Synthesis and cytotoxic activity of some derivatives of alkyl piperidine

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A**bstract**: Synthesis of novel phenacyl derivatives of alkyl piperidine as cytotoxic agents *via* simple and single step reaction procedure is going to be reported here. Twelve new compounds were successfully synthesized in moderate yield and in solid form. Their synthesis was confirmed by TLC, melting point, CHN analysis and through different spectral studies such as UV, IR, Mass and proton NMR. The advantages of this synthetic route are simple operation, mild reaction conditions and good yields. These newly synthesized derivatives were extensively explored for their cytotoxicity by brine shrimp lethality assay.

K**eywords**: Alkyl piperidine derivatives, phenacyl halides, synthesis and cytotoxic activity.

INTRODUCTION

Heterocyclic compounds gain importance in the chemical research on the basis of their pharmacological and other biological activities (Fazal Mohamed, *et al*., 2011). The aim of my research is to enhance the research work of our group involved in the synthesis of biologically active piperidine derivatives since last two decades (Saify, *et al.*, 1997- 2005). Nowadays, scientists are also deeply concerned with the synthesis of more and more new medicinal compounds to cater the need of increasing population. As a logical further step in the field of medicinal chemistry we have become interested in the synthesis of pharmacologically active piperidine derivatives from Piperidine-2-methanol (Fig. Ia) and Piperidine-2-ethanol (Fig. Ib) through simple condensation of these alkyl piperidine and substituted phenacyl halides. I believe that the resultant derivatives would be a good addition in the field of medicines and therapeutics (Sarwat Jahan, *et al*., 2010).

(a) (b)

Fig. 1: a) Piperidine-2-methanol, b) Piperidine-2-ethanol

MATERIALS AND METHODS

All chemicals (reagents) were purchased from Sigma (Aldrich, London) Chemical Company. Reagent grade solvents such as acetone, ethyl alcohol methyl alcohol and chloroform, etc were obtained from E. Merck and distilled three times prior use to ensure extra purity and stored under argon over appropriate dehydrating agents such as molecular sieve or KOH pellets. Other solvents such as n-hexane, ethyl acetate, benzene, diethyl ether etc obtained from E. Merck were also of reagent grade. Reactions were performed in oven-dried glassware's under positive pressure of argon or nitrogen. Anhydrous solvents were transferred by oven-dried syringes.

^{1}H-NMR spectra were measured in CD_3OD, D_2O, d_6-DMSO and $CDCl_3$ on Bruker SF 300, 400 and 500 spectrometers operating at 300, 400 and 500 megahertz (MHz) using tetramethylsilane (TMS) as internal standard. Low-resolution mass spectra (LRMS) were recorded under electron impact (EI) conditions using MAT 112, MAT 113 D Eva system and JMS 600H double focusing mass spectrometer connected to MAT-188 data system with PDP 11/34 DEC computer system. Infra red (IR) spectra were recorded in solid potassium bromide (KBr) disc on Vector 22 spectrophotometer. Ultraviolet (UV) spectra were measured in methanol on CECIL CE 7200, spectrophotometer with scan speed 10nm/s.

Synthesis and spectral analysis of derivatives of alkyl piperidine

(I): Piperidine 2- methanol, or Piperidine-2-yl-methanol (Parent molecule)

White crystals having melting point 68-70^{o}C, UV $\lambda_{max}^{(MeOH)}$ nm (ε_{max}): 192.5 (2381); IR ν_{max} (KBr) cm^{-1}: 3400, 3250, 2800, and 750; EIMS *m/z*: 115 (5), 125.9 (11), 111 (7), 98 (6), 69 (6), 57 (7), 56 (37), 55 (19), 54 (7) 53 (3); ^{1}H-NMR (300 MHz, DMSO) δ: 1.7 (3H, t, J=6 Hz, 21 Hz, H-4), 1.44 (1H, s, H-3), 2.89 (1H, s, H-6,), 3.78 (3H, t, J=6 Hz, 15 Hz, H-2), 3.02 (2H, d, J=6 Hz, H-2, 1), 1.86 (2H, d, J=9 Hz, H-5), 2.48 (1H, s, H-3); Anal. Found C (72.72%), H (13.13%) and N (14.14%).

