Synthesis and Biological screening of 4-(1-pyrrolidinyl) Piperidine Derivatives as Effective Analgesics

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Summary: A variety of 4(1-pyrrolidinyl) piperidine analogs **2-6** with variable substituents on phenyl ring of phenacyl moiety were synthesized and evaluated for their analgesic inhibitory potential by tail flick method revealed significant analgesic activity. The synthetic compounds exhibit analgesic inhibitory potential was ranging from significant to highly significant activity. Compounds were evaluated by thermal stimuli (tail immersion method) at the dose of 50 mg/kg of body weight. The compounds **2-5** showed significant and highly significant analgesic activity. Pethidine was used as reference drug. Same compounds tested at the dose of 75mg/kg of body weight showed toxicity.

The size of the substituent, electron donating or withdrawing affect of substituents as well as the position of substituent on phenyl ring affected the activity. These compounds could be considered to develop a new class of analgesics.

Key words: Piperidine, Pyrrolidine and Analgesic.

Introduction

The piperidine containing compounds possessed versatile activities like antibacterial [1] antifungal [2], anti-acetylcholinesterase [3], anti-inflammatory [4], histamine H1 antagonists [5], antidiabetic and for obesity activity [6]**.** It prompted us to synthesize new derivatives by incorporating different phenacyl halide and acetonaphthone containing moiety in 4-(1-pyrrolidinyl) piperidine. The presence of these groups is expected to impart potential CNS activities. A number of attempts were made to discover potent analgesics without undesirable effects in which piperidine and pyrrolidine moiety played important role [7, 8].

It was, therefore, proposed to synthesize some promising analgesic agents. These synthesized compounds were evaluated for analgesic activity. Some of the compounds were found to possess highly significant activity.

Scientists have designed tests for analgesics in animals and correlate their data with human clinical observations. These testing methods were widely used which showed a high correlation with human data reproducible, simple to run, and sufficiently sensitive to detect both weak and potent analgesics. Application of a thermal stimulus, usually to a rodent of sufficient intensity and duration, caused an escape attempt on the part of the animal within a reproducible period of time referred to as latency. Analgesic drugs extended the latency for the escape reaction. In tail flick procedure, the rodent's tail was exposed to a radiant heat or hot water stimulus; the response was tail withdrawal. These assays were quite good for narcotics and less dependable for narcotic antagonist analgesics and nornarcotics [9].

In the last few decades, there have been extensive researches on piperidine and pyrrolidine ring containing compounds to evaluate their potential as pharmacologically and biologically active agents. Piperidine and pyrrolidine was a widely used building block and chemical reagent in the synthesis of organic compounds [10-78].

Piperidine derivatives synthesis was reported by Saify *et. al*. [79-88]. We report in this paper the synthesis and analgesic activity of 4-(1-pyrrolidinyl piperidine) and its derivatives.

Result and Discussion

Piperidine and pyrrolidine nucleus containing compounds had tremendous potential to exhibit analgesic activity. 3-phenyl piperidine derivatives with significant analgesic activities were reported [11, 12]. The most active members had N-phenacyl or phenethyl substituents. New compounds were designed and synthesized by optimizing the 4-amino-piperidine template which might have a

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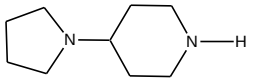
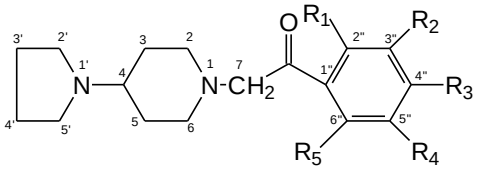
potential to be developed as a novel analgesic agent [13].

