K. 0000-0002-4428-5066; G.Ç. 0000-0002-3026-4563.

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ABSTRACT

A novel series of benzoyl hydrazones (2a-j) were synthesized and evaluated, *in vitro*, for antimicrobial activity against selected bacteria and fungi. The structures of the compounds were established by IR, ^{1}H-NMR, ^{13}C-NMR (APT), electrospray ionization mass spectrometry (ESI-MS) and microanalysis (C, H, N). All of the tested compounds, except for compound 2h, displayed weak antibacterial properties against *Staphylococcus epidermidis* ATCC 12228 and *Staphylococcus aureus* ATCC 29213. Compounds 2a, 2b, 2e, 2f and 2i further exhibited marginal antifungal activity against *Candida parapsilosis*.

Keywords: Hydrazone, cyclohexanone, antibacterial activity, antifungal activity

INTRODUCTION

The spread of antibiotic-resistant bacteria is one of the biggest threats to global health. Mortality, morbidity and medical costs due to antibiotic resistance are increasing worldwide. A growing list of infections such as tuberculosis, pneumonia, gonorrhoe, blood poisoning and foodborne diseases, are becoming harder to treat as the antimicrobial agents become less effective (WHO 2018). Every year, around 214,000 deaths in newborns are attributable to drug-resistant pathogens, especially in low- and middle-income countries (Laxminarayan et al. 2016). Despite a growing clinical need, the development of new antibacterial agents to deal with the threat is insufficient. Only two novel antibiotic classes have been discovered in the last 20 years (oxazolidinones and lipopeptides) both of which provide coverage against Gram-positive bacteria (Luepke et al. 2017; Tacconelli et al. 2018). The approval rate of U.S. Food and Drug Administration (FDA) for new antibiotics has fallen to dismally low levels during the past 30 years (Shlaes et al. 2013). There is an urgent need for new antibiotics with activity against resistant microorganisms.

Hydrazide-hydrazones, R_1R_2C=N-NR_3COR_4 (R_{1-4}=alkyl, aryl or H), are well known as compounds with a wide range of antimicrobial properties (Popiolek 2017). Several *N*-aroylhydrazones derived from aryl- and heteroaryl hydrazides are emerging in the literature as potential antibacterial agents with wide spectra of activity against both Gram-(+) and Gram-(-) bacteria (Vicini et al. 2002; Gürkök et al. 2009; Moldovan et al. 2011; Xavier et al. 2012; Pieckzonka et al. 2013; Qing Ge et al. 2014; Kaplancıklı et al. 2014; Morjan et al. 2014; Nastasa et al. 2015; Tatar et al. 2016; Sridhar et al. 2016). Some of these derivatives have also been reported to have an inhibitory effect on fungi, especially on Candida species (Vicini et al. 2002; Gürkök et al. 2009; Xavier et al. 2012; Kaplancıklı et al. 2014; Nastasa et al. 2015). In an early report, Backes et al. identified a series of N'-(2-hydroxybenzylidene) benzohydrazides with potent antifungal activity against two human pathogenic species, *Candida albicans* and *Candida glabrata*, at low µM concentrations (Backes et al. 2014).

In this study we report the synthesis and structural characterization of novel *N*-benzoylhydrazones which were obtained by the condensation of 2-hydroxy-4-methoxybenzohydrazide and appropriate cyclohexanone derivatives. These new compounds

Address for Correspondence :
Gökçe CİHAN-ÜSTÜNDAĞ, e-mail: gokcechn@istanbul.edu.tr

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were evaluated for *in vitro* antibacterial and antifungal activity against a variety of pathogenic bacteria and fungi species.

MATERIALS AND METHODS

Chemistry

Melting points were determined in open capillary tubes with a Buchi B-540 melting point apparatus and were uncorrected. Microanalyses were performed on a Thermo Finnigan Flash EA 1112 elemental analyzer. IR spectra were recorded in KBr discs (υ_{max} in cm^{-1}) on a Shimadzu IRAffinity-1 FTIR spectrophotometer. ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (APT) (DMSO-d_6) spectra were run on VarianUNITYINOVA (500 MHz) instrument. Chemical shifts were reported as δ (ppm) relative to TMS as internal standard and coupling constants (*J*) were given in hertz (Hz). MS (ESI+/-) were determined on a Finnigan LCQ Advantage Max mass spectrometer (br.: broad/distorted, cyc.: cyclohexylidene, ar.: aromatic).

