Behavioral and neurochemical profile of some novel phenacyl based isonipecotamide derivatives

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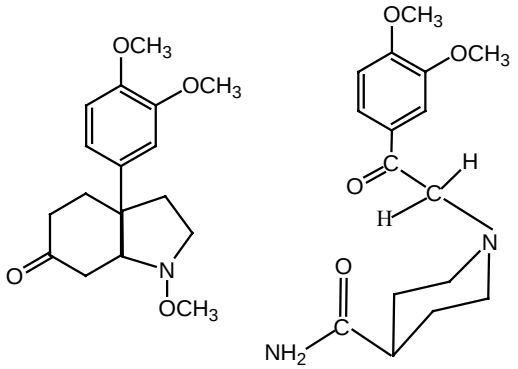
Abstract: Study of natural products led to the development of new molecules of potential biological activity. Piperidine nucleus constitutes one of the components of various alkaloids and drugs. During the course of our project regarding the synthesis of derivatives of piperidine carboxamide to study the effects of these compounds as anti-depressive agents, some of the compounds exhibited significant effects at all three doses, through open field activity thus establishing a direct relationship between dose and locomotion. Moreover, these compounds have also shown the decreased level of 5-HT alone with increased level of dopamine as an indication of their antagonism towards 5-HT receptor.

Keywords: Neurochemical Profile, neurotransmitters, biogenic amines, catecholamine, indolamine, antagonism, depression.

INTRODUCTION

Depression is a major disease affecting 13-20% of the population world wide (Licinio and Wong 1999). The mood elevating properties of *Seeletium fortuosum* have been attributed to mesembrine **(A)**, an alkaloid with potent selective serotonin (5-HT) reuptake activity (Van Vyke and Gericke 2000) and one of the synthesized compounds **(B)** resembles this alkaloid.



(A) **(B)**

Arecoline is a component of *Areca catechu*, widely used as a chewing nut with a reputation of having anti-tumor, anti-oxidant and anti-depressant activities and also having acetylcholine-estrase (ACE) inhibitory activity (Gilani *et al*., 2006). As arecoline is a component of beetle nut and itself a member of piperidine ring system, it is proposed that N-substituted derivatives of piperidine carboxamide would exhibit antidepressant activity via serotonin re-uptake mechanism on the basis of SAR.

The biogenic amines (i.e. 5-hydroxytrptamine) control different behaviors related to central nervous system in man and animals. These amines act as neurotransmitters and behave as buffers or mediators for passing the nerve impulses across the synaptic cleft. Deficiency of these chemicals at the receptor site may cause different behavioral disorders (Schildkraut, 1978) such as depression, extreme excitation, sleep exhilaration, awake-fullness, etc. Out of these disorders "depression" is very common and reported as "common cold" (Wells *et al*., 1989). Most of the drugs which are synthetic in nature are employed for the treatment of these disorders either as antipsyschotic or serotonergic. In this context piperidine based analogs have been developed and were found effective in the treatments of various neurological disorders (Barlocco *et al*., 2001; Michael *et al*., 1999).

Considerable works have been carried out on piperidine derivatives as neuroactive agents from the past decade and were found to be potent as 5-HT_1A and 5-HT_2A receptors antagonists (Carr *et al*., 2000; Lavielle *et al*., 2001; Luca *et al*, 2005; Annemarie *et al*., 2009). These substances were evaluated in treating various conditions of psychoses and schizophrenia. In exploring CNS active agents, alkyl and aryl derivatives of piperidine have been synthesized and proved to be effective in the treatment of psychiatric illness and other related disorders (Shipe *et al*., 2008, Dutta *et al*., 1998; Jordi *et al*., 1996). However with reference to Parkinson and Alzheimer's diseases, it was reported (Choi *et al*., 2000, Wagner *et al*., 2004; Cole and Vasser, 2008) that piperidine compounds exhibited a ten-fold increase in dopamine DA receptor reuptake inhibition at the DAT (dopamine transporter).