Considering these different derivatives of 4-(1-pyrrolidinyl) piperidine were synthesized. N-substituted phenacyl derivatives of 4(1-pyrrolidinyl) piperidine were obtained by N-alkylation compound with acetonaphthone and phenacyl halides having chloro, methoxy, flouro and bromo substitution on the aromatic ring using acetone as solvent. Stirring was done for 24 hours at room temperature and or at 52-54^{0}C. Reaction procedure was monitored by taking TLC of the reaction mixture after suitable intervals and visualized by U.V. light. Iodine vapours were also employed for the detection of spots. Most of the products appeared in good yield and were in solid form. 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). ^{1}H-NMR was performed in CD_3OD and D_2O at 300 MHz. Signals at δ 7-9 showed the presence of aromatic hydrogen while a sharp singlet at δ 3-6 confirmed the presence of CH_2 of the chain of the phenacyl moiety. Peaks at δ 1-3 showed the presence of remaining aliphatic hydrogen.



[**1**] 4 - (1 - Pyrrolidinyl) piperidine

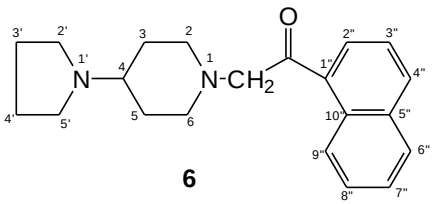


2) R3 = Cl R2, R3, R4, R5 = H X = Br

3) R3 = OCH3 R2, R3, R4, R5 = H X = B

4) R3 = Br R2, R3, R4, R5 = H X = Br

5) R3 = F R2, R3, R4, R5 = H X = Br



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All the newly synthesized derivatives of 4-(1-pyrrolidinyl) piperidine **1** and its derivatives **2-6** were screened for their analgesic activity. The results of analgesic activities are displayed in Table-1**.**

The parent compound 4-(1-pyrrolidinyl) piperidine **1** investigated for analgesic activity by tail immersion method was found devoid of any activity, but its derivatives **2-5** showed analgesia of significant and highly significant levels [89].

Compound **2** showed analgesia with delayed onset of action after 120 mins of drug administration. This effect increased and compound showed highly significant activity after 150 mins. This activity persisted for 30 mins and then activity disappeared completely. Analgesia produced by this compound was comparable with pethidine at 90 and 150 mins and showed greater TFLD value at 150 mins. Compounds **3** and **4** showed highly significant ($p<0.01$) analgesia after 30 mins which persisted for 30 mins and disappeared completely.

Flouro-phenacyl (para-flouro) derivative 1-[2-(4′′-floro-phenyl)-2-oxo-ethyl]-4-pyrrolidin-1′-yl-piperidinium bromide (**5**) tested at the dose of 50mg/kg, was found active with short onset of action and showed analgesia only after 30 mins. This significant analgesia became highly significant at 60 mins. This highly significant activity dropped to significant activity at 90 mins and then vanished completely. This compound exhibited greater analgesic activity in comparison with pethidine with higher TFLD values at 30, 60, 90, 120, 150 and 180 mins.

Among the synthesized analogues, except compound **6,** all the compounds showed analgesic activity. Compound **5** had the long duration of action, while **3-5** had short duration of action. All compounds **2-5** except **6** exhibited highly significant activity (graph **1-7**).

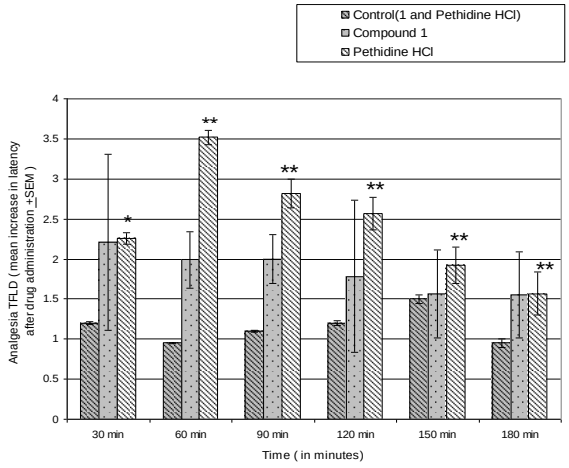
SAR studies of all compounds revealed that most of the analogues of 4-(1-pyrrolidinyl) piperidine **1** were found with analgesic activity. All these compounds had para substitution at phenyl ring. A minor change in functional group at para position of phenyl ring was affecting the activity in terms of onset, level and duration of action.