(Ia): 2-hydroxymethyl-1-[(3-nitro-phenyl)-2-oxoethyl]-piperidinium bromide

Piperidine-2-methanol (1.15 Gm) was dissolved in 10 mL of distilled hot acetone and kept for stirring on a magnetic

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stirrer (Hot plate made in UK by Bibby Sterlin Limited). 2-bromo-3'-nitro acetophenone (2.4 Gm) dissolved in 10 mL of distilled acetone. Clear, colorless solution of 2-bromo-3'- nitro acetophenone transferred into a flask containing piperidine-2-methanol with constant stirring at room temperature for six hours and color changed to brown clear solution. Reaction progress was monitored by thin layer chromatography (6:4, ethanol: chloroform) and then refluxed on a water bath for 72 hours at 100°C. Crystals appeared in the reaction flask were filtered and washed with acetone. The product thus obtained was purified through recrystallization by using methanol and ether to yield 27.81% golden yellow shiny crystals. The pure compound was dried in vacuum desiccator over anhydrous calcium sulphate. Melting point was determined (179-180°C); UV $\lambda_{max}^{(MeOH)}$ nm (ε_{max}): 262.5 (541); IR ν_{max} (KBr) cm^{-1}: 3500, 3200, 2900, 2700, 1718, 1500 and 750; EIMS *m/z*: 276 (15), 260(8), 246(9), 149(11), 110(19), 95(69), and 54(65); ^{1}H-NMR (400 MHz, DMSO) δ: 2.89 (1H, s, H-1"), 3.48 (2H, t, J=4 Hz, 8 Hz, H-3"), 1.80 (2H, t, J=8 Hz, 28 Hz, H-4), 1.83 (2H, d, J=12 Hz, H-5), 1.86 (2H, d, J=12 Hz, H-2), 3.00 (2H, d, J=8 Hz, H-6), 1.44 (2H, s, H-3), 2.48 (1H, s, H-2"), 7.93 (1H, d, J=4 Hz, H-4'), 8.30 (1H, t, J=8 Hz, 0.8 Hz, H-6'), 7.82 (1H, t, J=4 Hz, 12 Hz, H-5'), 8.36 (1H, s, H-2', 3'); Anal. Found C (48.17%), H (5.63%), and N (7.46%).

(Ib): 2-hydroxymethyl-1-[(4-bromo-phenyl)-2-oxoethyl]-piperidinium bromide

Piperidine-2-methanol (1.15 Gm) was dissolved in 10 mL of distilled acetone followed by stirring. 2, 4'-di bromo acetophenone (2.77 Gm) was dissolved in 20 mL of distilled acetone and transferred into a flask containing piperidine-2-methanol with continuous stirring for six hours at room temperature. The color of the reaction mixture changed to brown clear solution, which was monitored by thin layer chromatography [6:4, ethanol (6 mL & chloroform (4 mL)] and kept for refluxing on a water bath at 100°C for 24 hours. White crystals appeared which were filtered, washed and recrystallized in methanol and ether, dried in desiccator to yield 41.01% having melting point 195°C; UV $\lambda_{max}^{(MeOH)}$ nm (ε_{max}): 257.5 (305); IR ν_{max} (KBr) cm^{-1} : 3600, 3500, 2900, 1720, 1625, 1500 and 760; EIMS *m/z*: 311 (2), 186 (6), 184 (7), 158 (2), 156 (57), 154 (58), 132 (1), 110 (32), 95 (36), 75 (64), 56 (16), 54 (46) and 51 (16); ^{1}H-NMR (400 MHz, DMSO) δ: 2.89 (1H, s, H-1"), 3.48 (2H, t, J=4 Hz, 8 Hz, H-3"), 1.80 (2H, t, J=8 Hz, 28 Hz, H-4), 1.83 (2H, d, J=12 Hz, H-5), 1.86 (1H, d, J=12 Hz, H-2), 3.00 (1H, d, J=8 Hz, H-6), 1.44 (1H, s, H-3), 2.48 (1H, s, H-2"), 7.67 (1H, d, J=4 Hz, H-2), 7.65 (2H, d, J=4 Hz, H-6, 1), 7.42 (2H, d, J=8 Hz, H-3), 7.40 (2H, d, J=8 Hz, H-5, 4); Anal. Found C (42.78%), H (5.10%), and N (3.14%).

