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Synthesis, E/Z isomerization, and antimicrobial studies of different structured novel ketone derivatives

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Abstract: In this study, five novel ketones' (**k1- k5**), five new hydrazones (**h1-h5**) and five new semicarbazones (**s1-s5**) were synthesized. The synthesized compounds were identified and their E/Z isomerization were studied by FT-IR, ^{1}H-NMR, ^{13}C-NMR, mass spectrometric and chromatographic methods. These mentioned hydrazones and semicarbazones were investigated for their antimicrobial activities. Seven bacterial species three fungal species were tested. Ciprofloxacin and fluconazole were used as standard compounds. The MIC values were determined. The relationship between structure and antimicrobial actvity was reported. It was found that hydrazones exhibited better activity than that of semicarbazones. Besides, acetylacetone as a diketone yielded the known 1-(2,4-dinitrophenyl)-3,5-dimethyl-1H-pyrazole (**p1**) which showed higher antimicrobial activity than hydrazones and semicarbazones against *Klebsiella pneumoniae* ATCC 4352, *Proteus mirabilis* ATCC 14153, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Candida parapsilosis* ATCC 22019, and *Candida tropicalis* ATCC 750.

Keywords Diazo compounds; Substituent effect; Heterocycles; Antimicrobial activity; Pyrazole

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INTRODUCTION

Ketones and their derivatives are valuable compounds in organic synthesis. Among them, hydrazones and semicarbazones have many applications in organic, analytic, and medicinal chemistry (1-3). They can form stable complexes with transition metal ions, protect and purify carbonyl compounds by making them highly stable (4). Due to the literature survey many hydrazones and semicarbazones show biological activities such as antimicrobial, anticonvulsant (5), analgesic, antiinflammatory, antitubercular, antitumor properties (6), pesticide effcets and are plant growth regulators (7). With the aim to obtain new antimicrobial agents, novel five hydrazones (**h1-h5**) (Table 2) and five semicarbazones (**s1-s5**) (Table 3) were synthesized by starting from their corresponding ketones (**k1- k5**) (Table 1). **k1-k4** were obtained by Friedel Crafts

acylation. Three of them are original. Tolyl undecyl ketone, bromophenyl undecyl ketone, and chlorophenyl undecyl ketone are original ones. According to the literature, all the synthesized hydrazones (**h1-h5**) and semicarbazones are novel compounds. Hydrazones includes azomethine group which enables the formation of pyrazoles. 1-(2,4-dinitrophenyl)-3,5-dimethyl-1H pyrazole was synthesized because pyrazoles and substituted pyrazoles have considerable biological importance as being anticancer, anti-inflammatory, anticonvulsant, antiviral and antiprotozoal (8-9).

The above mentioned new hydrazones, semicarbazones and pyrazole (Table 4) (10) were characterized by FT-IR, ^{1}H-NMR, ^{13}C-NMR, mass and chromatographic methods. Their E/Z isomerization was analyzed. Their antimicrobial activities were tested against seven species of bacteria and three species of fungi by using ciprofloxacin and fluconazole as standards. Their MIC values were determined. These studies let us to examine the relationship between structure and antimicrobial activity. Hydrazones were more active than semicarbazones. 1-(2,4-dinitrophenyl)-3,5-dimethyl-1H pyrazole was tested against more and different microbials in this study and found as the most effective compound (11-12).

Table 1 Ketones as starting compounds.

Ketone's code	Full name
k1	Phenyl undecyl ketone
k2	Tolyl undecyl ketone
k3	Bromophenyl undecyl ketone
k4	Chlorophenyl undecyl ketone
k5	Cyclopropyl phenyl ketone

Table 2 Synthesized hydrazones.

Hydrazone's code	Full name
h1	Phenyl undecyl ketone 2,4-dinitrophenylhydrazone
h2	Tolyl undecyl ketone 2,4-dinitrophenylhydrazone
h3	Bromophenyl undecyl ketone 2,4-dinitrophenylhydrazone
h4	Chlorophenyl undecyl ketone 2,4-dinitrophenylhydrazone
h5	Cyclopropyl phenyl ketone 2,4-dinitrophenylhydrazone

Table 3 Synthesized semicarbazones.

Semicarbazone'code	Full name
s1	Phenyl undecyl ketone semicarbazone
s2	Tolyl undecyl ketone semicarbazone
s3	Bromophenyl undecyl ketone semicarbazone
s4	Chlorophenyl undecyl ketone semicarbazone
s5	Cyclopropyl phenyl undecyl ketone semicarbazone

Table 4 Synthesized pyrazole.

Pyrazole's code	Full name
p1	1-(2,4-dinitrophenyl)-3,5-dimethyl-1H pyrazole

MATERIALS AND METHODS

Chemicals and Devices

Chemicals were supplied from Merck and Aldrich. Reactions' statuses were monitored by TLC (silica gel 60 F_{254}, n-hexane/EtOAc, 1:1)

FT-IR data were obtained by using an ATR type Bruker Vertex 70 spectrometer. The NMR spectra were recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C using Me_4Si as the internal standard in $CDCl_3$ or DMSO. GC-MS were recorded on Shimadzu QP2010 Plus. A Buchi melting point B-540 apparatus was used for melting point determinations.