Derivatives of 4-(1-pyrrolidinyl) piperidine with good results could be placed among the compounds which had potential to become good analgesic agents. Some of the compounds could be selected for further studies regarding analgesic activity because of their significant results.

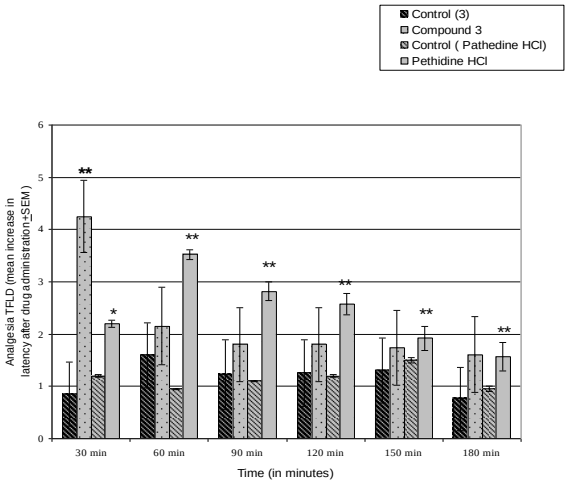
Table-1: Analgesia produced by compounds 1-6 and pethidine HCl (standard drug) in mice against thermal stimuli (tail immersion method).

Compound No/ Name	Dose : mg / Kg	Mean increase in tail flick latency after drug administration (TFLD) + SEM (s) Treatment : i.p					
		30 min	60 min	90 min	120 min	150 min	180 min
Control (1) and pathedine HCl	----	1.2+ 0.02	0.95+0.01	1.1 + 0.01	1.2+0.03	1.5+ 0.05	0.95+ 0.05
1	50	2.21+ 1.1	1.99+0.35	2.0 + 0.30	1.78+0.95	1.561+ 0.55	1.55+ 0.54
Contol(2)	----	1.59+ 0.53	1.43+0.53	1.45 + 0.53	1.71 + 0.55	1.87+ 0.54	1.70+ 0.46
2	50	1.57+ 0.64	1.98+ 0.65	2.287+ 0.65	3.35+ 0.45*	4.50+ 0.45**	2.507+ 0.61
Control (3)	----	0.85+ 0.61	1.6+0.62	1.23 + 0.66	1.26+0.64	1.31+ 0.62	0.76+ 0.61
3	50	4.25+ 0.69**	2.15+ 0.74	1.80+ 0.71	1.80+ 0.71	1.74+ 0.72	1.607+ 0.72
Control (4)	----	1.631+ 0.52	1.75+ 0.54	1.92+ 0.54	1.27+ 0.53	1.36+ 0.53	1.45+ 0.52
4	50	6.31+ 0.61**	2.632+ 0.61	1.97+ 0.61	1.81+ 0.61	1.62+ 0.65	1.43+ 0.65
Control (5)	----	1.34+0.73	0.58+0.77	0.87+0.70	0.62+0.726	0.79+1.69	0.79+0.69
5	50	4.67+ 0.98*	4.34+ 0.98**	4.13+ 0.98*	2.303+ 0.98	2.03+ 0.98	2.05+ 0.98
Control (6)	----	0.90+0.79	0.79+0.79	0.81+0.79	0.82+0.79	0.83+0.79	0.77+0.8
6	50	1.3+ 0.69	1.56+ 0.69	1.2+0.70	1.12+ 0.70	1.03+ 0.71	1.02+ 0.71
Pethidine HCl	----	2.26+ 0.07*	3.52+ 0.09**	2.82+ 0.18**	2.57+ 0.20**	1.92+0.23**	1.57+ .27**