(Ic): 2-hydroxymethyl-1-[(4-flouro-phenyl)-2-oxoethyl]-piperidinium bromide

2-bromo-4'-fluoro acetophenone (2.17 Gm) was dissolved in 15 mL of distilled acetone. To this, a solution of piperidine- 2-methanol (1.15 Gm) in distilled acetone was mixed with constant stirring at room temperature for six hours. Cleared brown colored solution was monitored by thin layer chromatography [ethanol (6 mL) and chloroform (4 mL)] and refluxed on a water bath at 100°C for 8 hours. Brown crystals appeared in the reaction flask were filtered, washed with acetone and recrystallized in methanol and ether, dried in desiccator to yield 21.71% having melting point 148°C; UV $\lambda_{max}^{(MeOH)}$ nm (ε_{max}): 255 (435); IR $(KBr)_{\nu max}$ cm^{-1}: 3600, 3400, 2950, 1710, 1615, 1500 and 800; EIMS *m/z*: 250 (9), 234 (6), 150 (16), 121 (13), 120 (10), 111(48), 95 (54), 83 (34) and 54 (78); ^{1}H-NMR (300 MHz, DMSO) δ: 2.89 (1H, s, H-1"), 3.48 (2H, t, J=4 Hz, 8 Hz, H-3"), 1.80 (2H, t, J=8 Hz, 28 Hz, H-4), 1.83 (2H, d, J=12 Hz, H-5), 1.86 (1H, d, J=12 Hz, H-2), 3.00 (1H, d, J=8 Hz, H-6), 1.44 (1H, s, H-3), 2.48 (1H, s, H-2"), 7.33-7.27 (2H, t, J=6 Hz, 15 Hz, H-3'), 7.53 (1H, s, H-1'), 7.51 (2H, d, J=3 Hz, H-2'), 7.50 (2H, d, J=3 Hz, H-6'); Anal. Found C (36.50%), H (4.92%) and N (2.71%).

(Id): 2-hydroxymethyl-1-[(4-nitro-phenyl)-2-oxoethyl]-piperidinium bromide

2-bromo-4'-nitro acetophenone (2.4 Gm) was dissolved in 10 mL of distilled acetone, which was mixed in a flask containing piperidine-2-methanol (1.15 Gm) dissolved in 10 mL of acetone with constant stirring at room temperature for six hours. Color of the reaction mixture became brown colored cleared solution and monitored by thin layer chromatography [6:4, ethanol (6 mL) and chloroform (4 mL)]. This was refluxed on a water bath at 100°C for 18 hours. Brown precipitates were appeared in the reaction flask were filtered and recrystallized by dissolving in methanol and ether to yield 35.56% having melting point 175°C; UV $\lambda_{max}^{(MeOH)}$ nm (ε_{max}): 265 (564); IR $(KBr)_{\nu max}$ cm^{-1}: 3600, 3300, 2900, 1712, 1625, 1500 and 795; EIMS *m/z*: 278 (2), 260 (3), 247 (4), 128 (15), 112 (17), 104 (15), 95 (13), 84 (66), 83 (58), 82 (35), 68 (26), 57 (30), 56 (33) and 54 (74); ^{1}H-NMR (400 MHz, MeOD) δ: 2.89 (1H, s, H-1"), 3.48 (2H, t, J=4 Hz, 8 Hz, H-3"), 1.80 (2H, t, J=8 Hz, 28 Hz, H-4), 1.83 (2H, d, J=12 Hz, H-5), 1.86 (1H, d, J=12 Hz, H-2), 3.00 (1H, d, J=8 Hz, H-6), 1.44 (1H, s, H-3), 2.48 (1H, s, H-2"), 7.82 (2H, d, J=8 Hz, H-2'), 7.84 (1H, d, J=8 Hz, H-1', 6'), 8.29 (2H, d, J=8 Hz, H-3'), 7.71 (1H, s, H-4'), 8.31(1H, d, J=8 Hz, H-5'); Anal. Found C (52.41%), H (6.63) and N (3.69).

(Ie): 2-hydroxymethyl-1-[(4-methoxy-phenyl)-2-oxoethyl]-piperidinium bromide

Piperidine-2-methanol (1.15 Gm) dissolved in 10 mL of distilled acetone was added to a solution containing *α*-bromo-*p*-methoxy acetophenone (2.29 Gm) in 12 mL of distilled acetone with continuous stirring at room temperature for six hours. During stirring the color of the reaction mixture changed to black cleared solution which was monitored by thin layer chromatography [ethanol (6 mL) and chloroform (4 mL)] and refluxed on a water bath for 18 hours at 100°C. Black precipitates formed were filtered, recrystallized by dissolving in methanol and ether