Alkyl and phenyl substituted ketone synthesis

Phenyl undecyl ketone (k1), Tolyl undecyl ketone (k2), Bromophenyl undecyl ketone (k3) and Chlorophenyl undecyl ketone (k4)

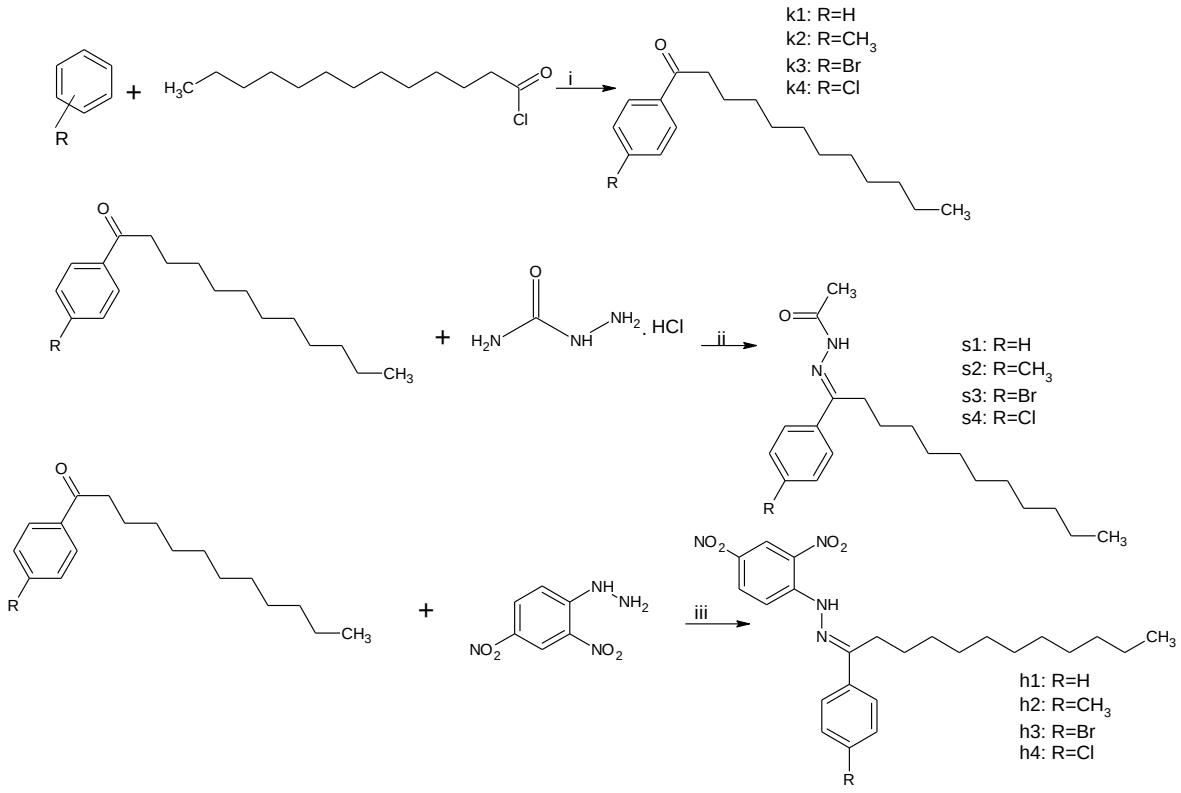
were synthesized by Friedel Crafts acylation as follows; 56 mmol benzene for k1; toluene for k2; bromobenzene for k3; chlorobenzene for k4; and 19 mmol anhydrous $AlCl_3$ were cooled on an ice bath. 19 mmol dodecanoic acid chloride was added from a separatory funnel. HCl discharging was completed in an hour by heating on a hot water bath. Benzene phase was rinsed with NaOH and water and then dried over $MgSO_4$. The ketone was purified on column chromatography with acetone /petroleum ether (1:9) (13). Cyclopropyl phenyl ketone (k5) was purchased and used for the synthesis of h5-s5, and acetyl acetone (k6) was also purchased and used for synthesis of p1.

Hydrazone and pyrazole synthesis

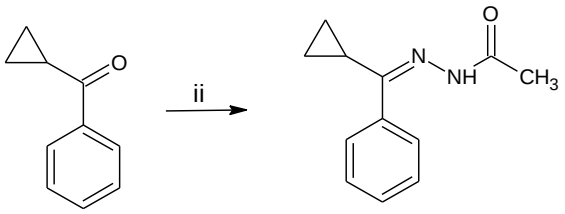
1.0 mmol (gram) ketone and 1.0 mmol 2,4-dinitrophenyl hydrazine were refluxed for 60 h in 20 mL of n-propanol. Reaction was monitored by TLC. n-propanol was evaporated from the rotary evaporator. Crude hydrazones (h1-h5) were recrystallized from n-propanol. p1 was also synthesized according to this procedure and recrystallized from methanol.

Semicarbazone synthesis

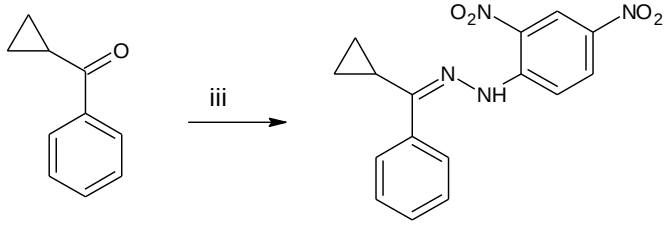
0.5 g semicarbazide.HCl, 0.8 g NaOAc, and 0.5 g of ketone were dissolved in 5 mL of water. 0.5 mL of ethanol was then added. The mixture was shaken well and kept on a hot water bath for 1 h. The mixture was then cooled to room temperature and poured into the ice-water mixture. Crystals were obtained and recrystalized from ethanol again.



