Behavioral studies of the derivatives of alkyl piperidine

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ABSTRACT

Derivatives of alkyl piperidine were synthesized and were evaluated for potential anti-depressant and anti-psychotic activities in albino mice. The derivatives **Ia-If** and **IIa-IIf**, containing nitro, fluoro, chloro, bromo and methoxy groups possessed significant activity in open field test when tested at the dose of 50 mg/kg body weight. It is also evident that the number of nitro groups, their positions in the phenyl ring and the other functional groups has relationships among them to impart certain activity to the molecules to which they are attached. The structures of the synthesized compounds were confirmed through different spectral techniques EI-MS, ^{1}HNMR, IR and UV.

Key words: Alkyl piperidine derivatives and behavioral activity.

INTRODUCTION

Any change in the levels of neurotransmitters influence the behavior like locomotion, attitude, gripping, exploration etc. Various approaches such as latency to move and number of square crossed are the methods of choice to study locomotion and exploratory behavior [1-8] .

Open field activity test method is a precise established method to investigate the behavioral changes in mice [9]. This method has been used for the measurement of behavioral activity of small animals for the determination of behavior [10].

Substituted alkyl piperidine has been a rich source of numerous pharmacologically active drug substances since several decades [11-15]. Due to their often biological activities, optically active piperidine alkaloids containing a stereogenic carbon atom at the 2-position are an important group of natural products and they have been the target of a number of synthetic strategies [16-20].

It was also reported that series of N-phenyl piperidine analogs were active and very potent versus wild-type HIV-1 and a broad range of NNRTI-resistant mutant viruses [21].

Therefore a huge amount of efforts have been devoted to their construction by synthetic chemists all over the world [22-34].

Different patterns of behavioral disorders include anxiety, confusion, depression, agitation and insomnia which are due to the deficiency or increase in biogenic amines or impair neurotransmission. Any change in the levels of neurotransmitters influence the behavior like locomotion, attitude, gripping, exploration etc. Various approaches such as latency to move and number of square crossed are the methods of choice to study locomotion and exploratory behavior.

EXPERIMENTAL

MATERIALS

White Albino mice of either sex (locally bred) weighing between 20-30 gm, purchased from Agha Khan Medical University and Hospital, Karachi were employed for behavioral activity. All solvents such as DMSO and ethyl alcohol were of analytical grade. Disposable insulin syringes were used for intra peritoneal route.

INSTRUMENTS

Behavioral studies were performed in an open field apparatus mentioned below in the Research Institute of Pharmaceutical Sciences, Faculty of Pharmacy, and University of Karachi, Pakistan.

Determination of Behavioral Studies

Open field activity method

The open field apparatus composed of a square area 76×76 cm with walls 42 cm high. Floor of the apparatus was divided by lines into 25 equal squares. The mice 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 [35]

RESULTS AND DISCUSSIONS

Different patterns of behavioral disorders include anxiety, confusion, depression, agitation and insomnia, which are due to the deficiency or increase in biogenic amines or impair neurotransmission [36] [37].

Open field activity test method is a precise established method to investigate the behavioral changes in mice [9]. This method has been used for the measurement of behavioral activity of small animals for the determination of behavior [38] [31][32] [39] and[13] [40] and [41].

Mixed strains of albino mice administering six derivatives **(Ia, Ib, Ic, Id, Ie** and **If)** of compound **I** and six derivatives (**IIa, IIb, IIc, IId, IIe** and **IIf**) of compound **II** when exposed to open field test for 5 minutes after 30 minutes of administration through intra peritoneal route showed variable results.

The results of parent molecules (compound **I** and compound **II**) are shown in tables 1, and 2, respectively while tables 1a to 1f and tables 2a to 2f presented the results of their derivatives (50 mg/kg) with corresponding figures.