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Fedouloff and coworkers (1998) reported 5-HT_4 receptor antagonist from isonipecotamide. The work was followed by other scientists to develop piperidine derivatives useful in the treatment of several CNS disorders (Hossnor *et al*., 1998; Mathias *et al*., 2010; Vinaya *et al*., 2010)**.**

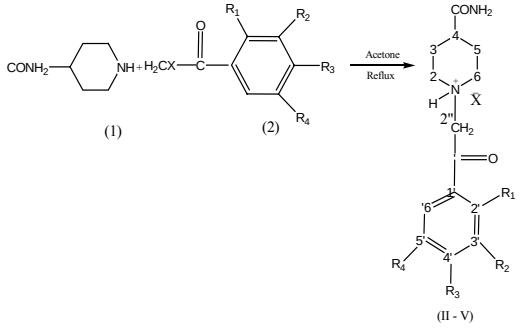
In search of structure-activity relationship a series of novel nipecotamide and isonipecotamide derivatives have been synthesized by our group (Saify, *et al*., 1999; Saify *et al*., 2005; Shamim *et al*., 2006; Mushtaq *et al*, 2007) and were studied for different pharmacological activities. Most of these compounds exhibited pronounced results. In view of the above noted significant results, our group carried out the synthesis of some more new phenacyl derivatives of isonipecotamide. These newly synthesized compounds were subjected for their behavioral effects as well as for neurochemical estimations.

MATERIALS AND METHODS

The chemicals and solvents used were purchased from Sigma Co., (Aldrich, London) and Merck. The melting points of the compounds were recorded in glass capillary tubes on Gallenkamp melting point apparatuses and were uncorrected. Structures of these newly synthesized compounds were elucidated by different spectral studies.

^{1}H NMR spectra were taken in $CDCl_3$ and DMSO-d_6 on Broker AM 300 and Broker AM 400 NMR spectrometers operating at 300 and 400 MHz. The chemicals shift were recorded in PPM (δ) and coupling constant (*J*) were in Hz. Mass spectra were measured on Finnegan MAT 112 11/34 computer system.

General method for synthesis of compounds (II – V)



(II) $R^2 = R^3 = OH$, $R^1 = R^4 = R^5 = H$, X = Cl
(III) $R^2 = NO_2$, $R^1 = R^3 = R^4 = R^5 = H$, X = Br
(IV) $R^3 = NO_2$, $R^1 = R^2 = R^4 = R^5 = H$, X = Br
(V) $R^3 = Br$, $R^1 = R^2 = R^4 = R^5 = H$, X = Br

Isonipecotamide (1) and corresponding substituted phenacyl halides (2) were dissolved in acetone in equimolar quantities separately in conical flask heated on water bath and then mixed together in a round bottom flask. The reaction mixtures were stirred on magnetic stirrer for about 4 to 5 hours. Precipitates of products appeared on mixing the reactants and in some cases after refluxing on water bath and setting aside at room temperature. The completion of reaction was monitored by TLC using $CHCl_3$-MeOH as solvent system in varying ratios. The resulting products were filtered and washed with warm acetone to remove the unreacted starting materials. The products thus obtained were recrystallized from methanol at least three times to ensure purity and to improve color and shape of the crystals.

1-[2-(3,4-dihydroxyphenyl)-2-oxoethyl)]**-4-carboxamide piperidinium chloride (II)**

Gray crystalline powder (40%) were obtained from methanol with m.p. 222-224°C. The spectra of the condensed product (II) displayed the characteristic absorption band at λ_{max}.(KBr)cm^{-1} 3384($CONH_2$), 2931(OH, broad), 1668(C=O), 1429(CH_2). Absorptions at 1587, 1102 and 756-634 confirmed the aromatic system while its ultraviolet spectrum exhibited the maxima at λ_{max} 309, 351.2 and 389.6 nm. The EIMS of (II) showed M+ 279 which gave the mass measurement of the molecular formula $C_{14}H_{19}N_2O_4$. The 1HNMR spectrum of (II) showed signals at δ: 1.51 (m, 1H, *H*-4), 1.61 (m,4H, *H*-3,5), 2.03 (m, 4H, *H*-2, 6), 3.62(s 2H, H-2□), 6.79 (d, *J*=8.14 Hz, 1H, *H*-5′), 7.38 (dd, *J*, 1.93Hz, 5.3 Hz, 1H, *H*-6′), 7.42(d, *J*=2.72 Hz, 1H, *H*-2′), 7.21 (s,1H, H-O*H*), 6.72 (s, 1H, H-O*H*), indicating the aromatic ring. Thus on the basis of the above spectroscopic data, the condensed product (II) was formulated as 1-[2-(3,4-dihydroxy-phenyl)-2-oxo-ethyl]-4-carboxamide piperidinium chloride.