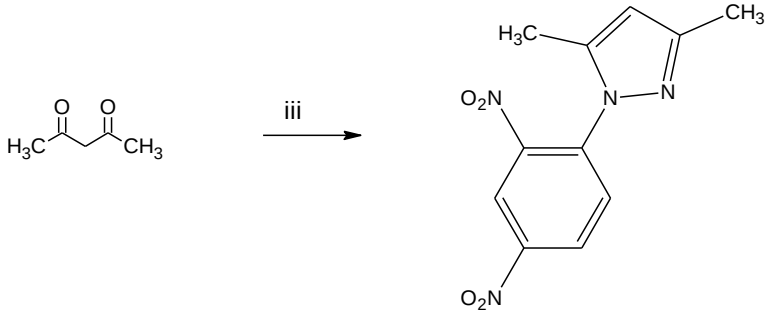
Scheme 1. General overview to the reactions.



k5: Cyclopropyl phenyl ketone s5: Cyclopropyl phenyl ketone semicarbazone



k5: Cyclopropyl phenyl ketone h5: Cyclopropyl phenyl ketone 2,4-dinitrophenylhydrazone



k6: Acetyl acetone p1: 1-(2,4-dinitrophenyl)-3,5-dimethyl-1H pyrazole

i) $AlCl_3$ cooled on ice bath; HCl discharging on hot water bath; rinsed with NaOH; dried over $MgSO_4$.
ii) NaOAc, H_2O, ethanol on hot water bath for 1 h; cooled to room temperature; poured into ice-water mixture.
iii) n-Propanol, reflux, 60 h

Physical and spectral data of compounds h1-h5, s1-s5, and p1

h1 (**Phenyl undecyl ketone 2,4-dinitrophenylhydrazone):** Orange solid; yield: 343 mg, (78 %); mp:101-102°C; FT-IR (ATR): ῡ=3297, 2895, 2812, 1591, 1554, 1468, 1287, 1245, 1098, 902, 836, 791 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO): δ=0.828 ppm (t, 3H, J=6.83, CH_3), δ=1.216 -1.628 ppm (m,16H, J=7.23, $(CH_2)_8$), δ=1.314 ppm (p, 2H, J=6.35, (E) β CH_2), δ=1.416 ppm (t, 2H, J=7.32, (E) α CH_2), δ=1.582 ppm (p, 2H, J=8.29, (Z) β CH_2), δ=2.896 ppm (t, 2H, J=8.29, (Z) α CH_2), δ=6,977-7.479 ppm (m, alkyl substituted benzene protons), δ=8.095 ppm (d, J=9.27, NO_2 substituted benzene protons), δ=7.930-7.949 ppm (m, J=1.95, 3.42, 1.46, 2.93, NO_2 substituted benzene protons) δ=8.899 ppm (s, (E), NH), δ=11.257 ppm (s, (Z), NH) ^{13}C-NMR (500 MHz, DMSO): δ=12.818 ppm (CH_3), δ=20.961-28.055 ppm ($(CH_2)_8$), δ=20.168 ppm (α CH_2), δ=115.366-143.457 ppm (aromatic benzene carbons), δ=155.418 - ppm (C=N); Anal. calcd. for $C_{24}H_{31}N_4O_4$: C, 65.16; H, 7.69; N, 12.67. Found: C, 65.21; H, 7.61; N, 12.63; MS (m/z): 98, 99, 299, 357, 441 (M^+)

h2 (Tolyl undecyl ketone 2,4-dinitrophenylhydrazone): Bright pomegranate flower colored solid; yield: 299 mg, (66 %); mp: 122-123 °C; FT-IR (ATR): ῡ=3318, 3110, 2921, 2851, 2287, 2108, 1613, 1584, 1495, 1416, 1255, 1102, 1057, 1016, 919, 848, 818 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO): δ=0.829 ppm (t, 3H, J=7.32, CH_3), δ=1.218 -1.630 ppm (m, 16 H, J=7.81, $(CH_2)_8$), δ= 1.310 ppm (t, 2H, J=6.83, (E) α CH_2), δ=1.409 ppm (p, 2H, J=7.32, (E) β CH_2), δ=1.568 ppm (p, 2H, J=7.81, (Z) β CH_2), δ=2.874 ppm (t, 2H, J=8.29, (Z) α CH_2), δ=7.288-7.849 ppm (d,d, 4H, J=7.81, 7.83, CH_3 substituted aromatic protons), δ=8.084 ppm (d, J=9.76, protons next to the NH), δ=8.406 ppm (dd, J=2.93, 2.44, protons next to the NO_2), δ=8.899 ppm (sd, J=2.44, proton between two NO_2),