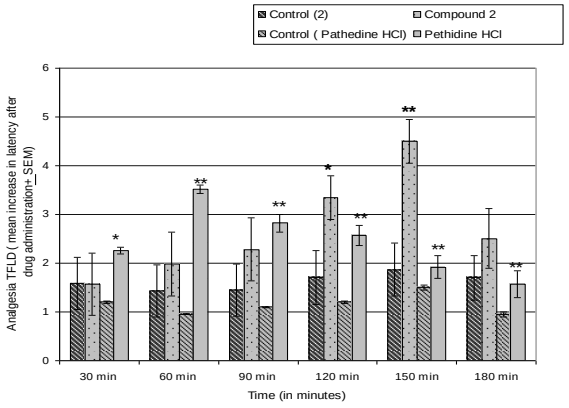
Significant differences by student "t" test *p<0.05, **p<0.01as compared to control n = 5
* = significant
** = highly significant



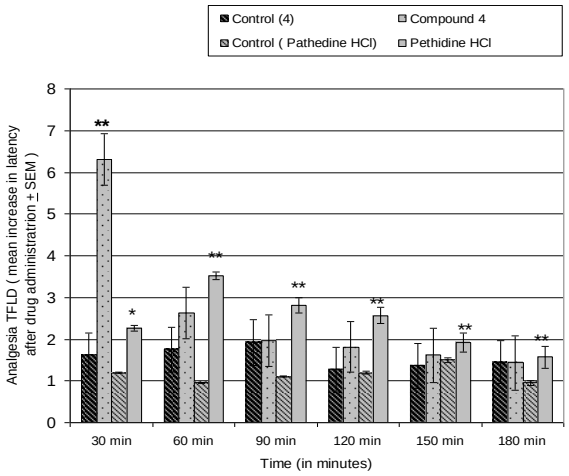
Graph-1: Analgesic effects of 4-(1-pyrrolidinyl) piperidine (**1**) and pethidine HCl (standard drug).



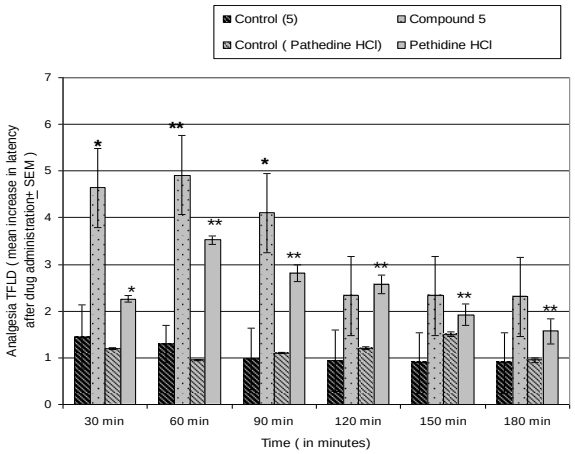
Graph 3: Analgesic effects of compound **3** and pethidine HCl (standard drug).



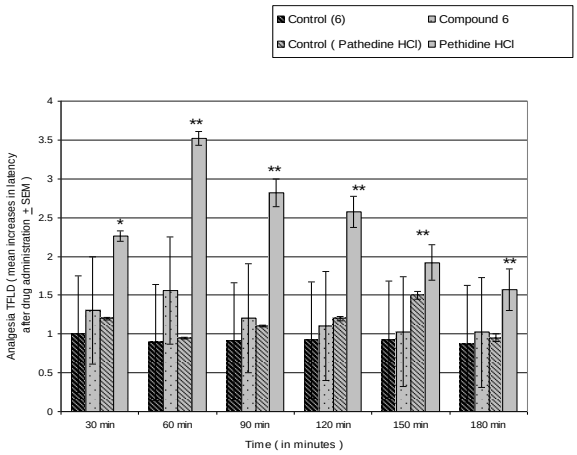
Graph-2: Analgesic effects of compound **2** and pethidine HCl (standard drug).



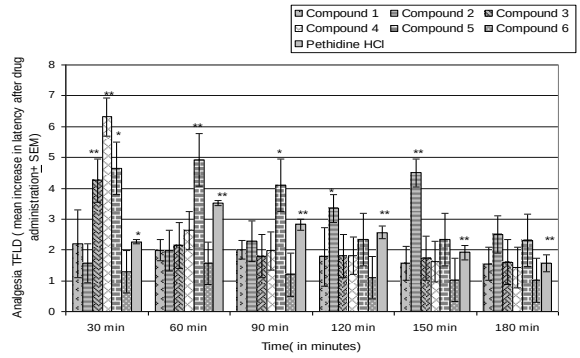
Graph-4: Analgesic effects of Compound 4 and pethidine HCl (standard drug).