1-[2-(3-nitrophenyl)-2-oxo-ethyl]-4-carboxamide piperidinium bromide (III)

Off-white shiny crystals obtained from aqueous methanol (40%), m.p. 243-244°C.The spectra of the condensed product (III) showed the characteristic absorption band at λ_{max}.(KBr)$cm^{-1,}$ 3370($CONH_2$), 3180, 2920(C-H), 1700 (C=C), 1650(C=O). Aromatic system is indicated by the peaks at 1520, 1345, 820, 730 and 670. While it's ultraviolet spectrum exhibited the maxima at λ_{max} 191.8, 246, and 354.2 nm. The EIMS of (III) showed M+ 292 which gave the mass measurement of the molecular formula $C_{14}H_{18}N_3O_4$. The 1HNMR spectrum of (III) showed signals at δ: 2.49 (m, 1H, *H*-4), 2.54 (m,4H, *H*-3,5), 3.15 (m, 4H, *H*-2, 6), 5.19(s 2H, H-2□), 7.90-7.96 (dd, *J*=7.3 Hz, 10.8 Hz, 1H, *H*-5′), 8.57 (d, *J*, 2.8 Hz, 1H, *H*-6′), 8.42(d, *J*=9.2 Hz, 1H, *H*-4′), 8.6 (d, J=1.2 Hz, 1*H*, H-2′), Thus on the basis of the above spectroscopic data the condensed product (III) was formulated 1-[2-(3-nitro-phenyl)-2-oxo-ethyl]-4-carboxamide piperidinium bromide.

1-[(2-(4-nitrophenyl)-2-oxoethyl)-4-carboxamide]-piperidinium bromide (IV)

Mustard crystalline powder obtained from aqueous methanol (45%), m.p. 210-211°C. IR, λ_{max} (KBr)$cm^{-1,}$ 3386($CONH_2$), 3268, 3199(C-H), 2941(C=C), 1519

(C=O). Aromatic system is indicated by the peaks at 1345, 853, 730 and 610. While it's ultraviolet spectrum exhibited the maxima at λ_{max} 203 and 262 nm. The EIMS of (IV) showed M+ 292 which gave the mass measurement of the molecular formula $C_{14}H_{18}N_3O_4$. The 1HNMR spectrum of (III) showed signals at δ: 2.21 (m, 1H, *H*-4), 2.02 (m,2H, *H*-5), 2.96 (m, 2H, *H*-3), 3.42 (m, 2H, *H*-2), 3.30 (m, 2H, *H*-6), 6.57 (s 2H, H-2□), 8.28 (d, *J*=8.69 Hz, 2H, *H*-2′,6′), 8.37 (d, *J*, 8.69 Hz, 2H, *H*-3′,5′). Thus on the basis of the above spectroscopic data the condensed product (IV) was formulated as 1-[2-(4-nitro-phenyl)-2-oxo-ethyl]-4-carboxamide piperidinium bromide.

1-[(2-(4-bromophenyl)-2-oxoethyl)]-4-carboxamide-piperidinium bromide (V)

Creamy crystalline powder obtained from aqueous methanol (72%), m.p. 246°C. IR, λ_{max} (KBr)$cm^{-1,}$ 3414($CONH_2$), 2949(C-H), 2941(C=C), 1691 (C=O) 585 (Br). Aromatic system is indicated by the peaks at 1229, 968, 809 and 649. While it's ultraviolet spectrum exhibited the maxima at λ_{max} 203 and 262 nm. The EIMS of (V) showed M+ 326 which gave the mass measurement of the molecular formula $C_{14}H_{19}N_2O_2Br$ The 1HNMR spectrum of (V) showed signals at δ: 2.13 (m, 2H, *H*-5), 2.10 (m, 1H, *H*-4), 2.74 (m, 2H, *H*-3), 2.95 (m, 2H, *H*-6), 3.27 (m, 2H, *H*-2), 6.51 (s 2H, H-2□), 7.25 (d, *J*=9.02 Hz, 2H, *H*-3′,5′), 8.22 (d, *J*, 9.04 Hz, 2H, *H*-2′,6′). Thus on the basis of the above spectroscopic data the condensed product (V) was formulated as 1-[2-(4-bromo-phenyl)-2-oxo-ethyl]-4-carboxamide piperidinium bromide.