δ=11.251 ppm (s, 1H, NH) ^{13}C-NMR (500 MHz, DMSO): δ=14.625 ppm (CH_3), δ=21.601-29.847 ppm ($(CH_2)_8$), δ=31.979 ppm (α CH_2), δ=117.144-145.250 ppm (aromatic benzene carbons), δ=157.325 -ppm (C=N); Anal. calcd. for $C_{25}H_{34}N_4O_4$: C, 66.08; H, 7.49; N, 12.33. Found: C, 66.04; H, 7.46; N, 12.37; MS (m/z):100, 425, 455 (M^+)

h3 (Bromophenyl undecyl ketone 2,4-dinitrophenylhydrazone): Bright orange colored solid; yield: 431 mg, (83 %); mp: 119-120 °C; FT-IR (ATR): ῡ=3304 (NH), 2921, 2852, 1590, 1536, 1499, 1262, 1331, 1004, 907, 836 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO): δ=0.832 ppm (t, 3H, J=6.83, CH_3), δ=1.215 – 1.234 ppm (m, 16 H, J=9.76, $(CH_2)_8$), δ= 1.319 ppm (t, 2H, (E) α CH_2), δ=1.418 ppm (p, 2H, J=6.83, (E) β CH_2), δ=1.584 ppm (p, 2H, (Z) β CH_2), δ=2.883 ppm (t, 2H, J=7.81, (Z) α CH_2), δ=7.672-7.888 ppm (d,d, 4H, J=8.29, 8.79, (2H, 2H) chlorine substituted aromatic protons), δ=8.093 ppm (d, 1H, J=9.76, next to the NH), δ=8.419 ppm (d, 1H, J=2.44, next to the NO_2), δ=8.907 ppm (s, proton between two NO_2), δ=11.245 ppm (s, 1H, NH) ^{13}C-NMR (500 MHz, DMSO): δ=14.825 ppm (CH_3), δ=20.968-27.994 ppm ($(CH_2)_8$), δ=30.168 ppm (α CH_2), δ=115.393-143.323 ppm (aromatic benzene carbons), δ=154.285 -ppm (C=N); Anal. calcd. for $C_{24}H_{31}BrN_4O_4$: C, 55.49; H, 5.97; N, 10.79. Found: C, 55.53; H, 5.92; N, 10.77; MS (m/z):180, 351, 420, 442, 520 (M^+)

h4 (Chlorophenyl undecyl ketone 2,4-dinitrophenylhydrazone): Orange colored solid; yield: 374 mg, (79 %); mp: 106-107 °C; FT-IR (ATR): ῡ= 3305, 3220, 3121, 2920, 2851, 2106, 1614, 1588, 1498, 1491, 1420, 1330, 1305, 1261, 1132, 1091, 1057, 855, 835 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO): δ=0.892 ppm (t, 3H, J=7.32, CH_3), δ=1.279 -1.332 ppm (m,16 H, J=7.81, $(CH_2)_8$), δ= 1.384 ppm (p, 2H, J=7.32, (E) β CH_2), δ=1.481 ppm (t, 2H, J=7.81, (E) α CH_2), δ=1.630 ppm (p, 2H, J=7.81, (Z) β CH_2), δ=2.945 ppm (t, 2H, J=8.29, (Z) α CH_2), δ=7.59-7.61 ppm (dd,dd, J=2.93, 1.95, 4.88, 2.44, 4H, (2H, 2H) chlorine substituted aromatic protons), δ=8.02 ppm (d, 1H, J=1.95, next to the NH), δ=8.40 ppm (d, 1H, 2.93, next to the NO_2), δ=9.01 ppm (s, proton between two NO_2), δ=11.25 ppm (s, 1H, NH) ^{13}C-NMR (500 MHz, DMSO): δ=14.622 ppm (CH_3), δ=22.768-29.817 ppm ($(CH_2)_8$), δ=31.979 ppm (α CH_2), δ=110.00-145.131 ppm (aromatic benzene carbons), δ=155.975 ppm (C=N); Anal. calcd. for $C_{24}H_{31}ClN_4O_4$: C, 60.63; H, 6.53; N, 11.79. Found: C, 60.59; H, 6.51; N, 11.82; MS (m/z): 99, 236, 401, 475 (M^+)

h5 (Cyclopropyl phenyl ketone 2,4-dinitrophenylhydrazone): Bright orange solid; yield: 153 mg, (47 %); mp: 190-191 °C; IR (ATR): ῡ= 3301, 3117, 2926, 2859, 2292, 2121, 1625, 1587, 1483, 1402, 1322, 1263, 1117, 853, 802 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ=0.69 ppm (d, 2H, J=5.37, CH_2), δ=0.89 ppm (t(ddd), J=5.37, 6.34, 4.89, 3.41, 7.81, 1H, (E) CH), δ= 1.33 ppm (d, t, J=5.86, 4.88, 2H, CH_2), δ=1.73 ppm (t, 1H, J=6.35, 1.95, 5.85, 2.44, (Z) CH), δ=7.18-7.52 ppm, J=6.83 (cyclopropyl substituted aromatic protons), δ=7.82-8.27 ppm, J=9.25, 11.71, 11.76 (NO_2 substituted aromatic protons), δ=9.09 ppm (s, 1H, (E) NH), δ=12.04 ppm (s, 1H, (Z) NH). ^{13}C-NMR (500 MHz, $CDCl_3$): δ=76.78 ppm (CH_2), δ=22.04 ppm (CH_2), δ=77.30 ppm (CH), δ=123.56-132.55 ppm (cyclopropyl substituted aromatic carbons) δ=136.23-144.63 ppm (NO_2 substituted aromatic benzene carbons), δ=155.65 ppm (C=N); Anal. calcd. for $C_{16}H_{14}N_4O_4$: C, 58.90; H, 4.29; N, 17.18. Found: C, 58.92; H, 4.35; N, 17.13; MS (m/z): 115, 177, 232, 278,309, 327 (M^+)