Piperidine-2-methanol (**I**) and piperidine-2-ethanol (**II**) were tested at the dose of 50mg/kg body weight showed significant exploratory behavior and locomotion in the open field test (**figure 1** and **figure 2**).

Derivatives of parent **I** showed variable responses (**figure 1a** and **figure 1b**). Compounds **Ia** and **Ib** exhibited the similar response. It means introduction of nitro and bromo groups at *meta* position in the phenyl ring attenuated the activity initially present in the parent compound.

Compounds **Ic (figure 1c)**, **Id** (**figure 1d)**, **Ie (figure 1e)** and **If** (**figure 1f**) represented highly significant activity and also increased the activity of locomotion and exploration as compared to control.

The results obtained among the derivatives of piperidine-2-methanol, interestingly it is evident that the attachment of nitro, bromo, flouro and chloro groups at position 4 (*para* position) in the phenyl ring made the derivatives successful to produce exploration and locomotory behavior significantly. They have potentiated the effects of their parent compounds.

Compounds **IIa** (**figure 2a**), **IIc** (**figure 2c**) and **IId** (**figure 2d**) were less effective to change the exploratory and locomotion activity as compared to control.

Compounds **IIb** (**figure 2b**), **IIe** (**figure 2e**) and **IIf** (**figure 2f**) showed very significant increase in the activity of locomotion and exploration as far as the control is concerned.

Comparing the derivatives of parent **I**, its *para* nitro derivative exhibited pronounced activity whereas, results of the derivatives of parent **II** having nitro groups at different positions revealed variable results. The derivative having nitro group at *meta* and *para* positions (**IIc** and **IId** respectively), there is less significant activity while the derivative having nitro group at *ortho* position (**IIe**) attained highly significant activity. The results revealed that position of nitro group in the phenyl ring may have definite effect on the activity. Compound having two nitro groups at *meta* positions (**IIf**) was responsible to cause more pronounced change in the behavior.

Comparing all the derivatives containing nitro groups **Ia, IIc, IId, IIe** and **IIf,** fluoro compounds **Ic** and **IIb**,

chloro compound **If**, bromo compounds **Ib** and **IIa** and methoxy compound **Ie**, compounds **Ic** and **IIb** (*para* flouro) possessed significant activity in open field test when tested at the dose of 50 mg/kg body weight. It is also evident that the compound having two nitro groups at *meta* positions **IIf** exhibited highly significant activity.

CONCLUSION

It was concluded that the derivatives evaluated for behavioral effects through open field test method, showed hyperactivity in mice which would be useful in the elevation of mood and can act as neuroleptics.

Table – 1

Effect of piperidine 2-methanol (I) on behavior in open field test

Compound	Dose mg / kg	Number of Square crossed in 5 minutes
Control	-	48.00 ± 11.02
I	50	79.00 * ± 17.04

n / groups = 10

Significant different by student's t- test: *p< 0.05, **p<0.01 as compared to control

Table – 1a

Effect of piperidine derivative (Ia) on behavior in open field test

Compound	Dose mg / kg	Number of Square crossed in 5 minutes
Control	-	85.20 ± 1.21
Ia	50	73.30 ** ± 4.26

n / groups = 10

Significant different by student's t- test: *p< 0.05, **p<0.01 as compared to control

Table – 1b

Effect of piperidine derivative (Ib) on behavior in open field test

Compound	Dose mg / kg	Number of Square crossed in 5 minutes
Control	-	80.00 ± 1.23
Ib	50	65.50 *** ± 7.47

n / groups = 10

Significant different by student's t- test: *p< 0.05, **p<0.01 as compared to control

Table – 1c

Effect of piperidine derivative (Ic) on behavior in open field test

Compound	Dose mg / kg	Number of Square crossed in 5 minutes
Control	-	38.00 ± 9.21
Ic	50	73.80 ** ± 12.81

n / groups = 10

Significant different by student's t- test: *p< 0.05, **p<0.01 as compared to control