Pharmacological Activity

Male wistar rats (locally bred) weighing 200-300 g. purchased from HEJ Research Institute of Chemistry, University of Karachi. Animals were kept individually in plastic cages in the same environmental condition with free access to water and standard rodent diet for three days before experimentation. Rats were randomly assigned as control and standard groups taking seven animals in each group. The compounds (I, II-V) dissolved in DMSO/water were injected to the test animals intraperitonially at the dose of 50, 100 and 200mg/kg body weight. The vehicle/distilled water was injected to the control animals by the same route. Behavioral data was satisfactorily analysed by *student't test* while neurochemical data was subjected to 1-way ANOVA. Posthoc comparison was done by Newman Keuls statistics.

Open field Activity

The open field apparatus consisted of a square area of 76 × 76 cm with walls 42 cm high. The floor of the apparatus was divided by lines into 25 equal squares. The rats were exposed to the novel environment i.e. the open field after 30 minutes of receiving injection. The activity was scored as number of squares crossing with all four paws for five minutes and latency to move (Haleem *et al*., 1994).

Neurochemical estimations

Extraction Procedure for Brain:

Extraction media was prepared by mixing 3.4 ml perchloric acid, 0.1 g sodium metabisulfite, 0.001g ethylene-diamine-tetra-acectate (EDTA) and 0.01g cysteine. Volume was made upto 1-lit with distilled water, following decapitation 1 hour after the saline or test compounds injection. Brains were taken out within 1 minute then dipped in ice-cooled saline. The brain samples were kept at -70°C until analysis.

Brain samples were homogenized in 5 volumes of extraction medium and centrifuged at 60-70 RPM for 15 minutes. The clear supernatant was decanted into eppendorf tubes and centrifuged again for 10 minutes. Clear supernatant thus obtained was taken in another set of tubes for further study.

The brain extracts consisting of 5-hydroxytryptamine (5-HT), 5-hydroxy indole acetic acid (5-H1AA), homo-vanillic acid (HVA) and dopamine (DA) were analysed by HPLC-EC method at 0.8 V electrode potential. A 5U,-shim-pack clc ODS, 4.6mm 1D, 15 cm separation column was used for the analysis. The solvent system employed was methanol (14%) acetyl sodium sulphate (0.015%) and EDTA (0.005%) in 0.1M phosphate buffer (pH=2.9) Samples, from vehicle (saline and DMSO) and test compounds from injected animals were run one after another by using a 20 µl. loop injection.

STATISTICAL ANALYSIS

Behavioral data was statistically analysed by student 't' test. Neurochemical data was subjected to 1-way ANOVA. Posthoc comparison was done by Newman keuls statistic. P values >0.05 were considered non-significant.

RESULTS

Table 1 and fig. 1 showed the dose-response study of the parent compound, isonipecotamide (I) and the derivatives II, III, IV and V at three different doses, 50, 100 and 200mg/kg body weight on behavior by open field test.on rats. The compound I had significantly affected the latency of animals to move in the given doses except at the dose of 100mg/kg. However, the overall effect of the parent compound on open field activity was negligible.

The results of the brain extracts of experimental animals showing concentration of catecholamines and indolamines were given in tables 2-6.