s1 (Phenyl undecyl ketone semicarbazone): Creamy-white colored solid; yield: 193 mg, (61 %); mp: 98.5-99 °C; IR (ATR): ῡ= 3470, 3349, 3262, 3057, 2953, 2849, 1681, 1578, 1462, 1377, 1261, 1233, 1208, 968, 720, 688, 517 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO): δ=0.834 ppm (t, 3H, J=6.83, CH_3), δ=1.122 - 1.355 ppm (m,16 H, J=7.32, $(CH_2)_8$), δ=1.371 ppm (t, 2H, J=7.809, 4.88, (E) α CH_2) δ=2.697 ppm (t, 2H, J=6.83, 8.29, (Z) α CH_2), δ=6.420 ppm (s, 2H, NH_2), δ=7.306 – 7.797 ppm, J=7.32, 2.44, 2.93, 1.46 (m, m benzene ring protons), δ=9.453 ppm (s, 1H, NH) ^{13}C-NMR (500 MHz, DMSO): δ=12.633 ppm (CH_3), δ=22.776-29.706 ppm ($(CH_2)_8$), δ=31.983 ppm (α CH_2), δ=126.706-138.193 ppm (aromatic benzene carbons), δ=147.942 ppm (C=N), δ=157.966 ppm (C=O); Anal. calcd. for $C_{20}H_{32}N_2O$: C, 75.95; H, 10.13; N, 8.86. Found: C, 75.98; H, 10.17; N, 8.81; MS (m/z):180, 239, 287, 317 (M^+)

s2 (Tolyl undecyl ketone semicarbazone): Gelly lemon yellow colored solid; yield: 234 mg, (71 %); mp:101-103 °C; FT-IR (ATR): ῡ= 3470, 3185, 2920, 2850, 1680, 1656, 1572, 1457, 1325, 1186, 1097 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ=0.92 ppm (t, 3H, 5.37, 4.88, 1.95, 1.96, terminal CH_3), δ=1.213 -

1.328 ppm (m,16 H, J=3.9, 13.18, 6.83, 6.84, 7.32, 5.37, $(CH_2)_8$), δ=1.38 ppm (p, 2 H, J=6.34, 7.32, 7.81, 7.32, 6.83, (E) β CH_2), δ=1.55 ppm (p, 2 H, J=5.86, 5.37, 5.37, (Z) β CH_2), δ=1.63 ppm (s, 2H, NH_2), δ=2.39 ppm (s, 3H, tolyl CH_3), δ=2.59 ppm (t, 2H, J=8.3, 7.81, (E) α CH_2), δ=2.93 ppm (t, 2H, J=7.81, 7.32, (Z) α CH_2), , δ=7.57 ppm (dd, J=1.95, 1.46, 6.83, 2H aromatic protons), δ=7.81 ppm (d, J=6.34, 1H aromatic proton), δ=7.87 ppm (d, J=4.88, 1H aromatic proton), δ=7.94 ppm (s, 1H, NH) ^{13}C-NMR (500 MHz, $CDCl_3$): δ=12.13 ppm (terminal CH_3), δ=22.67-29.91 ppm ($(CH_2)_8$), δ=31.92 ppm (α CH_2), δ=38.55 ppm (tolyl CH_3), δ=126.04-199.93 ppm (aromatic benzene carbons), δ=200.36 ppm (C=N), δ=222.775 ppm (C=O); 822. Anal. calcd. for $C_{21}H_{34}N_2O$: C, 76.36; H, 10.30; N, 8.48. Found: C, 76.35; H, 10.34; N, 8.43; MS (m/z): 258, 287, 300, 331 (M^+)

s3 (Bromophenyl undecyl ketone semicarbazone): White solid; yield: 288 mg, (73 %); mp: 120-121°C; FT-IR (ATR): ῡ= 3469, 3260, 3133, 2921, 2851, 1682, 1577, 1458, 1406, 1314, 1093, 987, 913 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ=0.796 ppm (t, 3H, J=6.83, CH_3), δ=0.796 - 1.186 ppm (m,16 H, $(CH_2)_8$), δ=1.296 ppm (p, 2H, J=7.32, 7.75, 7.81, (E) β CH_2), δ=1.424 ppm (t, 2 H, J=7.81, 8.29, (E) α CH_2) δ=1.565 ppm (s, 2H, (Z) β CH_2), δ=2.268 ppm (s, 2H, (Z) α CH_2), δ=2.541 ppm (t, 2H, J=8.3, NH_2), δ =7.400 ppm (dd, dd, J=2.44, 4.40, 7.32, 2.44 aromatic protons, 4H), δ=8.972 ppm (s, 1H, NH) ^{13}C-NMR (500 MHz, $CDCl_3$): δ=13.086 ppm (CH_3), δ=21.675-28.743 ppm ($(CH_2)_8$), δ=30.913 ppm (α CH_2), δ=122.489-148.726 ppm (aromatic benzene carbons), δ=157.300 ppm (C=N), δ=176.950 ppm (C=O); Anal. calcd. for $C_{20}H_{31}BrN_2O$: C, 60.76; H, 7.85; N, 7.09. Found: C, 60.71; H, 7.88; N, 7.03 MS (m/z): 232, 303, 334, 352, 396 (M^+)

