SYNTHESIS AND BEHAVIOURAL STUDY OF 4-(1-PYRROLIDINYL) PIPERIDINE AND ITS DERIVATIVES

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ABSTRACT:
In continuation of our research work on piperidine molecule some new nitro and methoxy and 3, 4- dihydroxy phenacyl derivatives (II-V) of 4-(1-Pyrrolidinyl) Piperidine (I) were synthesized and tested for behavioural activity using open field experimental model. Results indicate a significant change in activity for parent compound at 50 and 100 mg/kg of body weight. Derivatives showed varied results for exploration and motility behaviour.

Keyword: Piperidine, pyrrolidin, behavioural activity, open field activity, phenacyl derivative.

INTRODUCTION
Anxiety, confusion, depression, agitation and insomnia are the examples of different patterns of behavioural disorders, which are the results of deficiency or increase in biogenic amines or impair neurotransmission (Schildkraut, 1978 and Wells et al., 1989).

Different approaches such as latency to move and number of square crossed are both pharmacologically and behaviourally valid method to study locomotion and exploratory behaviour (Porsolt et al., 1977; 1978; 1991; Willner, 1984; Shalyapina et al., 2003; Klejbor et al., 2003; Dere et al., 2003 and Mogilnicka, 1986).

Present investigation of behavioural changes in rats was done by using a precise established method of open field activity (Hall, 1934). The open field test has been used for the measurement of behavioural activity of small animals for the determination of behaviour (Archer, 1973). Activity was monitored as number of square crossed by animal in a novel enivronment for 5 minutes.

EXPERIMENTAL SECTION
General:
Reagents were purchased from Aldrich Chemical Company. All solvents were reagents grade. Reactions were monitored by TLC using pre-coated silica gel, GF-254 and were visualized under ultraviolet light at 254 nm and 366 nm on HP-UVIS Desaga (Heidelberg). Iodine vapors were also employed for the detection of spots. All melting points were recorded on Gallenkamp melting point apparatus and are uncorrected. Solid calcium sulphate from E. Merck was used for drying reaction product after workup. Ultraviolet (UV) spectra were recorded in methanol on a Hitachi U-3200 spectrophotometer. Infra Red (IR) spectra were measured on a Shimadzu IR 460 spectrophotometer using KBR disc. Mass spectra (MS) were determined on Varian Massen spectrometer MAT 311A spectro-meter. Nuclear magnetic resonance (1HNMR) spectra were recorded in DMSO-d6/MeOD on Bruker AM-400 and 500 spectrometer operating at 400 and 500 MHz.
TLC and final pure product was confirmed by taking point and then instrumental methods were used to confirm the structure of the product. In IR spectra compounds gave peaks at 3400-3700 cm\(^{-1}\) (NH), 2900-2500 cm\(^{-1}\) (C-H), 1600-1700 cm\(^{-1}\) (C=O), 1500-1600 cm\(^{-1}\) (C=C), 13-1400 cm\(^{-1}\) (CH\(_2\)). Chemical shifts in \(^1\)HNMR are reported in ppm. Selected data are reported as: chemical shift, multiplicity (s = singlet, d = doublet, dd = doublet of a doublet, t = triplet, m = multiplet), number of protons (1H = one proton, 2H = two proton, nH = n proton), coupling constant, \(J=(HZ)\), and assignment = \(H\)-n. Signals were at \(\delta\) 7-9 showing the presence of aromatic hydrogen while a sharp singlet at \(\delta\) 3-6 confirmed the presence of CH\(_2\) of the chain of the phenacyl moiety. Peaks at \(\delta\) 1-3 showing the presence of remaining aliphatic hydrogens.

**General Method of synthesis (II-V):**

To a stirred solution of I (1.48g, 1mmole) in acetone (15-20ml) was added successively substituted phenacyl halide (2.44g, 1mmole) dissolved separately in 15-20 ml acetone. Reaction mixture stirred for 48 hours at room temperature. The process of reaction was monitored through thin layer chromatography. The crude solid product was filtered and washed with acetone. The product thus obtained was purified through recrystallization by using ethanol and ether. The pure compound was dried in desiccator over anhydrous calcium sulphate. Melting point was recorded and spectral data were obtained to confirm the structure of compound.
**Table**

Effect of 4-(1-Pyrrolidinyl) Piperidine (I) and its derivatives on behaviour in open field test