and left for crystallization to yield 38.25% having melting point 162°C; UV $\lambda_{max}^{(MeOH)}$ nm (ε_{max}): 232.5 (425); IR ν_{max} (KBr) cm^{-1}: 3550, 3450, 2950, 1715, 1640, 1500 and 730; EIMS *m/z*: 262 (3), 246 (16), 244 (36), 230 (4), 215 (2), 175 (3), 163 (12), 162 (9), 136 (7), 134 (81), 120 (11), 112 (11), 110 (31), 95 (69), 83 (48), 57 (26), and 54 (24); ^{1}H-NMR (300 MHz, MeOD) δ: 2.89 (1H, s, H-1"), 3.48 (2H, t, J=4 Hz, 8 Hz, H-3"), 1.80 (2H, t, J=8 Hz, 28 Hz, H-4), 1.83 (2H, d, J=12 Hz, H-5), 1.86 (2H, d, J=12 Hz, H-2), 3.00 (2H, d, J=8 Hz, H-6), 1.44 (1H, s, H-3), 2.48 (1H, s, H-2"), 7.47 (2H, d, J=6 Hz, H-5'), 6.99 (2H, d, J=1.8 Hz, H-6'), 3.80 (1H, s, H-1'), 6.96 (1H, s, H-2'), 7.44 (2H, d, J=6 Hz, H-3', 4'); Anal. Found C (46.93%), H (5.32%) and N (7.91%).

(If): 2-hydroxymethyl-1-[(4-chloro-phenyl)-2-oxoethyl]-piperidinium bromide

Piperidine-2-methanol (1.15 Gm) dissolved in 10 mL of distilled acetone with constant stirring at room temperature for six hours. To this was added 2-bromo-4'-chloroacetophenone (2.33 Gm) dissolved in 10 mL of distilled acetone, cleared yellow colored solution became brown, monitored by thin layer chromatography and refluxed on a water bath at 100°C for 33 hours. Brown crystals appeared were filtered, washed with acetone, recrystallized in methanol and ether to yield 10.23% having melting point 160-165°C; UV $\lambda_{max}^{(MeOH)}$ nm (ε_{max}): 257.5 (312); IR ν_{max} (KBr) cm^{-1}: 3595, 3450, 2980, 1718, 1625, 1500 and 725; EIMS *m/z*: 267 (2), 266 (9), 249 (7), 235 (2), 219 (2), 167 (8), 139 (29), 111 (95), 110 (36), 97 (13), 83 (44), 82 (31), 68 (28), 55 (64), 53 (7), and 51 (4); ^{1}H-NMR (300 MHz, DMSO) δ: 2.89 (1H, s, H-1"), 3.48 (2H, t, J=4 Hz, 8 Hz, H-3"), 1.80 (2H, t, J=8 Hz, 28 Hz, H-4), 1.83 (2H, d, J=12 Hz, H-5), 1.86 (2H, d, J=12 Hz, H-2), 3.00 (1H, d, J=8 Hz, H-6), 1.44 (1H, s, H-3), 2.48 (1H, s, H-2"), 7.46 (2H, m, H-3', 5'), 7.49 (1H, s, H-2'), 7.48 (1H, s, H-6'), 7.44 (1H, s, H-4') 7.42 (1H, s, H-1'); Anal. Found C (48.32%), H (5.38%) and N (3.38%).

II: Piperidine 2-ethanol or Piperidine-2-yl-ethanol (Parent molecule)

White amorphous powder having melting point 38–40°C; UV $\lambda_{max}^{(MeOH)}$ nm (ε_{max}): 279.5 (284); IR ν_{max} (KBr) cm^{-1}: 3400, 3300, 2800, and 800; EIMS *m/z*: 130 (80), 129 (16), 128 (34), 112 (14), 110 (15), 99 (7), 98 (32), 96 (9), 86 (13), 85 (84), 83 (8), 82 (31), 81 (8), 72 (55), 70 (32), 69 (11), 68 (21), 57 (25), 55 (67), 54 (31) and 53 (13); ^{1}H-NMR (300 MHz, DMSO) δ: 2.26 , 2.49 and 2.71 (1H, s, H-1), 3.52 (3H, d, J=3 Hz, H-2), 1.54-1.63 (4H, m, H-3), 1.44 (1H, s, H-5), 1.29-1.39 (4H, dd, J=6 Hz, 12 Hz, H-4), 2.84 (2H, d, J=6 Hz, H-6); Anal. Found C (74.33%), H (13.27%) and N (12.38%).

(IIa): 2-hydroxy-ethyl-1-[(4-bromo-phenyl)-2-oxoethyl]-piperidinium bromide.