s4 (Chlorophenyl undecyl ketone semicarbazone): Creamish white solid; yield: 259 mg, (74 %); mp: 106-107 °C; FT-IR (ATR): ῡ= 3533, 3485, 3416, 3174, 3089, 2919, 2849, 1696, 1605, 1553, 1463, 1422, 1317, 1093, 1146, 1010, 834 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO): δ=0.829 ppm (t, 3H, J=7.32, 6.83, CH_3), δ=1.212 – 1.330 ppm (m,16H, $(CH_2)_8$), δ=1.258 ppm (p, 2H, (E) β CH_2), δ=1.330 ppm (t, 2H, (E) α CH_2), δ=2.154 ppm (p, 2H, (Z) β CH_2), δ=2.701 ppm (t, 2H, J=6.34, 7.81 (Z) α CH_2), δ=6.471 ppm (s, 2H, NH_2), δ =7.376-7.837 ppm (dd, dd, J=1.95, 8.78, aromatic protons, 4H), δ=9.526 ppm (s, 1 H, NH) ^{13}C-NMR (500 MHz, DMSO): δ=14.625 ppm (CH_3), δ=22.780-29.714 ppm ($(CH_2)_8$), δ=31.987 ppm (α CH_2), δ=128.525-137.034 ppm (aromatic benzene carbons), δ=146.703 ppm (C=N), δ=157.893 ppm (C=O); Anal. calcd. for $C_{20}H_{31}N_2O$: C, 68.38; H, 8.83; N, 7.98. Found: C, 68.42; H,8.85; N, 7.91; MS (m/z): 170, 264, 286, 329, 351 (M^+)

s5 (Cyclopropyl phenyl ketone semicarbazone): White solid; yield: 119 mg, (53 %); mp: 169-170°C; FT-IR (ATR): ῡ= 3372, 3342, 3273, 3216, 3005, 1665, 1596, 1466, 1400, 1363, 1298, 1147, 1086, 1036, 966, 930 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ=0.62 ppm (dt, J=1.5, 3, 4, 2 H, CH_2), δ=1.136 ppm (td, 2H, J=4, 1.5, CH_2), δ= 1.57 ppm (m, H, CH), δ=1.78 ppm (s, 2 H, NH_2), δ=7.72 ppm (aromatics, J=6.83, 2.44, 1.46, 5.86, 5H), δ=8.53 ppm (s, 1 H, NH). ^{13}C-NMR (500 MHz, $CDCl_3$): δ=76.79 ppm (CH_2), δ=77.04 ppm (CH_2), δ=77.30 ppm (CH), δ=127.10-136.70 ppm (aromatic carbons), δ=148.84 ppm (C=N), δ=156.78 ppm (C=O); Anal. calcd. for $C_{12}H_{14}N_2O$: C, 71.29; H, 6.93; N, 13.86. Found: C, 71.31; H, 6.96; N, 13.82; MS (m/z): 130, 159,173, 203 (M^+)

p1 (1-(2,4-dinitrophenyl)-3,5-dimethyl-1H pyrazole): (11) Brown colored solid; yield: 165 mg, (63 %); mp: 105-106 °C; FT-IR (ATR): ῡ= 3080, 2993, 1630, 1608, 1522, 1381 $cm^{-1;}$ ^{1}H-NMR (500 MHz, $CDCl_3$): δ=2.15 ppm (d, 3H, CH_3), δ=2.72 ppm (d, 3H, CH_3), δ=6.00 ppm (s, 1H, CH), δ=7.66 ppm (d, 1H, J=8.29, aromatic), δ=7.76 ppm (s, 1H, aromatic), δ=8.45 ppm (d, 1H, J=2.44, aromatic), δ=8.50 ppm (d, 1H, J=1.95, aromatic), δ=8.68 ppm (d, aromatics, J=2.44, ^{13}C-NMR (500 MHz, $CDCl_3$): δ=31.34 ppm (CH_3), δ=36.19 ppm (CH_3), δ=108.80 ppm (CH), δ=120.88-146.11 ppm (aromatic carbons), δ=152.09 ppm (C-N), δ=162.57 ppm (C=N); Anal. calcd. for $C_{11}H_{10}N_4O$: C, 50.38; H, 3.82; N, 21.37. Found: C, 66.04; H, 7.46; N, 12.37; MS (m/z): 95, 102, 169, 186, 199, 232, 264 (M^++1)

Antimicrobial activity

Antimicrobial activity against *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, *Candida tropicalis* ATCC 750 was determined by the microbroth dilution method according to the

recommendations of Clinical Laboratory Standarts Institute (CLSI). Mueller Hinton broth (Difco, Detroid, USA) was used for bacterial species and RPMI- 1640 (Sigma) was used for Candida species throughout the experiments. Serial two fold dilutions ranging from 5000 to 1.22 µL were prepared in the medium. The inoculum was prepared using a 4-6 h broth culture of each bacterial 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 of $5x10^5$ cfu/mL for bacteria and $5x10^3$ cfu/mL for yeast in the test tray. The trays were covered and placed into plastic bags to prevent evaporation. Microplates were incubated for 18-24 h at 35 °C for bacteria and 46-50 h at 35 °C for yeast. The MIC value was evaluated as the lowest concentration of the compound that the visible proliferation has not occurred. Ciprofloxacin and fluconazole were included throughout the experiments in the study as standard antimicrobials for bacteria and fungi. The results for both antimicrobials were found according to the CLSI criteria. (14-15)