DISCUSSION

Animal models of depression provide a new approach to study depression in animals that are both pharma-

cologically as well as behaviorally valid (Porsolt *et al*., 1978**).** It had been reported that a variety of clinically effective antidepressants such as imipramine, nialamide and viloxazine reduced the immobility time of animals and reduction in immobility reflects their antidepressant properties (Porsolt *et al*., 1977). Behavioral studies such as latency to move and number of squares crossed play important role for the evaluation of antidepressant drugs (Porsolt *et al*., 1991). It has been suggested that the observed immobility signifies behavioral despair resembling a state of mental depression (Willner, 1984)**.** It is well established that the locomotor test plays an important role in evaluation of antidepressant agents as locomotor compounds can show positive effects in the antidepressants tests (Porsolt *et al*., 1993, Willner, 1991)**.**

In the light of above findings, a precise method was established to determine the effect of test compounds on the mobility and open field activity of the synthesized compounds in rats. The open field activity was monitored

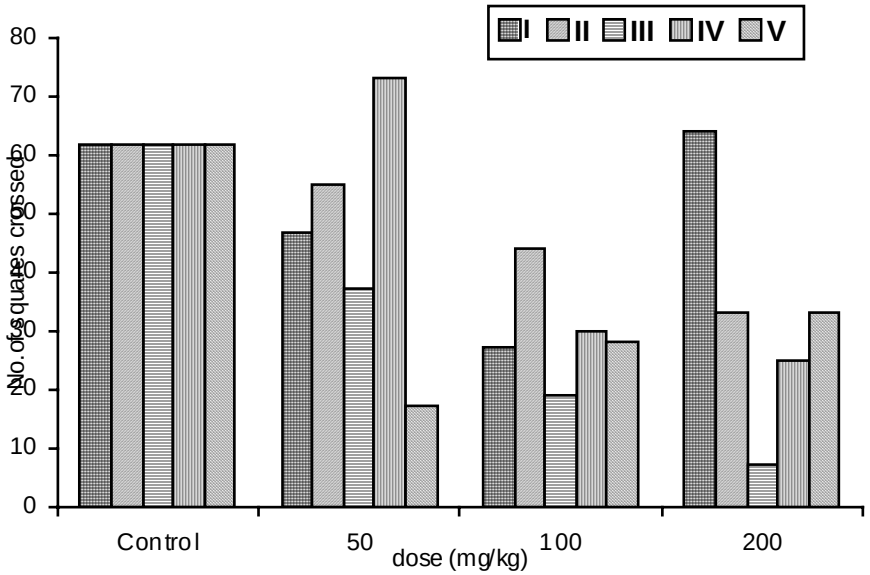


Fig. 1: Dose-response study of isonipecotamide derivatives (I, II, III, IV and V) through open-field activity in rats
Values are mean ± S.D. (n=7) 30 minutes after injection.
Significant differences by student 't' test: **P<0.01, as compared to control.

Table 1: Dose-response study of (I), (II), (III), (IV) and (V) through open field activity in rats

Treatment	Dose (mg/kg)	No. of square crossed	t-test	Latency to move (sec.)	t-test
Control	–	67.68±26.6		2.25±0.96	
I	50	46.7±17.3	1.3	1.25±0.5	1.04
	100	27.3±1.3	3.13	*1±0	6.25 P<0.05
	200	64.3±19.2	2.7	1.25±0.5	2.0
II	50	*55±2	8.5 P<0.05	1±0	0
	100	44±2.5	2.3	1±0	0
	200	33±1.52	3.2	1±0	1.7
III	50	17±1.7	5.9	1±0.33**	15.2 P<0.01
	100	28±1.8**	11.9 P<0.01	1±0.35**	14.3 P<0.01
	200	33±1.7**	15.1 P<0.01	1.9±0.29**	14.1 P<0.01
IV	50	37±1.2**	20.3 P<0.01	4±0.28*	10.7 P<0.05
	100	19±1.7	4.7	1±0	0
	200	7±1.85*	2.2	1±0	0
V	50	73.25±2.7**	23.1 P<0.01	*4±0.37	8.1 P<0.05
	100	30±2.1*	9.17 P<0.05	1±0	0
	200	25±1.26	4.75	1±0	0

Values are mean±S.D. (n=7), 30 minutes after injection.
Significant differences by student t-test when *P<0.05 and highly significant when **P<0.01 as compared to control rats.