2', 4'-di bromo acetophenone (1.30 Gm) dissolved in 10 mL of distilled acetone followed by continuous stirring to which piperidine-2-ethanol (0.5 Gm) dissolved in 10 mL of distilled acetone was added at room temperature for six hours. Cleared colorless solution became yellow during stirring which was monitored by thin layer chromatography and refluxed on a water bath at 100°C for 96 hours. Yellow precipitates appeared were filtered, washed with acetone and recrystallized in methanol and ether to yield 35.58% having melting point 116°C; UV $\lambda_{max}^{(MeOH)}$ nm (ε_{max}): 257.5 (1446); IR ν_{max} (KBr) cm^{-1}: 3400, 3300, 2800, 1725, 1675, 1500 and 725; EIMS *m/z*: 325 (2), 343 (3), 227 (7), 200 (4), 184 (8), 183 (94), 167 (3), 157 (20), 155 (22), 139 (4), 125 (7), 111 (11) 109 (7), 97 (13), 85 (11), 83 (12), 71 (16), 57 (19), and 51 (3); ^{1}H-NMR (300 MHz, MeOD) δ :3.29 (1H, s, H-3"), 2.26 (1H, s, H-2), 1.44 (1H, s, H-3), 1.54 (4H, m, H-4, 5), 1.87 (1H, s, H-6), 7.92 (2H, d, J=6 Hz, H-1'), 7.64 (2H, d, J=6Hz, H-2'), 7.89, (2H, d, J=6 Hz, H-5'), 7.61 (2H, d, J=6 Hz, H-6'), 7.71 (1H, s, H-3'), 7.74 (3H, t, J=6 Hz, 15 Hz, H-4'); Anal. Found C (55.46%), H (5.64%) and N (4.34%).

(IIb): 2-hydroxy-ethyl-1-[(4-fluoro phenyl)-2-oxoethyl]-piperidinium bromide

2-Bromo-4′-Fluoro acetophenone (1.05 Gm) dissolved in 10 mL of distilled acetone and piperidine-2-ethanol (0.5 Gm) dissolved in 10 mL of distilled acetone was mixed together with constant stirring at room temperature for six hours. Color of the reaction mixture changed to yellow cleared solution, monitored by thin layer chromatography, which was kept for refluxing on a water bath at 100°C for 96 hours. Yellow precipitates appeared in the reaction flask were filtered, washed with acetone and recrystallized in methanol and ether to yield 17.93% having melting point 100-101°C; UV $\lambda_{max}^{(MeOH)}$ nm (ε_{max}): 240 (2082); IR ν_{max} (KBr) cm^{-1}: 3590, 3500, 2900, 1722, 1620, 1500 and 795; EIMS *m/z*: 264 (1), 248 (4), 220 (3), 203 (3), 142 (24), 140 (11), 136 (6), 128 (3), 125 (5), 124 (57), 112 (6), 110 (4), 109 (18), 98 (26), 96 (18), 95 (72), 94 (12), 84 (72), 80 (10), 75 (52), 69 (12), 63 (3), 56 (17), 50 (9) and 46 (3); ^{1}H-NMR (300 MHz, DMSO) δ: 3.29 (s, 1H, *H*-3"), 2.26 (s, 1H, *H*-2), 1.44 (s, 1H, *H*-3), 1.54 (m, 4H, *H*-4, 5), 1.87 (1H, s, H-6), 8.07 (2H, s, H-3'), 8.12 (2H, s, H-5'), 8.10 (2H, d, J=3 Hz, H-4'), 7.37 (2H, d, J=3 Hz, H-2'), 7.42 (2H, d, J=3 Hz, H-6'), 7.44 (2H, s, H-1'); Anal. Found C (60.02%), H (4.14%) and N (0.63%).

(IIc): 2-hydroxy-ethyl-1-[(3-nitro-phenyl)-2-oxoethyl]-piperidinium bromide

Piperidine-2-ethanol (0.5 Gm) dissolved in 10 mL of distilled acetone and 2-Bromo-3′-Nitroacetophenone (2.4 Gm) dissolved in 12 mL of distilled acetone was mixed together by continuous stirring at an ambient temperature for six hours and during stirring the color of the reaction mixture became yellow cleared solution which was refluxed on a water bath at 100°C for 118 hours. Off-white precipitates appeared in the reaction flask, monitored by thin layer chromatography, washed with warm acetone, filtered and recrystallized in methanol and ether, kept in vacuum desiccator for drying to yield 37.02%whose melting point was 132°C; UV $\lambda_{max}^{(MeOH)}$ nm

(ε_{max}): 246.5 (2320); IR ν_{max} (KBr) cm^{-1}: 3409, 3312, 2825, 1724, 1672, 1505 and 723; EIMS *m/z*: 292 (4), 248 (7), 174 (3), 154 (21), 150 (8), 126 (2), 123 (7), 113 (5), 98 (6) and 84 (12); ^{1}H-NMR (300 MHz, MeOD) δ: 3.29 (1H, s, H-3"), 2.26 (1H, s, H-2), 1.44 (1H, s, H-3), 1.54 (4H, m, H-4, 5), 1.87 (1H, s, H-6), 7.63 (2H, d, J=6 Hz, H-5'), 8.25 (2H, d, J=9 Hz, H-6'), 8.38 (3H, d, J=39 Hz, H-4'), 8.79 (5H, m, H- 1', 2', 3'); Anal. Found C (61.64%), H (6.84%) and N (9.58%).