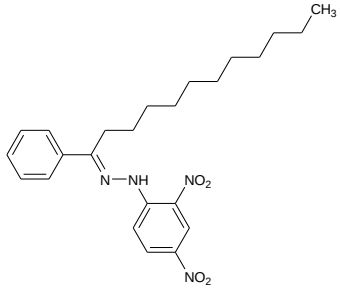
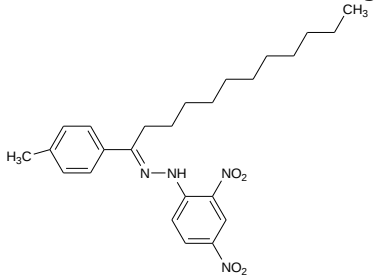
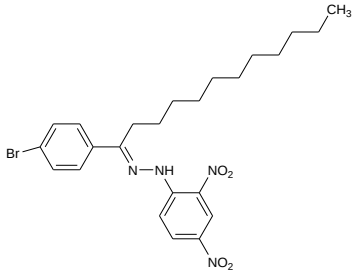
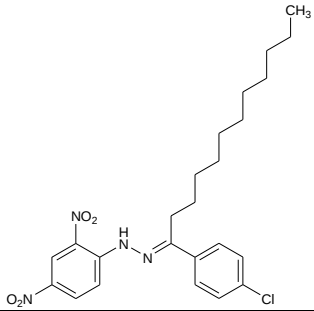
RESULTS AND DISCUSSION

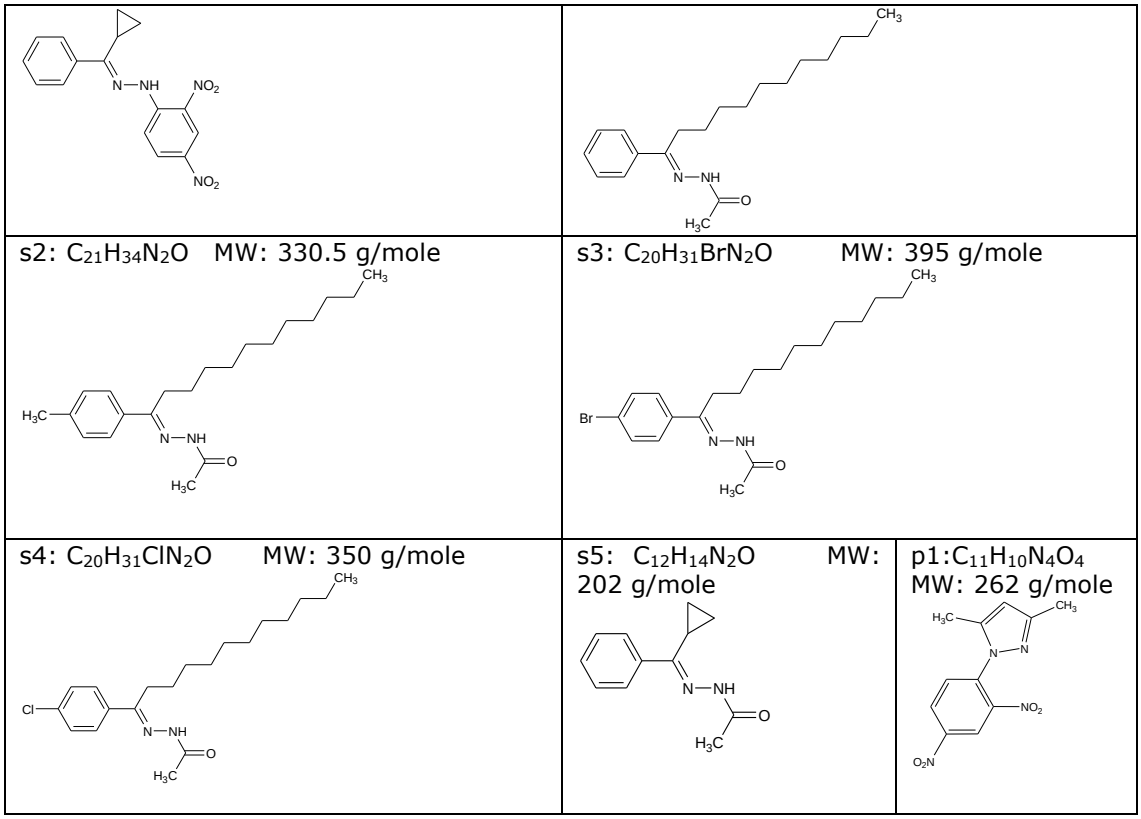
Chemistry

Novel long chain alkyl, phenyl and cyclopropyl containing semicarbazones (**s1-s5**) and 2,4-dinitrophenylhydrazones (**h1-h5**) were synthesized in this study, with the aim to investigate their anitimicrobial activities. Besides, a pyrazole ring carrying 2,4-dinitrophenylhydrazone **p1**(1-(2,4-dinitrophenyl)-3,5-dimethyl-1H pyrazole) was synthesized from the reaction of 2,4-dinitrophenylhydrazine with a diketone acetylacetone.

The semicarbazones and hydrazones were obtained from their corresponding ketones (**k1-k5**) prepared by Friedel-Crafts acylation. The substances (**h1-h5**) (**s1-s5**) and (**p1**) (Table 5) were checked with spectroscopic methods. Their E/Z isomerization was 1:1 as detected by their NMR spectra. The signals of isomer protons of the double bond resonated on different chemical shifts with same magnitude. Therefore the ratio of isomers was determined as 50%. E and Z isomers had different shielding effects in ^{1}H-NMR spectrum depending on the electronic densities in the molecule due to the location of the substituents. These were seen and determined in the upper or lower fields according to the shielding effects. (16) C=N groups of synthesized semicarbazones and hydrazones showed peaks around 1550 cm^{-1} in the FT-IR spectra.