Table 2: Dose-response study of isonipecotamide (I) on catecholamine and indoleamine levels (ng/g) in rat brain, 1 hour after the injection

Neurotrans-mitters	Control	Dose (mg/kg body weight)			ANOVA (Diff. 3,12)	
		50	100	200	F	P
DA	230.68 ±33.7	174.68*±27.6	217.72±40.4	210.31±45.4	1.97	P<0.05
DOPAC	150.8 ±42.9	192.93±43.5	198.34±42.1	130.23±38.50	2.49	–
HVA	85.0 ±15.0	150.0**±40.0	191.0**±26.0	180.0**±45.0	7.9	P<0.01
5-HT	189.43 ±39.0	156.01±23.0	147.17±14.0	161.66±29.3	1.72	–
5-HIAA	270.26 ±31.9	345.20±102.5	284.14±69.4	282.57±31.2	1.05	P<0.05

Difference significant by Newman-Keuls test were **P<0.01, *P<0.05 from control following one-way ANOVA. Values are mean±S.D. (n=7).

Table 3: Dose-response study of 1-[(2-(3,4-dihydroxyphyenyl)-2-oxoethyl)]-4-carboxamide-piperidinium chloride (II) on catecholamine and indoleamine levels (ng/g) in rat brain, 1 hour after the injection

Neurotrans-mitters	Control	Dose (mg/kg body weight)			ANOVA (Diff. 3,12)	
		50	100	200	F	P
DA	77.37±25.0	112.65*±15.0	206.42**±83.0	332.17**±100.3	13.5	P<0.01
DOPAC	43.30±16.3	49.26±20.4	97.35**±20.9	129.42**±19.6	20.2	P<0.01 P<0.05
HVA	28.18±9.0	36.89*±5.0	72.9*±21.3	343.7**±95.8	18.9	P<0.01 P<0.05
5-HT	2.05±0.10	2.30±0.22	3.03±0.14	44.76**±15.3	8.7	P<0.05
5-HIAA	115.2±16.6	62.98**±21.2	128.3*±26.7	196.57*±24.7	11.0	P<0.05

Difference significant by Newman-Keuls test were **P<0.01, *P<0.05 from control following one-way ANOVA. Values are mean±S.D. (n=7).

Table 4: Dose-response study of 1-[(2-(3,4-nitrophyenyl)-2-oxoethyl)-4-carboxamide] piperidinium chloride (III) on catecholamine and indoleamine levels (ng/g) in rat brain, 1 hour after the injection

Neurotrans-mitters	Control	Dose (mg/kg body weight)			ANOVA (Diff. 3,12)	
		50	100	200	F	P
DA	351.73±62.0	400.65*±36.3	425.24*±45.0	1030.46**±150.0	2.2	P<0.05
DOPAC	49.06±8.0	45.88±21.2	69.21*±16.1	156.14±15.4	3.1	P<0.05
HVA	73.34±20.2	43.71*±7.55	55.81*±10.3	89.76*±5.4	2.0	–
5-HT	40.13±4.5	38.58±2.73	32.91±9.99	105.13**±15.4	5.9	P<0.05
5-HIAA	129.00±165	121.30±13.6	133.32±6.6	238.55**±10.2	9.7	P<0.01

Difference significant by Newman-Keuls test were **P<0.01, *P<0.05 from control following one-way ANOVA. Values are mean±S.D. (n=7).

in a novel environment for 5 minutes. Statistical analysis was done by the method of student 't' test.

It was evident from the results shown in table 1 that the compound II at the dose of 50 mg/kg had significantly affected the number of squares crossing (P<0.05). It was interesting to note that compound (IV) i.e. *m*-nitro phenacyl derivative showed highly significant effects at the doses of 50 mg (P<0.01) on square crossing and in latency to move (P<0.05) but at the higher dose i.e., 200 mg/kg, effect was non significant. It was seen that the effects on latency to move was observed at a dose of 50 mg/kg and at the dose of 100 mg/kg there was no change in the test animals as compared to control. Almost similar behavior was observed by the compound (V) at a dose of 50 mg/kg on both the parameters. At the dose of 100 mg/kg, the effects became less pronounced as compared to that of 50mg/kg dose while the effect was vanished at the dose of 200 mg/kg Effects produced by the compound III were highly significant, indicated by squares crossing and significant effects on latency to move at the doses of 100 and 200 mg/kg.