General procedure for the synthesis of 2-hydroxy-4-methoxy-N'-[(non)substituted cyclohexylidene]benzohydrazides (**2a**-**j**)

A mixture of 2-hydroxy-4-methoxybenzohydrazide (0.003 mol) and an appropriate cyclohexanone (0.003 mol) in absolute ethanol (20 mL) was refluxed on a water bath for 5-6 h. After cooling, the product was precipitated by adding distilled water. The solid thus obtained was filtered off and recrystallized from ethanol.

N'-cyclohexylidene-2-hydroxy-4-methoxybenzohydrazide (**2a**)

Light brown crystals (81%); mp 200-201°C; IR(KBr): υ_{max} 3311 (N-H), 1633 (C=O), 1612 (C=N), 1587, 1562, 1544, 1519 (C=C); ^{1}H-NMR (DMSO-d_6/500MHz): δ 1.59-1.68 (6H, m, CH_2-cyc.), 2.31-2.35 (4H, m, CH_2-cyc.), 3.77 (3H, s, 4-OCH_3-ar.), 6.46 (1H, d, *J=2.5*, H3-ar.), 6.51 (1H, dd, *J=8.5, 2.5*, H5-ar.), 7.86 (1H, d, *J=8.5*, H6-ar.), 10.87 (1H, s, NH/OH), 12.26 (1H, s, NH/OH); ^{13}C-NMR (APT) (DMSO-d_6/125 MHz): δ 25.48, 26.06, 27.21, 28.13, 35.36 (CH_2-cyc.), 55.79 (4-OCH_3-ar.), 101.69, 106.61 (C3,C5-ar.), 109.62 (C1-ar.), 131.11 (C6-ar.), 160.50, 163.71, 164.42 (C2,C4-ar.,C=N,C=O). MS (ESI+) m/z (%): 263.2 ($[M+H]^+$, 55.0), 285.2 ($[M+Na]^+$, 100). Anal. Calcd for $C_{14}H_{18}N_2O_3$ (262.30): C, 64.10; H, 6.92; N, 10.68. Found: C, 63.74; H, 7.10; N, 10.32.

2-hydroxy-4-methoxy-N'-(4-methylcyclohexylidene)benzohydrazide (**2b**)

Beige crystals (78%); mp 204-205°C; IR(KBr): υ_{max} 3280 (N-H), 1651 (C=O), 1616 (C=N), 1604, 1535, 1504 (C=C); ^{1}H-NMR (DMSO-d_6/500 MHz): δ 0.92 (3H, d, *J=6.8*, 4-CH_3-cyc.), 1.04-1.20 (2H, m, CH_2-cyc.), 1.67-1.71 (1H, m, CH-cyc.), 1.80-1.87 (2H, m, CH_2-cyc.), 2.01 (1H, td, *J=13.7, 5.4*, CH_2-cyc.), 2.26 (1H, td, *J=13.2, 4.9*, CH_2-cyc.), 2.42 (1H, br. d, *J=13.7*, CH_2-cyc.), 2.73 (1H, br. d, *J=14.1*, CH_2-cyc.), 3.77 (3H, s, 4-OCH_3-ar.), 6.46 (1H, d, *J=2.4*, H3-ar.), 6.51 (1H, dd, *J=8.8*, 2.4, H5-ar.), 7.86 (1H, d, *J=8.8*, H6-ar.), 10.86 (1H, s, NH/OH), 12.27 (1H, s, NH/OH); ^{13}C-NMR (APT) (DMSO-d_6/125 MHz): δ 21.73 (4-CH_3-cyc.), 27.27 (CH_2-cyc.), 31.49 (CH-cyc.), 33.97, 34.57, 35.16 (CH_2-cyc.), 55.80 (4-OCH_3-ar.), 101.69, 106.58 (C3,C5-ar.), 109.60 (C1-ar.), 131.09 (C6-ar.), 160.58, 163.72, 164.02, 164.42 (C2,C4-ar.,C=N,C=O). MS (ESI+) m/z (%): 277.1 ($[M+H]^+$, 100). Anal. Calcd for $C_{15}H_{20}N_2O_3$ (276.33): C, 65.20; H, 7.30; N, 10.14. Found: C, 65.03; H, 7.50; N, 9.88.