Graph-5: Analgesic effects of compound **5** and pethidine HCl (standard drug).



Graph 6: Analgesic effects of compound **6** and pethidine HCl (standard drug).



Graph-**7**: Analgesic effects of compound **1-6** and pethidine HCl (standard drug).

Experimental

General Procedure

4-(1-Pyrrolidinyl) piperidine (0.7713g, 0.5mmole), transferred in a conical flask, 15-20ml acetone was added to dissolve it successively. 2-bromo-4'-chloroacetophenone (1.1675g, 0.5mmole) dissolved separately in 15-20 ml acetone and poured in the same flask gradually. When both solutions were mixed, precipitate appeared spontaneously. The mixture was stirred vigorously for a further 2 hours at room temperature. The process of reaction was monitored through thin layer chromatography. The crude solid product was filtered and washed twice with acetone. The product thus obtained was purified through recrystallization by using warm ethanol and ether. The pure compound was dried in desicator over anhydrous calcium sulphate. Melting point was recorded and spectral data were obtained to confirm the structure of compound.

Reagents, Chemicals and Instruments

Reagents were purchased from Aldrich Chemical Company. All solvents were reagents grade. Reactions were monitored by thin layer chromatography using pre-coated silica gel, GF-254 and analytical thin layer chromatography was performed on silica gel (Kieselgel 60, 254, E. Merck) precoated 0.25 mm plates. Visualization was accomplished with ultraviolet light at 254 and 365 nm UVP UVLS-26 Series (Cambridge). Iodine vapours were also employed for the detection of spots. All melting points were recorded on Gallenkamp melting point apparatus and were uncorrected. Solid calcium sulphate from E. Merck was used for drying reaction product after workup. Ultraviolet spectra were recorded in methanol on a Hitachi U-3200 spectrophotometer. Infra Red spectra were measured on a Shimadzu IR 460 spectrophotometer using KBR disc. Mass spectra were determined on Varian Massen spectrometer MAT 311A spectrometer. Nuclear magnetic resonance spectra were recorded in D_2O and CD_3OD on AVANCE AV 300 spectrometer operating at 300MHz.

Animals

Male Albino mice weighing between 20-30g, purchased from Aga Khan Medical University and Hospital, Karachi, used in the study. Groups of six 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.

Drugs

Test Compounds were dissolved in water for injection. Pethidine (50 mg/kg) was used as a

standard drug. Test compounds were injected intraperitoneally to the test groups at the doses of 50mg/kg body weight. Pethidine HCl was also administered in the same dose to the standard group. Control groups receiving only vehicle always ran parallel to the compound treated groups.

Assay for Analgesic Activity

The compounds were tested for their antinociceptive effect against thermal stimuli (tail flick method) according to the method of Distasi *et al***.** [90].

Each mouse was held in a suitable restrainer with whole tail extending out. An area of the tail 2-3cm in length was marked and immersed in a water bath thermostatically maintained at 51°C. Baseline latency (reaction time) was obtained with three measurements, after each measurement a cutoff time of 180 seconds was used to prevent tissue damage. The mean of these three measurements was the pre-drug latency time.

Pethidine HCl was also administered at the same dose to the standard group. Control groups were supplied with only vehicle. Readings were taken at 30, 60, 90, 120, 150 and 180 mins after administration of compounds, mean of the three readings was considered as the post drug reaction time. Tail flick latency difference (TFLD) or mean increase in latency after compound administration was used to measure the analgesia produced by test and standard drugs. TFLD was calculated as:

Analgesia TFLD = (Post-drug TFL – Pre-drug TFL)

Statistical Analysis

Analgesic activity was expressed as TFLD±SEM in terms of seconds. Statistical analysis was performed using student t-test and values were considered significant or highly significant when $P<0.05$ or $P<0.01$ respectively. All statistical procedures were performed according to the method of Alcaraz and Jimenez [91].