By comparing the compounds (III) and (IV) *(meta* and *para* nitro respectively), it can be seen that *m*-intro compound exhibited open field activity at the dose of 100 mg/kg while *p*-derivative was devoid of this activity at the same dose but interestingly the same compound

Table 5: Dose-response study of 1-[(2-(4-nitrophyenyl)-2-oxoethyl)-4-carboxamide piperidinium bromide (IV) on catecholamine and indoleamine levels (ng/g) in rat brain, 1 hour after the injection

Neurotrans-mitters	Control	Dose (mg/kg body weight)			ANOVA (Diff. 3,12)	
		50	100	200	F	P
DA	88.1±2.46	284.26**±6.6	384.92**±18.3	447.22**±42.3	19	P<0.01
DOPAC	8.69±1.64	65.95*±6.1	128.41**±11.5	69.17**±4.16	24.8	P<0.01
HVA	31.9±3.4	53.42*±4.5	307.61**±6.44	241.9**±10.4	101.8	P<0.01
5-HT	95.60±9.6	16.74*±1.0	4.85**±0.95	3.85**±0.66	43.8	P<0.05
5-HIAA	180.62±3.61	185.29±10.39	210.15**±9.5	162.76±3.27	14.3	P<0.01

Difference significant by Newman-Keuls test were **P<0.01, *P<0.05 from control following one-way ANOVA. Values are mean±S.D. (n=7).

Table 6: Dose-response study of 1-[4-bromophenyl)-2-oxoethyl)-4-carboxamide-piperidinium bromide (V) on catecholamine and indoleamine levels (ng/g) in rat brain, 1 hour after the injection

Neurotrans-mitters	Control	Dose (mg/kg body weight)			ANOVA (Diff. 3,12)	
		50	100	200	F	P
DA	84.0±11.3	94.43±13.0	221.03±33.3	438.46*±119.0	2.3	P<0.01
DOPAC	10.25±4.5	34.37*±12.1	33.33*±14.0	149.24**±10.2	23.2	P<0.01 P<0.05
HVA	67.5±25.3	178.84*±30.5	158.16*±40.2	330.48**±82.0	34.0	P<0.01
5-HT	100.08±16.22	86.88±25.3	2.04**±0.50	71.57*±30.2	19.8	P<0.01 P<0.05
5-HIAA	177.08±34.9	189.26±25.7	175.26±37.0	333.81*±76.5	8.7	P<0.05

Difference significant by Newman-Keuls test were **P<0.01, *P<0.05 from control following one-way ANOVA. Values are mean±S.D. (n=7)

demonstrated highly significant activity at the dose of 50mg/kg. From these results we can interpret that the *p*-position of $–NO_2$ group was responsible to give the open field activity at the lowest dose as compared to the *m*-derivative.

Considering the effects produced by the above mentioned compounds, the possible mechanisms involved in the behavioral change of animals can be discussed in the following manner.

Behavioral studies in animal models reveal that a decrease of catecholamine and 5HT (serotonin) turnover is responsible for the depressant and anti-anxiety effects of most of the compounds ((Baldessarini, 1996). Considerable attention had been given on the role of dopamine (DA) and 5-Hydroxytryptamine (5HT) in certain behavioral states (Inoue, 1993). Findings showed that the drugs that reduce central monoamine transmissions produced sedation and hypo activity.

Neuroleptic and antidepressant drugs affect motor activity and hypermotility was produced by amphetamine and metamphetamine **(**Bradbury *et al*., 1983, Baldessarini, 1986**).** Nearly, all the neuroleptic agents used in psychiatry diminish spontaneous motor activity in animals as well as in human beings (Baldessarini, 1996). DA mainly controls the locomotion because it had been observed previously that, neuroleptics which are antagonists to DA receptors, decrease locomotion in animals (Shamoona *et al.,* 1999).

Latency to move and number of square crossed observed by compound I injected animals at different doses showed no significant change in locomotion while the compounds synthesized from this precursor, had shown significant effects in most of the animals at different doses and different locomotory effects were observed. Among all the four derivatives tested for open-field activity, compound III had shown highly significant effects at all three test doses establishing a direct relationship between dose and locomotion. Locomotion was increasing with dose as compared to control. Therefore, it can be suggested that these compounds were perhaps enhancing the release of DA from dopaminergic fibers.