N'-(4-ethylcyclohexylidene)-2-hydroxy-4-methoxybenzohydrazide (**2c**)

White crystals (86%); mp 166-168°C; IR(KBr): υ_{max} 3305 (N-H), 1633 (C=O), 1610 (C=N), 1580, 1550, 1516 (C=C); ^{1}H-NMR (DMSO-d_6/500 MHz): δ 0.89 (3H, t, *J=7.3*, 4-$CH_2\underline{C}H_3$-cyc.), 1.06-1.18 (2H, m, CH_2-cyc.), 1.25 (2H, quin., *J=7.3*, 4-$C\underline{H}_2CH_3$-cyc.), 1.44-1.48 (1H, m, CH-cyc.), 1.86-2.03 (3H, m, CH_2-cyc.), 2.25 (1H, td, *J=13.5, 4.9*, CH_2-cyc.), 2.43 (1H, br. d, *J=13.7*, CH_2-cyc.), 2.73 (1H, br. d, *J=14.2*, CH_2-cyc.), 3.77 (3H, s, 4-OCH_3-ar.), 6.46 (1H, d, *J=2.5*, H3-ar.), 6.51 (1H, dd, *J=9.0, 2.5*, H5-ar.), 7.86 (1H, d, *J=8.5*, H6-ar.), 10.86 (1H, s, NH/OH), 12.26 (1H, s, NH/OH); ^{13}C-NMR (APT) (DMSO-d_6/125 MHz): δ 11.95 (4-$CH_2\underline{C}H_3$-cyc.), 27.25, 28.63, 31.64, 32.72, 34.52 (CH_2-cyc., 4-$\underline{C}H_2CH_3$-cyc.), 38.06 (CH-cyc.), 55.79 (4-OCH_3-ar.), 101.69, 106.60 (C3,C5-ar.), 109.60 (C1-ar.), 131.10 (C6-ar.), 160.53, 163.71, 164.03, 164.72 (C2,C4-ar.,C=N,C=O). Anal. Calcd for $C_{16}H_{22}N_2O_3$ (290.36): C, 66.18; H, 7.64; N, 9.65. Found: C, 66.40; H, 7.80; N, 9.52.

2-hydroxy-4-methoxy-N'-(4-propylcyclohexylidene)benzohydrazide (**2d**)

White crystals (87%); mp 158-160°C; IR(KBr): υ_{max} 3304 (N-H), 1637 (C=O), 1612 (C=N), 1558, 1519 (C=C); ^{1}H-NMR (DMSO-d_6/500 MHz): δ 0.87 (3H, t, *J=7.3*, 4-$CH_2CH_2\underline{C}H_3$-cyc.), 1.07-1.22 (4H, m, 4-$CH_2\underline{C}H_2CH_3$, CH_2-cyc.), 1.28-1.34 (2H, m, 4-$C\underline{H}_2CH_2CH_3$-cyc.), 1.54-1.58 (1H, m, CH-cyc.), 1.85-2.02 (3H, m, CH_2-cyc.), 2.25 (1H, td, *J=13.8, 4.9*, CH_2-cyc.), 2.43 (1H, br. d, *J=14.2*, CH_2-cyc.), 2.73 (1H, br. d, *J=13.7*, CH_2-cyc.), 3.77 (3H, s, 4-OCH_3-ar.), 6.46 (1H, d, *J=2.5*, H3-ar.), 6.51 (1H, dd, *J=8.8, 2.5*, H5-ar.), 7.86 (1H, d, *J=8.5*, H6-ar.), 10.86 (1H, s, NH/OH), 12.26 (1H, s, NH/OH); ^{13}C-NMR (APT) (DMSO-d_6/125 MHz): δ 14.65 (4- $CH_2CH_2\underline{C}H_3$-cyc.), 20.12, 27.28, 32.02, 33.10, 34.54, 38.25 (CH_2-cyc., 4-$\underline{C}H_2CH_2CH_3$-cyc.), 36.04 (CH-cyc.), 55.78 (4-OCH_3-ar.), 101.69, 106.59 (C3,C5-ar.), 109.60 (C1-ar.), 131.09 (C6-ar.), 160.55, 163.71, 163.99, 164.71 (C2,C4-ar.,C=N,C=O). Anal. Calcd for $C_{17}H_{24}N_2O_3$ (304.38): C, 67.08; H, 7.95; N, 9.20. Found: C, 67.33; H, 8.11; N, 9.13.