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose mg/kg</th>
<th>Number of square crossed in 5 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>45.05±3.47</td>
</tr>
<tr>
<td>4-(1-Pyrrolidinyl)Piperidine (I)</td>
<td>100</td>
<td>84.83**±7.2</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>56.3±6.08</td>
</tr>
<tr>
<td>4-Pyrrolidin-1´-yl-1-[2-(2˝-nitro-phenyl)-2-oxo-ethyl]-piperidinium bromide (II)</td>
<td>100</td>
<td>90*±15.0</td>
</tr>
<tr>
<td>4-Pyrrolidin-1´-yl-1-[2-(3˝-nitro-phenyl)-2-oxo-ethyl]-piperidinium bromide (II)</td>
<td>100</td>
<td>30.61**±4.93</td>
</tr>
<tr>
<td>4-Pyrrolidin-1´-yl-1-[2-(2˝,4˝-dimethoxy-phenyl)-2-oxo-ethyl]-piperidinium bromide (IV)</td>
<td>100</td>
<td>48.36±18.5</td>
</tr>
<tr>
<td>4-Pyrrolidin-1´-yl-1-[2-(3˝,4˝-dihydroxy-phenyl)-2-oxo-ethyl]-piperidinium bromide (V)</td>
<td>100</td>
<td>72.41±9.0</td>
</tr>
</tbody>
</table>

n / group = 6

Significant differences by student 't' test: *p<0.05, **p<0.01 as compared to control.

![Graph](image)

Fig. 1: Showing open field activity of Compound I. Values are mean ± S.D. (n=6) 30 minutes after injection significant differences by student t-test *P<0.05 and **P<0.001.

4-Pyrrolidin-1´-yl-1-[2-(2˝-nitro-phenyl)-2-oxo-ethyl]-piperidinium bromide (II)

\[ ^1 \text{HNMR (MeOH, 500 MHz)} \delta: 7.55 \text{ (d, 1H, } J=7.87 \text{Hz, } H-3'). 7.35 \text{ (d, 1H, } J=8.11 \text{Hz, } H-6'), 7.10-7.06 \text{ (m, 1H, } H-5'), 7.01-6.98 \text{ (m, 1H, } H-4'), 4.86 \text{ (s, 2H, } H-7), 3.83 \text{ (d, 1H, } J=10.82 \text{Hz, } H-4), 3.01-2.74 \text{ (m, 8H, } H-2', H-5', H-2, H-6), 1.81-1.49 \text{ (m, 8H, } H-3', H-4', H-3, H-5); \]

**EIMS m/z:** 317 (M^+Br, C_{17}H_{23}N_3O_3), 154, 124, 110, 98, 80, 72, 57, 53; IR \[ \nu_{\text{max}} \text{ (KBr) cm}^{-1}: 3423, 3305, 2950, 1750, 1601, 1593, 1396, 1080, 812, 540; \]

UV \[ \lambda_{\text{max}} \text{ (MeOH) nm: 295, 201, 198}. \]

4-Pyrrolidin-1´-yl-1-[2-(3˝-nitro-phenyl)-2-oxo-ethyl]-piperidinium bromide (III)

\[ ^1 \text{HNMR (MeOD, 400 MHz)} \delta: 8.80 \text{ (s, 1H, } H-2'), 8.47-8.45 \text{ (m, 1H, } H-4'), 8.41-8.38 \text{ (m, 1H, } H-6'), 7.77 \text{ (t, 1H, } J=7.97 \text{Hz, } H-5'); 4.86 \text{ (s, 2H, } H-7), 4.06 \text{ (s, 1H, } H-4), 3.26-3.10 \text{ (m, }...
4H, H-2’, H-5’), 2.39-2.33 (t, 4H, J=10.32Hz, H-2, H-6), 2.14-2.10 (m, 4H, H-3’, H-4’), 1.96-1.80 (m, 4H, H-3, H-5); EIMS m/z: 317 (M-Br, C17H23N3O3), 300, 219, 203, 153, 124, 108, 70, 57; IR νmax (KBr) cm⁻¹: 3433, 3163, 2941, 2572, 1674, 1595, 1396, 1078, 717; UV λmax (MeOH) nm: 228, 204, 201.

*4-Pyrrolidin-1’-yl-1-[2-(2’,4’-dimethoxyphenyl)-2-oxo-ethyl]-piperidinium bromide (IV).* $^1$H NMR (MeOD, 500 MHz) δ: 8.00 (d, 1H, J=8.93Hz, H-6’), 6.68 (m, 2H, H-3’, H-5’), 4.84 (s, 6H, OCH3-2’, OCH3-4’), 4.68 (s, 2H, H-7), 3.90 (s, 1H, H-4), 3.30-3.17 (m, 4H, H-2’, H-5’), 3.14-3.11 (m, 4H, H-2, H-6), 2.45

Fig. 2: Showing open field activity of Compound II and III. Values are mean ± S.D. (n=6) 30 minutes after injection significant differences by student t-test*P<0.05 and **P<0.001.