(IId): 2-hydroxy ethyl 1-(4-nitro-phenyl)-2-oxoethyl piperidinium bromide

Piperidine-2-ethanol (0.5 Gm) dissolved in 10 mL of distilled acetone and 2-Bromo-4′-Nitroacetophenone (2.1 Gm) dissolved in 15 mL of distilled acetone was mixed by constant stirring at room temperature for six hours. The color of the solution became dark brown cleared solution kept for refluxing at 100°C for about 64 hours. Orange colored precipitates appeared, examined by thin layer chromatography, washed with acetone, filtered and recrystallized in methanol and ether and dried in vacuum desiccator to yield 27.63% having melting point 119-120°C; UV $\lambda_{max}^{(MeOH)}$ nm (ε_{max}): 235 (1740); IR ν_{max} (KBr) cm^{-1}: 3585, 3495, 2905, 1711, 1615, 1495 and 790; EIMS *m/z* : 293 (2), 244 (5), 178 (6), 151 (3), 148 (4), 124 (1), 121(4), 111 (11), 96 (3) and 88 (11); ^{1}H-NMR (300 MHz, MeOD) δ: 3.29 (1H, s, H-3"), 2.26 (1H, s, H-2), 1.44 (1H, s, H-3), 1.54 (4H, m, H-4, 5), 1.87 (1H, s, H-6), 8.12 (6H, m, H-1', 2', 6'), 8.30 (6H, m, H-3', 4', 5'); Anal. Found C (61.64%), H (6.84%) and N (9.58%).

(IIe): 2-hydroxy-ethyl-1-[(2-nitro-phenyl)-2-oxoethyl]-piperidinium bromide

2-Bromo-2′-Nitroacetophenone (1.2 Gm) and Piperidine-2-ethanol (0.5 Gm) dissolved separately in 10 mL of distilled acetone. When the two solutions were mixed with constant stirring, the colorless solution has changed to yellow cleared solution and was refluxed at 100°C for 92 hours. Off-white precipitates were formed followed by thin layer chromatography, washed with warm acetone, filtered and recrystallized in methanol and ether and dried in vacuum desiccator to yield 26.76% having melting point 139°C; UV $\lambda_{max}^{(MeOH)}$ nm (ε_{max}): 222 (1740); IR ν_{max} (KBr) cm^{-1}: 3575, 3450, 2910, 1710, 1612, 1465 and 780; EIMS *m/z*: 291 (6), 246 (7), 176 (4), 153 (8), 146 (3), 127 (2), 122(5), 109 (7), 98 (3) and 85 (11); ^{1}H-NMR (300 MHz, DMSO) δ: 3.29 (1H, s, H-3"), 2.26 (1H, s, H-2), 1.44 (1H, s, H-3), 1.54 (4H, m, H-4, 5), 1.87 (1H, s, H-6), 7.71 (2H, d, J=15 Hz, H-4'), 7.76 (2H, d, J=15 Hz, H-5'), 8.12 (4H, m, H-1', 6'), 8.30 (4H, m, H-2', 3'); Anal. Found C (61.64%), H (6.84%) and N (9.58%).

(IIf): 2-hydroxy-ethyl-1-[(3', 5', dinitro-phenyl)-2-oxoethyl]-piperidinium bromide