N'-(4-*tert*-butylcyclohexylidene)-2-hydroxy-4-methoxybenzohydrazide (**2e**)

White crystals (91%); mp 203-205°C; IR(KBr): υ_{max} 3302 (N-H), 1635 (C=O), 1609 (C=N), 1558, 1516, 1480 (C=C); ^{1}H-NMR (DMSO-d_6/500 MHz): δ 0.86 (9H, s, 4-$C(CH_3)_3$-cyc.), 1.13-1.24 (2H, m, CH_2-cyc.), 1.31-1.36 (1H, m, CH-cyc.), 1.88-1.97 (3H, m, CH_2-cyc.), 2.25 (1H, td, *J=13.2, 4.9*, CH_2-cyc.), 2.46 (1H, br. d, *J=13.7*, CH_2-cyc.), 2.81 (1H, br. d, *J=14.2*, CH_2-cyc.), 3.77 (3H, s, 4-OCH_3-ar.), 6.46 (1H, d, *J=2.5*, H3-ar.), 6.51 (1H, dd, *J=9.0, 2.5*, H5-ar.), 7.86 (1H, d, *J=8.5*, H6-ar.), 10.85 (1H, s, NH/OH), 12.27 (1H, s, NH/OH); ^{13}C-NMR (APT) (DMSO-d_6/125 MHz): δ 26.75, 27.25 (CH_2-cyc.), 27.82 (4-$C(\underline{C}H_3)_3$-cyc.), 32.62 (4-$\underline{C}(CH_3)_3$-cyc.), 34.98 (CH_2-cyc.), 46.80 (CH-cyc.), 55.78 (4-OCH_3-ar.), 101.69, 106.60 (C3,C5-ar.), 109.58 (C1-ar.), 131.07 (C6-ar.), 160.51, 163.71, 164.05, 164.67 (C2,C4-ar.,C=N,C=O). MS (ESI+) m/z (%): 319.2 ($[M+H]^+$, 100). Anal. Calcd for $C_{18}H_{26}N_2O_3$ (318.41): C, 67.90; H, 8.23; N, 8.80. Found: C, 67.53; H, 8.25; N, 8.83.

2-hydroxy-4-methoxy-N'-(3-methylcyclohexylidene)benzohydrazide (**2f**)

White crystals (95%); mp 167-169°C; IR(KBr): υ_{max} 3311 (N-H), 1622 (C=O), 1613 (C=N), 1581, 1545, 1508 (C=C); ^{1}H-NMR (DM-

SO-d_6/500 MHz): δ 0.94, 0.95 (3H, 2d, *J=6.3*, 3-CH_3-cyc.), 1.12-1.20 (1H, m, CH/CH_2-cyc.), 1.37-1.49 (1H, m, CH/CH_2-cyc.), 1.60-1.94 (4H, m, CH/CH_2-cyc.), 2.17 (1H, td, *J=13.5, 5.4*, CH_2-cyc.), 2.35-2.44 (1H, m, CH_2-cyc.), 2.65-2.71 (1H, m, CH_2-cyc.), 3.77 (3H, s, 4-OCH_3-ar.), 6.46, 6.47 (1H, 2d, *J=2.5*, H3-ar.), 6.50-6.52 (1H, m, H5-ar.), 7.87, 7.89 (1H, 2d, *J=8.8*, H6-ar.), 10.89 (1H, s, NH/OH), 12.27 (1H, s, NH/OH); ^{13}C-NMR (APT) (DMSO-d_6/125 MHz): δ 22.17, 22.35 (3-CH_3-cyc.), 24.81, 25.91, 27.51 (CH_2-cyc.), 32.77, 33.70 (CH-cyc.), 33.79, 34.83, 36.00, 43.31 (CH_2-cyc.), 55.78 (4-OCH_3-ar.), 101.69, 106.58 (C3,C5-ar.), 109.58, 109.68 (C1-ar.), 131.09, 131.18 (C6-ar.), 160.50, 160.60, 163.72, 163.91, 164.33 (C2,C4-ar.,C=N,C=O).). MS (ESI-) m/z (%): 275.5 ([M-H]$^-$, 100). Anal. Calcd for $C_{15}H_{20}N_2O_3$ (276.33): C, 65.20; H, 7.30; N, 10.14. Found: C, 65.30; H, 7.52; N, 10.06.