Table 5 Structures of the synthesized hydrazones, semicarbazones and pyrazole.

h1: $C_{24}H_{34}N_4O_4$ MW: 440 g/mole 	h2: $C_{25}H_{34}N_4O_4$ MW: 454 g/mole 
h3:$C_{24}H_{31}BrN_4O_4$ MW: 519 g/mole 	h4: $C_{24}H_{31}ClN_4O_4$ MW: 474 g/mole 
h5: $C_{16}H_{14}N_4O_4$ MW: 326 g/mole	s1: $C_{20}H_{32}N_2O$ MW: 316,5 g/mole



Antimicrobial activities

The structure-antimicrobial activity relationship in this study was explained due to the results of the antimicrobial studies summarized in Table 6.

Cyclopropyl and nitro groups were effective in antimicrobial activity. This cyclopropyl and two deactivating nitro groups increased the activity of **h5**. Nitro groups with negative charged oxygen atom accelerated the attack of **h5** against three bacterial and two fungal species. Methyl groups were also effective by making H-bridges with bacteria according to their hyperconjugative character. Methyl and nucleophilic bromo groups had diminished the population of bacteria and fungi. According to the obtained antimicrobial results (Table 6) synthesized hydrazones (**h1 -h5**) were more antimicrobial active than that of semicarbazones (**s1- s5**). Pyrazole was the most effective one. Hydrazones in this study have more phenyl ring than that of semicarbazones. 2,4-dinitro phenyl substituent is more effective than CO-NH_2 group, therefore **h1>s1**. Nitro group with two oxygens had played an important role here. Alkyl and unsubstituted phenyl ring had no effect, as seen with **s1**. **s1** showed no antimicrobial activity. 2,4-dinitro phenyl substituent made hydrazone **h1** only against one bacterial species. The activity range of semicarbazones is as follows; **s5>s2>s3>s4≥s1**. **s5** is the best one because it carries a cyclopropyl substituent instead of a long alkyl chain. ring. **s5** has two rings, one non-aromatic cyclopropyl ring and one aromatic phenyl ring. These ring configurations made **s5** the most effective substance. **s5** was effective one among the semicarbazones. **s5** was effective against four bacterial species. **h1** with long alkyl chain showed no activity. **h5** with phenyl, cyclopropyl and 2,4-dinitrophenyl substituents were more effective than **s5. h5** was active against three bacterial and two fungal species. The data of Table 6 supported that the activity range of the hydrazones **h1-h5** followed as **h5≥h2>h3>h4>h1**. **h5** with cyclopropyl ring was active against three bacteria and two fungi species. Tolyl group led **h2** to be equally active with **h5** only against five bacterial species. Long chain alkyl group of **h2** was a deactivating group but tolyl with electron donating CH_3 group activated **h2** in the range of **h2>h3>h4>h1**. Electron donating groups like CH_3 increased the activity and electron-withdrawing groups like bromo and chloro decreased the activity. No substituent carrying phenyl ring was the most uneffective one obtained with **h1**. The substance **p1** with heterocyclic pyrazole character was the most active against six bacteria and three fungi species. Heterocyclic pyrazole group showed an

importance in being a good antimicrobial agent.

CONCLUSION

The synthesized novel hydrazones were found to be better antimicrobial agents than synthesized semicarbazones. Their preparation is cheap and environmentally friendly. Among them, **h5** and **s5** were the best inhibitory substances against the mentioned species of bacteria and fungi. **p1** (1-(2,4-dinitrophenyl)-3,5-dimethyl-1H pyrazole) was proposed to be a good general antimicrobial source with its wide effective spectrum. Hydrazones are more stable than semicarbazones by more electron delocalization. This resonance ability made the hydrazones more active.

According to the literature report lipophilic long alkyl chain with its sp^3 hybridization decreased the activity (17). Substitutions of hydrazones and semicarbazones' structures were also effective due to the results obtained in Table 6. Cyclopropyl's sp^2 increased the activity as seen with **h5-h1** and **s5-s1**. Substitutions on phenyl ring were important, too. (18-21) Electron donating group increased the activity as obtained with **h2>h1** and **s2>s1**. Electron withdrawing groups decreased the activity as seen with **h3>h4>h1** and **s3>s4≥s1**. Phenyl ring with none substitution was the least active structure as **h1** and **s1**. **p1** with 2,4-dinitrophenyl and two methyl groups on pyrazole ring was the best resonance stable compound and therefore exhibited the best activity.

Table 6 Antimicrobial activities of hydrazones, semicarbazones and pyrazole.

	P. aeruginosa ATCC 27853	*E. coli* ATCC 25922	*K. pneumoniae* ATCC 4352	*P. mirabilis* ATCC 14153	*S. aureus* ATCC 29213	*S. epidermidis* ATCC 12228	*E. faecalis* ATCC 29212	*C. albicans* ATCC 10231	*C. parapsilosis* ATCC 22019	*C. tropicalis* ATCC 750
s1	-	-	-	-	-	-	-	-	-	-
s2	-	312.5	-	625	-	-	-	-	-	-
s3	-	-	-	-	625	-	1250	-	-	-
s4	-	-	-	-	-	-	-	-	-	-
s5	-	312.5	625	625	-	1250	-	-	-	-
h1	-	-	-	-	-	-	625	-	-	-
h2	312.5	625	-	-	1250	1250	625	-	-	-
h3	-	-	625	625	-	-	625	78.12	-	-
h4	-	625	-	-	-	-	625	-	-	-
h5	-	-	-	-	1250	1250	625	-	312.5	312.5
p1	-	625	312.5	625	2.44	1.22	312.5	4.88	39.06	19.53

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