Fig. 3: Showing open field activity of Compound IV and V. Values are mean ± S.D. (n=6) 30 minutes after injection significant differences by student t-test*P<0.05 and **P<0.001.
(d, 4H, J=13.3Hz, H-3', H-4'), 2.10, 2.02 (m, 4H, H-3, H-5); EIMS m/z: 332 (M'-HBr, C_{19}H_{28}N_{2}O_{3}), 154, 124, 110, 98, 82, 79, 56; IR \nu _{\text{max}} (KBr) cm\(^{-1}\): 3745, 3444, 2952, 1749, 1601, 1593, 1454, 1080, 812, 538; UV \lambda _{\text{max}} (MeOH) nm: 389, 293, 202.

4-Pyrrolidin-1'-yl-1-[2-(3''-dihydroxyphenyl)-2-oxo-ethyl]-piperidinium bromide (V). \(^1\)HNMR (MeOD, 500 MHz) \delta : 7.45-7.38 (m, 2H, H-2'', H-6''), 6.82 (d, 1H, J=8.08Hz, H-5''), 4.88 (s, 2H, OH-3, OH-4), 3.90 (s, 1H, H-4), 3.87 (s, 2H, H-7), 3.14-3.09 (m, 4H, H-2', H-5'), 2.32-2.11 (m, 4H, H-2, H-6), 2.05-1.99 (m, 4H, H-3, H-4), 1.86-1.81 (m, 4H, H-3, H-4), 1.86-1.81 (m, 4H, H-3, H-5); EIMS m/z: 304 (M'-HCl, C_{17}H_{24}N_{2}O_{3}), 167, 153, 124, 96, 70, 57; IR \nu _{\text{max}} (KBr) cm\(^{-1}\): 3679, 3361, 2933, 1749, 1656, 1512, 1394, 1278, 806, 621; UV \lambda _{\text{max}} (MeOH) nm: 279, 231, 206, 199.

**Pharmacology**

**Open Field Activity**

**Animals:**
Male Sprague-Dawley rats (locally bred) weighing 200-300 g purchased from Aga Khan University and Hospital. Animals were kept individually in plastic cages in the same environmental conditions with free access to water and standard rodent diet for about three days before experimentation. Rats were randomly assigned as control, test and standard groups taking six animals in each group.

**Drugs:**
Compounds were dissolved in water for injection and injected to the test animals intraperitoneally (i.p.) (100 mg/kg body weight). Water for injection was injected by the same route to the control animals.

**Method:**
The open field apparatus consisted of a square area 76 x 76 cm with walls 42 cm high. Floor of the apparatus was divided by lines into 25 equal squares. The rats were exposed to the open field after 30 minutes of receiving injection. The activity was scored as number of squares crossings with all four paws for 5 minutes (Haleem et al., 1994).

**RESULT AND DISCUSSION**
Table presented the behavioural study of parent compound 4-(1-Pyrrolidinyl)Piperidine (I) (100 mg/kg) and its derivatives (100mg/kg) respectively.

4-(1-Pyrrolidinyl)Piperidine (I) tested at 100mg/kg of body weight showed highly significant exploratory activity and locomotion in the open field test.

Animals given four derivatives (II, III, IV and V) when exposed to open field test for 5 minutes after 2 hours of administration (i.p.) showed variable results.

Compound 4-Pyrrolidin-1'-yl-1-[2-(2''-nitro-phenyl)-2-oxo-ethyl]-piperidinium bromide (II) showed highly significant activity by increasing motor and investigating activity when comparing with control.

4-Pyrrolidin-1'-yl-1-[2-(3''-nitrophenyl)-2-oxo-ethyl]-piperidinium bromide (III), significantly decreased the activity of locomotion and exploration. Both the compounds II and III are nitro derivatives of 4-(1-Pyrrolidinyl)Piperidine (I) with the only change in position of nitro group in phenyl ring and interestingly they showed reverse activity. The result of open field test of both the compounds revealed that by changing the position of nitro group in the phenyl ring the compound showed opposite activity i.e. ortho nitro derivatives increased the activity but when nitro group moved to meta position the activity significantly decreased in terms of motor and investigation activity.

4-Pyrrolidin-1'-yl-1-[2-(2''-dimethoxyphenyl)-2-oxo-ethyl]-piperidinium bromide (IV) and 4-Pyrrolidin-1'-yl-1-[2-(3'',4'')-
dihydroxy-phenyl)-2-oxo-ethyl]-piperidi-nium bromide (V) failed to change the exploratory and locomotion activity. Comparing all the derivatives only nitro compounds possessed activity while methoxy and hydroxy groups were unable to produce any change in behaviour in open field test when tested at the dose of 100mg/kg of body weight.

**CONCLUSION**

Results of the activity clearly demonstrate that presence of different functional groups and their position in the compounds are important. Further studies on these compounds are in progress to evaluate the effect on behaviour.

**REFERENCES**