2-hydroxy-4-methoxy-N'-(3,3,5-trimethylcyclohexylidene)benzohydrazide **(2g)**

White crystals (94%); mp 203-206°C; IR(KBr): υ_{max} 3304 (N-H), 1658 (C=O), 1627 (C=N), 1604, 1543, 1504 (C=C); ^{1}H-NMR (DMSO-d_6/500 MHz): δ 0.78, 0.83 (3H, 2s, 3-CH_3-cyc.), 0.93-0.95 (3H, m, 5-CH_3-cyc.), 0.99, 1.02 (3H, 2s, 3-CH_3-cyc.), 1.09-1.15 (1H, m, CH/CH_2-cyc.), 1.44, 1.48 (1H, br. 2d, *J=13.1*, CH/CH_2-cyc.), 1.72-1.81 (2H, m, CH/CH_2-cyc.), 2.03-2.12 (1H, m, CH/CH_2-cyc.), 2.38 (1H, br. d, *J=9.8*, CH/CH_2-cyc.), 2.74 (1H, br. d, *J=13.2*, CH_2-cyc.), 3.76, 3.77 (3H, 2s, 4-OCH_3-ar.), 6.46, 6.47 (1H, 2d, *J=2.5*, H3-ar.), 6.50-6.54 (1H, m, H5-ar.), 7.87, 7.89 (1H, 2d, *J=9.0*, H6-ar.), 10.92 (1H, s, NH/OH), 12.11, 12.32 (1H, 2s, NH/OH); ^{13}C-NMR (APT) (DMSO-d_6/125 MHz): δ 22.57, 22.74, 25.62, 25.65, 28.44 (3-$(CH_3)_2$, 5-CH_3-cyc.), 30.00, 32.18 (CH-cyc.), 34.00, 34.61, 35.65 (CH_2-cyc.), 43.20, 47.57, 47.66 (CH_2-cyc., C3-cyc.), 55.80 (4-OCH_3-ar.), 101.71, 101.77, 106.57, 106.64 (C3,C5-ar.), 109.48, 110.32 (C1-ar.), 131.00, 131.68 (C6-ar.), 159.61, 160.74, 161.95, 163.62, 163.74, 164.10, 164.31 (C2,C4-ar.,C=N,C=O). MS (ESI-) m/z (%): 303.6 ([M-H]$^-$, 100). Anal. Calcd for $C_{17}H_{24}N_2O_3$ (304.38): C, 67.08; H, 7.95; N, 9.20. Found: C, 67.36; H, 8.12; N, 9.08.

N'-[4-(acetylamino)cyclohexylidene]-2-hydroxy-4-methoxybenzohydrazide **(2h)**

White flakes (90%); mp 242-245°C; IR(KBr): υ_{max} 3342, 3298 (N-H), 1631 (C=O), 1609 (C=N), 1537, 1514, 1497 (C=C); ^{1}H-NMR (DMSO-d_6/500 MHz): δ 1.36-1.50 (2H, m, CH_2-cyc.), 1.81 (3H, s, 4-NHCO<u>C</u>H_3-cyc.), 1.87-1.94 (2H, m, CH_2-cyc.), 2.13-2.19 (1H, m, CH_2-cyc.), 2.35 (1H, td, *J=13.3, 4.9*, CH_2-cyc.), 2.44-2.49 (1H, m, CH_2-cyc.), 2.68 (1H, br. d, *J=15.1*, CH_2-cyc.), 3.77 (3H, s, 4-OCH_3-ar.), 3.82-3.90 (1H, m, CH-cyc.), 6.46 (1H, d, *J=2.5*, H3-ar.), 6.51 (1H, dd, *J=8.8, 2.5*, H5-ar.), 7.86 (1H, d, *J=7.5*, H6-ar.), 10.88 (1H, s, NH/OH), 12.26 (1H, s, NH/OH); ^{13}C-NMR (APT) (DMSO-d_6/125 MHz): δ 23.20 (4-NHCO<u>C</u>H_3-cyc.), 25.71, 31.18, 32.35, 32.96 (CH_2-cyc.), 46.40 (CH-cyc.), 55.80 (4-OCH_3-ar.), 101.69, 106.64 (C3,C5-ar.), 109.50 (C1-ar.), 131.10 (C6-ar.), 160.63, 163.24, 163.78, 164.11 (C2,C4-ar.,C=N,C=O), 168.92 (4-NH<u>C</u>OCH_3-cyc.). Anal. Calcd for $C_{16}H_{21}N_3O_4$ (319.36): C, 60.17; H, 6.63; N, 13.16. Found: C, 59.73; H, 7.05; N, 12.93.