1-[2-(4''-chloro-phenyl)-2-oxo-ethyl]- 4-Pyrrolidin-1'-yl-Piperidinium; bromide [**2**]: State: White crystalline powder, Yield= 80.5%, molecular formula: $C_{17}H_{24}ON_2ClBr$, molecular formula weight, 387.0742 a.m.u, solubility= methanol, ethanol, melting point= 237 ± 3°C, ^{1}H-NMR (D_20, 300 MHz) δ 7.75-7.78 (d, 2H, *J*= 8.8*Hz*, H-2″, H-6″), 7.44-7.41 (d, 2H, *J* = 8.29 Hz, H-3″ H-5″), 4.60 (s, 2H, H-7), 3.5-3.3 (m,, 1H, Hz, H-4), 2.97-2.88 (t, 4H, *J* =13.3Hz, H-2′, H-5′), 2.30-2.26 (d, 4H, , *J*=13.5Hz , H-2, H-6), 1.9-1.7 (m, 8H, H-3′, H-4′, H-3, H-5), EIMS *m/z*: 306 (M^+-HBr, $C_{17}H_{23}N_2OCl$), 292, 251, 223,208,191,180,167,158, 153,139,124, 110, 83, 70,55, HR-EIMS: 306.1499(M^+-HBr $C_{17}H_{23}N_2OCl$), Cal. 306.1527, IR ν_{max} (KBr) cm^{-1}: 3422, 2942, 2570,1651, 1592,1088,1012, 775,525, UV λ_{max} (MeOH) nm: 239,205.

1-[2-(4''-methoxy-phenyl)-2-oxo-ethyl]-4-pyrrolidin-1'-yl-piperidinium; bromide [**3**]: White crystalline powder, Yield = 81.5%, Molecular formula: $C_{18}H_{27}N_2O_2Br$, Molecular weight: 383.3233 a.m.u. solubility = methanol, ethanol, melting point= 258±2°C, ^{1}H-NMR (D_2O, 300 MHz) δ 7.74-7.71(d, 2H, *J*=11.5Hz, H-2″, H-6″), 6.86-6.83 (d, 2H, *J*= 8.96 Hz, H-3″ H-5″), 4.60(s, 3H, OCH_3-4″), 3.82 (s, 2H, H-7), 3.69 (s, 1H, H-4), 3.19-2.90 (m, 8H, H-2′, H-5 , H-2, H-6), 2.15-1.86 (*m*, 4H, H-3′, H-4′), 1.63-1.55 (m, 4H, H-3, H-5), EIMS *m/z*: 302.4 (M^+-HBr, $C_{18}H_{26}N_2O$), 167.2, 153, 135, 124, 121, 110, 98, 96,55, HR-EIMS: 302.1986 (M^+-HBr $C_{18}H_{26}N_2O$) *Calcd*. 302.1994, IR υ_{max} (KBr) cm^{-1}: 3733, 3506, 2923, 2603, 2360, 1676, 1602, 1510, 1454, 1265, 1226, 1016, 837, 572, 418, UV λ_{max} (MeOH) nm, 389, 276, 218, 201.

Synthesis of 1-[2-(4''-bromo-phenyl)-2-oxo-ethyl]-4-Pyrrolidin-1′-yl-piperidinium; bromide **[4]:** White crystalline powder, yield = 71.6%, molecular formula: $C_{17}H_{24}N_2OBr_2$ molecular weight = 432.1933 a.m.u, solubility = methanol, ethanol, melting point: decomposed at 259±2°C, EIMS *m/z:* 352 (M^+-Br, $C_{17}H_{24}N_2OBr$), 281, 254, 226, 198, 183, 167, 153, 139, 124, 11098, 85, 80, 70, 55, HR-EIMS: 352.2523 (M^+-Br, $C_{17}H_{24}N_2OBr$) Calcd. 352.2893, IR ν_{max} (KBr) cm^{-1} 3456, 2923, 2672, 1692, 1585,1454,1394,1224, 972, 576, 472, UV λ_{max} (MeOH) nm = 389, 256, 280.^{1}H-NMR D_2O, 300 MHz) δ 7.67-7.64(d, 2H, *J* = 8.58Hz, H-2″, H-6″), 7.56-7.53 (d, 2H, *J* = 0.02 *Hz*, H-3″, H-5″), 4.60 (s, 2H, H-7), 4.19 (d, 1H, *J* = 4.2 *Hz*, H-4), 3.44-2.91 (m, 8H, H-2′, H-5′, H-2, H-6), 1.88-1.71(m, 8H, H-3′, H-4′, H-3, H-5),