The primary mechanism underlying behavioral effects including locomotion stimulation was thought to be due to its ability to bind to dopamine receptors (Amir *et al*., 2000**).** In agreement with the dopamine hypothesis, piperidine derivatives had been found to inhibit dopamine reuptake and motor effects. It can also be suggested that besides the inhibition of dopamine reuptake, other mechanisms might also play a modulatory role in increasing the locomotion in animals.

Taking this fact into consideration that clinically employed phenacyl piperidine analogs might induce neurotoxic action on dopamine neurons in brain (Ross *et al*., 1986)**,** these compounds were also studied for their effects on the release of neurochemical transmitters along with the parent compound. Considerable progress had been made in identifying these chemical transmitters, in large part because of the microelectrode technique and immunoassay, immunohisto-chemical staining and radiochemical techniques as well as electron microscopy, spectrofluorometry and high performance liquid chromatography (HPLC) techniques (William *et al*., 1995)**.** In order to investigate the possible role of these piperidine derivatives in the release of neurochemical transmitters, HPLC technique has been employed.

From the tables 2-6 showing the release of neurotransmitters, it was observed that starting material. i.e., isonipecota-mide upon administration, decreased dopamine (DA) significantly at the dose of 50mg/kg and less significantly at dose of 100mg and 200/mg as compared to control. The level of DOPAC and HVA were however increased but effects were only significant in case of HVA at all test doses as compared to control. The level of 5-HT was decreased at the dose of 50mg and 100mg/kg and then it increased at the dose of 200 mg/kg while concentration of 5-H1AA was increased initially at the dose of 50mg/kg and then decreased at the dose of 100mg and 200mg/kg. However, the effects were not significant as compared to control.

On comparing the levels of DA for 3, 4-dihydroxy derivative (II), nitro compounds (III and IV) and bromo derivative (V), there was significant increase in the concentration of DA and its metabolites DOPAC & HVA. A marked decrease in the level of 5-HT could be observed at the dose of 100mg/kg. The 5-HT and 5-HIAA levels for the compound II were increased in the dose dependent manner but at the dose of 50mg/kg, the levels were less than that of the control. The derivatives (III IV & V) showed remarkable decrease in 5-HT levels mostly at the doses of 50mg and 100mg/kg and 5-H1AA concentration of these compounds was significantly increased. Hence it is evident that by changing the halide group at *para* position, activity was greatly changed or it can be said the nitro compound displayed more pronounced effect as compared to the bromo compound. In case of compound (III), (IV) and (V) the level of DA and its metabolites DOPA and HVA had increased. The enhancement of DOPA and HVA might be due to increase turnover of DA which might be due to increased activity of catecholamine synthesizing enzyme, tyrosine-hydroxylase (Sved and Fernstrom, 1981).

Significant increase in the level of 5-HIAA as in the case of compounds (III), (IV) and (V) compared to control group suggesting that these compounds might increase 5-HT turnover in brains (Friedman *et al*, 1972; Pinna *et al*., 2009). Hence, the decreased level of (5HT) along with increase level of (DA) by compounds (III)–(IV) were indicative of their antagonistic behaviour towards 5-HT receptor (Canton *et al*., 1994)**.** These findings suggested that these compounds might act as antischizophrenic agents because of decrease 5-HT function. It is further added that the low level of noradrenaline, 5-HT and dopamine in the central and peripheral nervous system are the causes of depression (Cooper *et al*., 1992, Schildkraut, 1978). It can further be extrapolated to mean that there is under activity of these neurochemical systems indicating their destruction by enzymes MAO and COMT (Zygmumt *et al*., 1987).

Comparing the newly synthesized compounds of isonipecotamide with standard drugs, it can be suggested that these compounds have significant CNS activity and might be used in various CNS disorders. It is further added that these compounds produced CNS activity in a dose dependent manner. However, a thorough study is required to establish a complete neurochemical profile of these compounds.

CONCLUSION

The present study suggests that these synthesized compounds (II-V) might be used in various CNS disorders as drugs in psychotic patients because of their effects on the enhancement of both serotonin and catecholamine metabolism. However intensive research is needed to establish their mechanism of actions.

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