N'-[2-(2-cyanoethyl)cyclohexylidene]-2-hydroxy-4-methoxybenzohydrazide **(2i)**

White crystals (69%); mp 147-149°C; IR(KBr): υ_{max} 3304 (N-H), 1649 (C=O), 1614 (C=N), 1543, 1508 (C=C); ^{1}H-NMR (DMSO-d_6/500 MHz): δ 1.32-1.40 (1H, m, CH/CH_2-sp.), 1.49-1.74 (5H, m, 2-C<u>H</u>$_2$$CH_2$CN-cyc. and/or CH/$CH_2$-sp.), 1.88-1.94 (1H, m, CH/CH_2-sp.), 2.04-2.11 (2H, m, 2-C<u>H</u>$_2$$CH_2$CN-cyc. or CH/$CH_2$-sp.), 2.37-2.41 (1H, m, CH/CH_2-sp.), 2.60-2.65 (3H, m, 2-CH_2C<u>H</u>$_2$CN-cyc., CH/CH_2-sp.), 3.77 (3H, s, 4-OCH_3-ar.), 6.47 (1H, d, *J=2.5*, H3-ar.), 6.52 (1H, dd, *J=8.5, 2.5*, H5-ar.), 7.87 (1H, d, *J=8.5*, H6-ar.), 10.94 (1H, s, NH/OH), 12.22 (1H, s, NH/OH); ^{13}C-NMR (APT) (DMSO-d_6/125 MHz): δ 14.78 (2-CH_2<u>C</u>H_2CN-cyc.), 24.04, 26.51, 27.45 (CH_2-cyc., 2-<u>C</u>$H_2$$CH_2$CN-cyc), 33.30, 41.88 ($CH_2$-cyc.), 43.20 (CH-cyc.), 55.80 (4-OCH_3-ar.), 101.71, 106.66 (C3,C5-ar.), 109.58 (C1-ar.), 121.31 (2-$CH_2$$CH_2$<u>C</u>N-cyc), 131.12 (C6-ar.), 160.57, 163.77, 164.10, 165.46 (C2,C4-ar.,C=N,C=O). Anal. Calcd for $C_{17}H_{21}N_3O_3$ (315.37): C, 64.74; H, 6.71; N, 13.32. Found: C, 64.29; H, 6.86; N, 13.46.

2-hydroxy-4-methoxy-N'-(4-phenylcyclohexylidene)benzohydrazide **(2j)**

White crystals (84%); mp 221-222°C; IR(KBr): υ_{max} 3263 (N-H), 1638 (C=O) 1607 (C=N), 1543, 1502 (C=C); ^{1}H-NMR (DMSO-d_6/500MHz): δ 1.60-1.75 (2H, m, CH_2-cyc.), 1.97-2.04 (2H, m, CH_2-cyc.), 2.16 (1H, td, *J=14.1, 5.4*, CH_2-cyc.), 2.46 (1H, td, *J=13.7, 4.9*, CH_2-cyc.), 2.56 (1H, br. d, *J=13.7*, CH_2-cyc.), 2.85-2.91 (2H, m, CH-cyc., CH_2-cyc.), 3.77 (3H, s, 4-OCH_3-ar.), 6.47 (1H, d, *J=2.5*, H3-ar.), 6.52 (1H, dd, *J=8.5, 2.5*, H5-ar.), 7.18-7.31 (5H, m, 4-C_6H_5-cyc.), 7.88 (1H, d, *J=9.0*, H6-ar.), 10.94 (1H, s, NH/OH), 12.27 (1H, s, NH/OH); ^{13}C-NMR (APT) (DMSO-d_6/125 MHz): δ 27.81, 33.24, 34.27, 35.13 (CH_2-cyc.), 42.93 (CH-cyc.), 55.80 (4-OCH_3-ar.), 101.71, 106.65 (C3,C5-ar.), 109.62 (C1-ar.), 126.61, 127.16, 128.84 (4-C_6H_5(C2-6)-cyc.), 131.17 (C6-ar.), 146.19 (4-C_6H_5(C1)-cyc.), 160.55, 163.46, 163.75, 164.04 (C2,C4-ar.,C=N,C=O). Anal. Calcd for $C_{20}H_{22}N_2O_3$ (338.40): C, 70.99; H, 6.55; N, 8.28. Found: C, 70.57; H, 6.67; N, 8.36.

Antibacterial and Antifungal Activity Assays

The *in vitro* antimicrobial activity of compounds **2a**-**j** was evaluated against four Gram negatif bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Proteus mirabilis* ATCC 14153), three Gram positive bacteria (*Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212) and three fungi (*Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019 and *Candida tropicalis* ATCC 750) using the microbroth dilution method according to the Clinical Laboratory Standards Institute (CLSI) recommendations (CLSI 1997; CLSI 2006) and compared with the standard drugs.