Table – 1d

Effect of piperidine derivative (Id) on behavior in open field test

Compound	Dose mg / kg	Number of Square crossed in 5 minutes
Control	-	75.00 ± 1.41
Id	50	96.04 *** ± 6.67

n / groups = 10

Significant different by student's t- test: *p< 0.05, **p<0.01 as compared to control

Table – 1e

Effect of piperidine derivative (Ie) on behavior in open field test

Compound	Dose mg / kg	Number of Square crossed in 5 minutes
Control	-	35.00 ± 4.96
Ie	50	53.04 * ± 9.86

n / groups = 10

Significant different by student's t- test: *p< 0.05, **p<0.01 as compared to control

Table – 1f

Effect of piperidine derivative (If) on behavior in open field test

Compound	Dose mg / kg	Number of Square crossed in 5 minutes
Control	-	66.00 ± 4.96
If	50	84.30 ** ± 6.40

n / groups = 10

Significant different by student's t- test: *p< 0.05, **p<0.01 as compared to control

Table – 2

Effect of piperidine 2-ethanol (II) on behavior in open field test

Compound	Dose mg / kg	Number of Square crossed in 5 minutes
Control	-	75.00 ± 3.54
II	50	50.60 * ± 13.26

n / groups = 10

Significant different by student's t- test: *p< 0.05, **p<0.01 as compared to control

Table – 2a

Effect of piperidine derivative (IIa) on behavior in open field test

Compound	Dose mg / kg	Number of Square

		crossed in 5 minutes
Control	-	85.00 ± 2.12
IIa	50	62.40 * ± 12.29

n / groups = 10

Significant different by student's t- test: *p< 0.05, **p<0.01 as compared to control

Table – 2b

Effect of piperidine derivative (IIb) on behavior in open field test

Compound	Dose mg / kg	Number of Square crossed in 5 minutes
Control	-	66.00 ± 4.25
IIb	50	89.90 * ± 13.02

n / groups = 10

Significant different by student's t- test: *p< 0.05, **p<0.01 as compared to control

Table – 2c

Effect of piperidine derivative (IIc) on behavior in open field test

Compound	Dose mg / kg	Number of Square crossed in 5 minutes
Control	-	63.00 ± 3.54
IIc	50	44.50 *** ± 5.64

n / groups = 10

Significant different by student's t- test: *p< 0.05, **p<0.01 as compared to control

Table – 2d

Effect of piperidine derivatives (IId) on behavior in open field test

Compound	Dose mg / kg	Number of Square crossed in 5 minutes
Control	-	57.00 ± 12.76
IId	50	36.60 *** ± 6.35

n / groups = 10

Significant different by student's t- test: *p< 0.05, **p<0.01 as compared to control

Table – 2e

Effect of piperidine derivative (IIe) on behavior in open field test

Compound	Dose mg / kg	Number of Square crossed in 5 minutes
Control	-	52.00 ± 4.96
IIe	50	76.40 ** ± 8.55

n / groups = 10

Significant different by student's t- test: *p< 0.05, **p<0.01 as compared to control

Table – 2f

Effect of piperidine derivative (IIf) on behavior in open field test

Compound	Dose mg / kg	Number of Square crossed in 5 minutes
Control	-	32.00 ± 4.25
IIf	50	74.60 *** ± 13.02

n / groups = 10

Significant different by student's t- test: *p< 0.05, **p<0.01 as compared to control

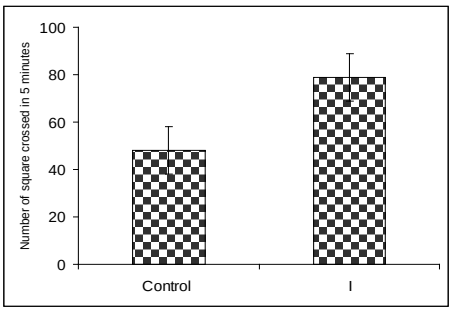

Fig 1: Showing open field activity of Compound I. Values are mean ± S. E. M. (n=10) 30 minutes after injection. Significant differences by student's t-test *p <0.05 and **p< 0.001.