Piperidine-2-ethanol (0.5 Gm) dissolved in 10 mL of acetone and to this was added a solution of 3', 5', dinitrobenzoyl chloride (1.15 Gm) dissolved in 16 mL of acetone with constant stirring for six hours at room temperature. White precipitates appeared during stirring which were monitored by thin layer chromatography and washed with acetone, filtered and recrystallized in methanol and ether and stored in vacuum desiccator to yield 25.94% having melting point 157-163°C; UV $\lambda_{max}^{(MeOH)}$ nm (ε_{max}): 224.5 (1708); IR ν_{max} (KBr) cm^{-1}: 3580, 3445, 2912, 1720, 1618, 1455 and 785; EIMS *m/z*: 337 (3), 266 (8), 251 (4), 220 (3), 206 (5), 183 (9), 168 (3), 138 (3), 123 (4), 113 (3), 93 (4) and 84 (5); ^{1}H-NMR (300 MHz, DMSO) δ: 3.29 (1H, s, H-3"), 2.26 (1H, s, H-2), 1.44 (1H, s, H-3), 1.54 (4H, m, H-4, 5), 1.87 (1H, s, H-6), 9.02 (6H, m, H-3', 4', 5', 6'), 9.31 (5H, m, H-1', 2'); Anal. Found C (50.32%), H (5.16%) and N (13.54%).

Determination of cytotoxic activity by brine shrimp lethality bioassay

Test Sample, *Artemia salina* (shrimp eggs), Sea Salt (38 g/L of D/W, pH 7.4), Hatching tray with perforated partition, Lamp to attract brine-shrimp larvae, Micropipette (5, 50, 500 µL), Vials tray, samples vials, Organic solvents (Methanol/Ethanol/Acetone/water), distilled water.

Methods

Storage of Artemia salina eggs

The eggs were stored at low temperatures (4°C), at which they remain viable for years. They are available from fish pet shops.

Hatching techniques

Half filled the hatching tray (a rectangular dish (22x32 cm) with filtered brine solution then sprinkle (50 mg) eggs of brine shrimp. Incubate at 37°C.

Sample preparation

Dissolve test sample (20mg) in 2 mL of respective solvent and transfer 5, 50 and 500 µL to vials (3vials/ concentration, 9 vials in total for one sample). The concentration will be 10, 100 and 1000 µg/mL respectively. Allow the solvent to evaporate over night. After 2-days of hatching and maturation as nauplii, place 10 larvae/vials, using a Pasteur pipette. Make the volume to 5 mL with seawater. Incubate at 25-27°C for 24 hours under illumination. Supplement other vials with solvent, and reference cytotoxic drug (Etoposide) serving as negative and positive controls, respectively. Analyze data with Finney computer program to determine LD_{50} values with 95% confidence intervals.

Shrimps can be used 48-72 hours after the initiation of hatching. After 72 hours, they should be discarded (Alves, *et al*., 2000; Kivac, *et al*., 2001; Carballo, *et al*., 2002; Mayer, *et al*., 1982 and Finney, *et al*., 1971).

RESULTS

Few derivatives of alkyl piperidine have been synthesized by simple condensation reaction which exhibited different promising biological and pharmacological activities. The

active site is the N of the nucleus where substitution was made easily in a single step.

Table 1: Brine Shrimp Lethality (cytotoxic) results for Piperidine-2-methanol and its phenacyl derivatives

S. No.	Comp. Code	Name of Compound	LD_{50} (µg/mL)
1	I	Piperidine 2- methanol	> 1000
2	Ia	2-hydroxymethyl-1-[(3-nitro-phenyl)-2-oxoethyl]-piperidinium bromide.	> 1000
3	Ib	2-hydroxymethyl-1-[(4-bromo-phenyl)-2-oxoethyl]-piperidinium bromide.	528.349
4	Ic	2-hydroxymethyl-1-[(4-fluoro-phenyl)-2-oxoethyl]-piperidinium bromide.	4.566
5	Id	2-hydroxymethyl-1-[(4-nitro-phenyl)-2-oxoethyl]-piperidinium bromide.	> 1000
6	Ie	2-hydroxymethyl-1-[(4-methoxy-phenyl)-2-oxoethyl]-piperidinium bromide.	45.267
7	If	2-hydroxymethyl-1-[(4-chloro-phenyl)-2-oxoethyl]-piperidinium bromide	33.65
8	Std. Drug	Etoposide	7.462

LD_{50} indicates the lethal dose

Table 2: Brine shrimp lethality (cytotoxic) results for Piperidine-2-ethanol and its phenacyl derivatives

S. No	Comp. Code	Name of Compounds	LD_{50} (µg/mL)
1	II	Piperidine 2-ethanol	465.321
2	IIa	2-hydroxy-ethyl-1-[(4-bromo-phenyl)-2-oxoethyl]-piperidinium bromide.	104.308
3	IIb	2-hydroxy-ethyl-1-[(4-fluoro phenyl)-2-oxoethyl]-piperidinium bromide.	3.006
4	IIc	2-hydroxy-ethyl-1-[(3-nitro-phenyl)-2-oxoethyl]-piperidinium bromide.	> 1000
5	IId	2-hydroxy-ethyl-1-[(4-nitro-phenyl)-2-oxoethyl]-piperidinium bromide.	> 1000
6	IIe	2-hydroxy-ethyl-1-[(2-nitro-phenyl)-2-oxoethyl]-piperidinium bromide.	> 1000
7	IIf	2-hydroxy-ethyl-1-[(3',5', dinitro-phenyl)-2-oxoethyl]-piperidinium bromide.	> 1000
8	Std. Drug	Etoposide	7.462