Serial twofold dilutions ranging from 2500 µg/mL to 1.22 µg/mL were prepared in the test medium, i.e. Mueller-Hinton broth for bacteria and RPMI-1640 medium for yeast strains. The inoculum was prepared using a 4-6 h broth culture of each bacteria type and 24 h culture of yeast strains adjusted to a turbidity equivalent to 0.5 McFarland standard, diluted in broth media to give a final concentration in the test tray of 5×10^5 cfu/mL for bacteria and 5×10^3 cfu/mL for yeast. The trays were covered and placed into plastic bags to prevent evaporation. The bacteria trays were incubated at 35°C for 18-20 h while the yeast-containing trays were incubated at 35°C for 46-50 h. The MIC was defined as the lowest concentration of compound giving complete inhibition of visible growth. As a control, antimicrobial effects of the solvents against the tested microorganisms were also investigated.

RESULTS AND DISCUSSION

Chemistry and Structural Characterization

The synthetic pathway for the preparation of the target hydrazones (**2a**-**j**) are illustrated in Scheme 1. Reactions occured readily under mild temperatures. The structures of the obtained compounds were established using IR, ^{1}H-NMR, ^{13}C-NMR (APT), electrospray ionization mass spectrometry (ESI-MS) and microanalytical data.

Scheme 1

IR spectra of the new hydrazone derivatives **2a**-**j** showed single N-H band in the 3263-3311 cm^{-1} region, while the IR spectrum of the starting hydrazide **(1)** exhibited three seperate N-H strechings at 3149, 3275 and 3319 cm^{-1}. The C=O groups of compounds **1** and **2a**-**j** absorbed in the 1635 and 1622-1658 cm^{-1} regions, respectively. No phenolic O-H streching vibrations were observed in the IR spectra of the hydrazide (**1**) or hydrazones (**2a**-**j**). Absence of the O-H bands in the expected regions is presumably due to the strong intramolecular hydrogen bonding between the phenolic O-H and C=O groups (Silverstein et al. 2005).

^{1}H-NMR spectra displayed the N-H and O-H resonances in the δ 10.85-10.94 ppm and δ 12.11-12.32 ppm regions as singlets. The resonances of the $-OCH_3$ group and aromatic hydrogens were observed in the δ 3.76-3.77 ppm and δ 6.46-7.89 ppm, respectively. The splitting patterns of the aromatic H3, H5, H6 hydrogens were in accordance with the 1,2,4-trisubstituted aromatic ring system. The aliphatic protons of the cyclohexane residue resonated at about δ 1.04-3.79 ppm region depending on the substituents on the ring system.

Carbon assignments were made on the basis of APT experiments which provided information about the number of protons attached to a ^{13}C atom. The quaternary C=N and C=O carbon resonances appeared as positive signals in downfield region together with the aromatic quaternary C2 and C4 carbon resonances (δ 159.61-165.46 ppm). Observation of upfield resonances (δ 11.95-47.66 ppm) assigned to the CH/CH_2 carbons of the cyclohexane residue further proved the intended conversion.

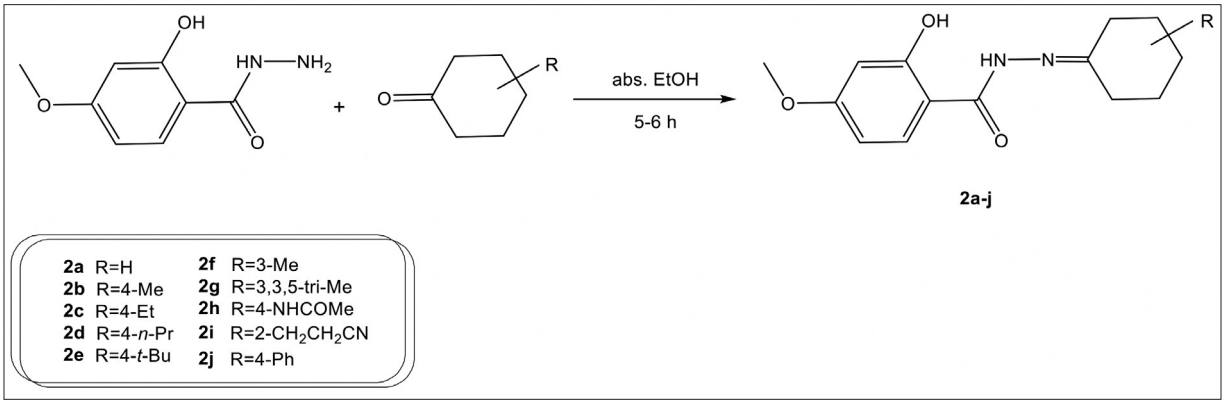
The proton spectra of compounds **2f** and **2g** displayed two sets of signals for some protons. Aromatic protons of **2f** and **2g** absorbed as two doublets (H3 and H6) and distorted multiplets (H5). Methyl substituents on the cyclohexane ring appeared as duplicated doublets for both compounds. The OH/CONH resonance about 12.00 ppm and the $-OCH_3$ resonance of compound **2g** were also detected as two separate singlets. Similarly, two signal sets appeared for most of the carbon atoms in the APT spectra of compounds **2f** and **2g**. The multiplicity in the signals pointed to the presence of two isomers due to the restricted rotation about the N=C double bond. It is assumed that the methyl substituents at 3- or 5-positions interrupt the symmetry of the molecules and give rise to the formation of *E* and *Z* isomers for compounds **2f** and **2g**.