*Synthesis of 1-[2-(4''-floro-phenyl)-2-oxo-ethyl]-4-Pyrrolidin-1'-yl-piperidinium bromide***[5]:** White crystalline powder, Yield: 77.2%, molecular formula: $C_{17}H_{24}N_2OFBr$, Molecular weight, 371.2877 a.m.u, solubility: methanol, ethanol, melting point =145±3°C, EIMS *m/z*: 290 (M^+-HBr, $C_{17}H_{23}N_2OF$), 221, 192, 167, 153, 124, 110, 98, 83, 70, 55, HR-EIMS, 290.1794 (M^+-HBr, $C_{17}H_{23}N_2OF$) Calculated 290.1794, IR ν_{max} (KBr) cm^{-1}:3425, 2959, 2798, 2573, 2479, 1651, 1606, 1508, 1227, 1039, 855, 493, UV λ_{max} (MeOH) nm = 227, 202. ^{1}H-NMR (CD_3OD,

300 MHz) δ 7.85-7.80(t, 2H, *J*= 5.4Hz, H″-2″, H-6″),7.10-7.04 (t, 2H, *J* = 8.8*Hz*, H-3″, H-5″), 4.60 (s, 2H, H-7), 3.91-3.88 (d, 1H, *J*= 8.4*Hz*, H-4), 3.31-2.94(m, 8H, H-2′, H-5′ H-2, H-6), 2.18-1.83 (m, 4H, H-3′, H-4′), 1.65-1.57 (m, 4H, H-3, H-5),

Synthesis of 1-[2-naphthalen-2-yl-2-oxo-ethyl]-4-pyrrolidin-1′-yl-piperidinium; bromide **[6]:** White crystalline powder, Yield: 73.2%, molecular formula: $C_{21}H_{27}N_2OBr$, molecular weight: 403.3560 a.m.u, solubility: methanol, ethanol melting point: 288±2°C, ^{1}H-NMR (CD_3OD, 300 MHz) δ 8.66 (s, 1H, H-10″), 8.08-7.94 (m, 4H, H-2″, H-8″, H-3″, H-5″), 7.69-7.66(t,1H, *J* = 3.7Hz, H-6″),7.63-7.60(t,1H, *J*=4.4Hz, H-7″), 4.7(s 2H, H-7), 3.63-3.4(m, 1H, H-4), 3.34-3.05 (m, 8H, H-2′, H-5, H-2, H-6), 2.40-2.38 (d, 4H, *J* =12.8Hz, H-3′, H-4′), 2.16-2.12 (d, 4H, *J*=17.6Hz, H-3, H-5), EIMS *m/z:*323(M^+-Br, $C_{21}H_{27}N_2O$), 322, 293, 253, 224, 167, 153, 141, 127, 110, 96, 80, 70, 55, HR-EIMS: 322.2045 (M^+-HBr, $C_{21}H_{26}N_2O$), Calcd. 322.2015, IR ν_{max} (KBr) cm^{-1}: 3423, 2953, 2925, 2611, 2482, 1691, 1626, 1453, 1394, 1277, 118, 1068, 933, 815, 752, 474, UV λ_{max} (MeOH) nm, 333, 294, 248, 208.

Conclusion

Enzyme played a pivotal role in modifying the naturally occurring substance. Attempts were being made in our lab to enhance the activity of existing bioactive compounds.

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