LD_{50} indicates the lethal dose

DISCUSSION

Natural products (extracts and pure compounds) and the synthetic compounds can be tested for their bioactivity by Brine shrimp lethality bioassay (Anna *et al*., 2011). Brine shrimp (*nauplii*) is used as a convenient monitor for screening and fractionation of new compounds. This bioassay is indicative of anticancer, antiviral, cytotoxicity and wide range of pharmacological activities of the compounds (Abdur-Rehman, *et al*., 2000). The approach in this assay is that toxicology is simply pharmacology at a higher dose, thus a single bioassay might lead to new pharmacological agent (Meyer, *et al.*, 1982).

The results of the brine shrimp lethality of piperidine-2-methanol and piperidine-2-ethanol along with their derivatives have been reported in the tables I and II. Activity is mentioned in terms of LD_{50} in microgram per milliliter.

From the data recorded in the table 1, it was shown that the parent molecule (compound I) did not display cytotoxic activity (LD_{50}>1000) while some of its derivatives exhibited promising cytotoxicity against *Artemia salina*. These compounds showed highly significant cytotoxic activity at the dose of 100µg/ml with LD_{50} 4.566 and 33.65 (Ic and If respectively), 65.267 and 528.349 (Ie and Ib respectively). It is evident that the compound Ic i.e., 2-hydroxymethyl-1-[(4-fluoro-phenyl)-2-oxoethyl]-piperidinium bromide exhibited highly potent effects (LD_{50} 4.566) as compared to the standard drug, etoposide. Rest of the compounds of this series were remained inactive (LD_{50}>1000).

Table 2 is showing the results for the parent II (piperidine-2-ethanol) and its newly synthesized derivatives (IIa-IIf). It is evident from the data that the parent molecule (compound II) itself showed cytotoxic activity against *Artemia salina* but to a very low degree (LD_{50} 665.321) and its only two derivatives (Compounds IIa and IIb) attained varying degree of cytotoxicity (LD_{50} 100.38 and 3.006 respectively) while others were failed to possess the same (LD_{50} >1000). It is also worth noting that the derivative IIb; 2-hydroxy-ethyl-1-[(4-fluoro phenyl)-2-oxoethyl]-piperidinium bromide exhibited the highly significant cytotoxic effects and was also proved to be more potent as compared to the standard drug.

During the structure activity relationship (SAR), it is interesting to note that the parent II possessed a low level of cytotoxicity and parent I was totally inactive. The only difference in their structures is one $–CH_2$ group which is in excess in parent II and just because of the carbon chain length, this compound may become active due to some unknown mechanism.

It is also worth mentioning that the halogenated derivatives showed pronounced cytotoxic activity and again highly significant cytotoxic activity was observed in the bromo and flouro derivatives of parent II. It can be stated that the cytotoxic behavior of parent II was potentiated in presence of halogens while other groups

like $-NO_2$ and $-OCH_3$ had attenuated the cytotoxicity of parent II. The results of flouro derivatives of both the parents were comparable (LD_{50} 4.566 and 3.006) for derivatives Ic and IIb respectively.

Among the halogenated compounds, flouro derivatives (compound Ic table I and compound IIb, table II) were found to be the most Cytotoxic active agents against *Artemia salina*. These two compounds Ic 2-hydroxymethyl-1-[(4-fluoro-phenyl)-2-oxoethyl]-piperidinium bromide and IIb 2-hydroxy-ethyl-1-[(4-fluoro phenyl)-2-oxoethyl]-piperidinium bromide were also found most potent in the brine shrimp bioassay when compared to the standard drug. It is also suggested that the flouro group contributed the cytotoxic activity. However, more exploitation is needed.

CONCLUSION

All the newly synthesized compounds were evaluated for cytotoxic activity. Most of the compounds showed significant lethal effects on *Artemia salina* while two compounds (Ic and IIb) revealed highly significant cytotoxic activities. It is also suggested that the flouro group present in these two compounds contributed the cytotoxic activity. Therefore, these two compounds may be selected as the lead compounds in future.

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