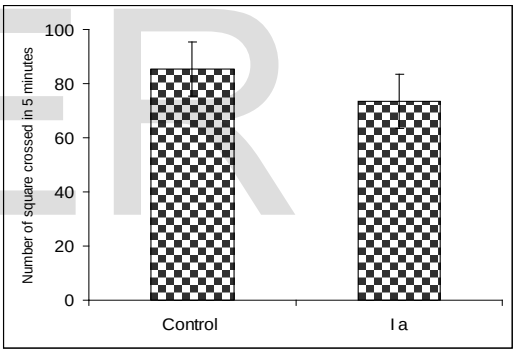

Fig 1a: Showing open field activity of Compound Ia. Values are mean ± S. E. M. (n=10) 30 minutes after injection. Signification differences by student's t-test *p <0.05 and **p< 0.001.

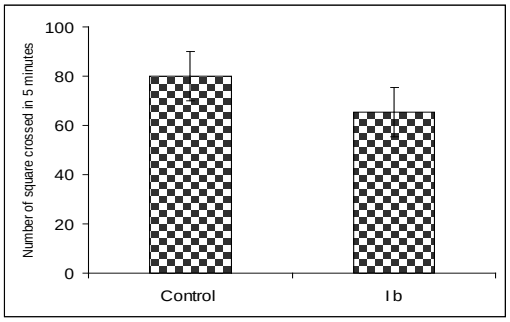

Fig 1b: Showing open field activity of Compound Ib. Values are mean ± S. E. M (n=10) 30 minutes after injection. Significant differences by student's t-test *p <0.05 and **p< 0.001.

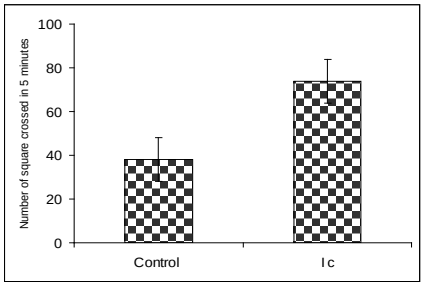

Fig 1c: Showing open field activity of Compound Ic. Values are mean ± S. E. M. (n=10) 30 minutes after injection. Significant differences by student's t-test *p <0.05 and **p<0.001.

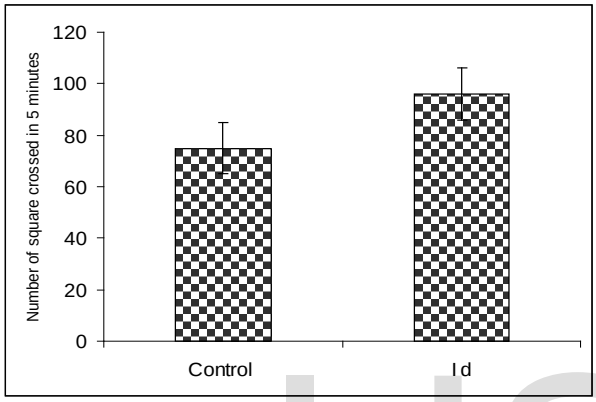

Fig 1d: Showing open field activity of Compound Id. Values is mean ± S. E. M. (n =10) 30 minutes after injection. Significant differences by student's t-test *p <0.05 and **p<0.001.

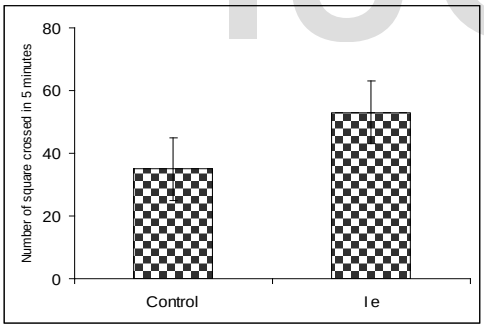

Fig 1e: Showing open field activity of Compound Ie. Values are mean ± S. E. M. (n=10) 30 minutes after injection signification differences by student's t-test *p <0.05 and **p< 0.001.