ESI-MS was used to verify the molecular weights of compounds **2a**, **2b**, **2e**, **2f** and **2g**. Compounds **2a**, **2b** and **2e** were analyzed under negative-ion ESI conditions while compounds **2f** and **2g** were analyzed under positive-ion ESI conditions. Deprotonated $[M-H]^-$ or protonated $[M+H]^+$ molecular ions observed in the ESI-MS confirmed the molecular weights of the compounds.

Antibacterial and Antifungal Activity

The antibacterial and antifungal activity of compounds **2a-j** was evaluated *in vitro* against the following strains: *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Proteus mirabilis* ATCC 14153, *Enterococcus faecalis* ATCC 29212, *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 29213, *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019 and *Candida tropicalis* ATCC 750. The compounds were prepared using twofold dilutions starting at 2500 µg/mL. The lowest concentration of compound giving complete inhibition of visible growth was referred as the MIC (minimum inhibitory concentration).

As shown in Table 1, *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 29213 and *Candida parapsilosis* ATCC 22019 were the most sensitive strains to the tested hydrazone derivatives. All of the tested compounds, except for compound **2h**, showed weak antibacterial activity against *Staphylococcus epidermidis* ATCC 12228 and *Staphylococcus aureus* ATCC 29213 with MIC values of 312.5-1250 µg/mL. Compounds **2a**, **2b**, **2e**, **2f** and **2i** further exhibited antifungal activity against *Candida parapsilosis*, showing complete inhibition at MIC val-



Scheme 1. Synthesis of **2a-j.**

Table 1. Antimicrobial properties of compounds 2a-j against selected bacteria and fungi

	MIC (μg/mL)[a]										
Microorganism	2a	2b	2c	2d	2e	2f	2g	2h	2i	2j	Reference antimicrobials
P. aeruginosa ATCC 27853	-[b]	-	-	-	625	-	-	-	-	-	2.4 (Ceftazidime)
E. coli ATCC 25922	-	-	-	-	-	-	-	-	-	--	4.9 (Cefuroxime-Na)
K. pneumoniae ATCC 4352	-	-	-	-	-	-	-	-	-	-	4.9 (Cefuroxime-Na)
P. mirabilis ATCC 14153	-	-	-	-	-	-	-	-	-	-	2.4 (Cefuroxime-Na)
E. faecalis ATCC 29212	-	-	-	-	-	-	-	-	-	-	128 (Amikacin)
S. epidermidis ATCC 12228	625	1250	1250	1250	625	1250	1250	-	1250	625	9.8 (Cefuroxime)
S. aureus ATCC 29213	625	1250	1250	1250	625	312.5	625	-	312.5	312.5	1.2 (Cefuroxime-Na)
C. albicans ATCC 10231	-	-	-	-	-	-	-	-	-	-	4.9 (Clotrimazole)
C. parapsilosis ATCC 22019	312.5	312.5	-	-	625	625	-	-	312.5	-	0.5 (Amphotericin B)
C. tropicalis ATCC 750	-	-	-	-	-	-	-	-	-	-	1 (Amphotericin B)

[a] Minimum inhibitory concentration: the lowest concentration of compound giving complete inhibition of visible growth.
[b] No activity at the highest concentration tested.

ues of 312.5 and 625 μg/mL. Neither of the test compounds displayed antimicrobial activity below 312.5 μg/mL.

Benzaldehyde phenylhydrazones incorporating an aromatic system linked to the hydrazide moiety have been extensively studied by different research groups (Kumar et al. 2010; Niazi et al. 2010; Tajudeen et al. 2013; Sapra et al. 2014; Backes et al. 2014). This type of compounds was found to be highly active against different bacteria and fungi species. Replacing the unsaturated aromatic ring with a saturated cyclohexane system seemed to have a negative effect on antimicrobial activity since compounds **2a**-**j** were found to be slightly active against the tested bacteria and fungi.

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