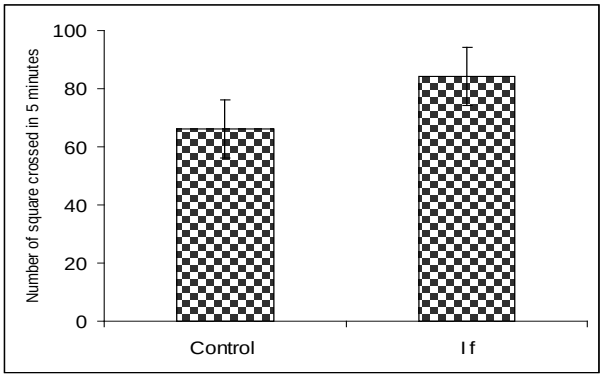

Fig1f: Showing open field activity of Compound If. Values are mean ± S. E. M. (n=10) 30 minutes after injection. Significant differences by student's t-test *p <0.05 and **p <0.001.

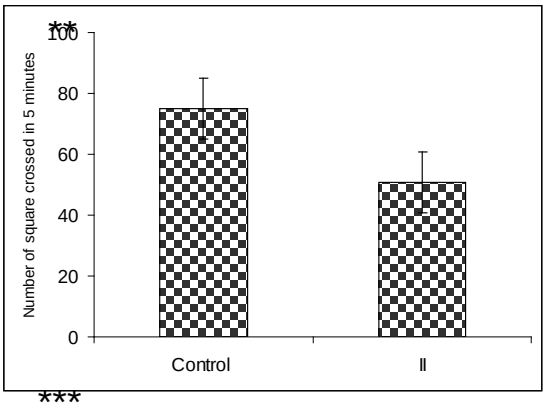

Fig 2: Showing open field activity of Compound II. Values are mean ± S. E. M. (n = 10) 30 minutes after injection signification differences by student's t-test *p <0.05 and **p< 0.001.

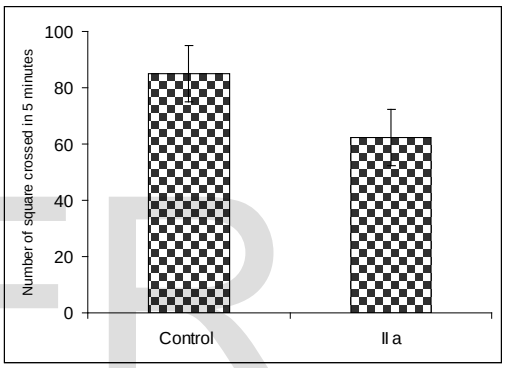

Fig 2a: Showing open field activity of Compound II a. Values are mean ± S. E. M. (n = 10) 30 minutes after injection signification differences by student's t-test * p <0.05 and **p< 0.001.

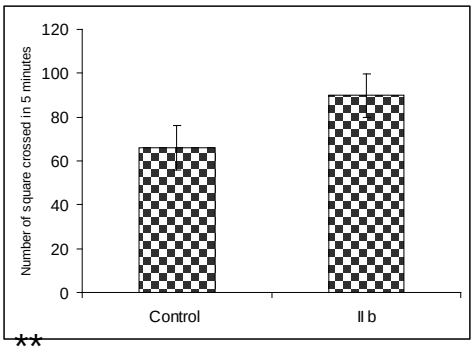

Fig 2b: Showing open field activity of Compound II b. Values are mean ± S. E. M. (n = 10) 30 minutes after injection signification differences by student's t-test *p <0.05 and **p< 0.001.

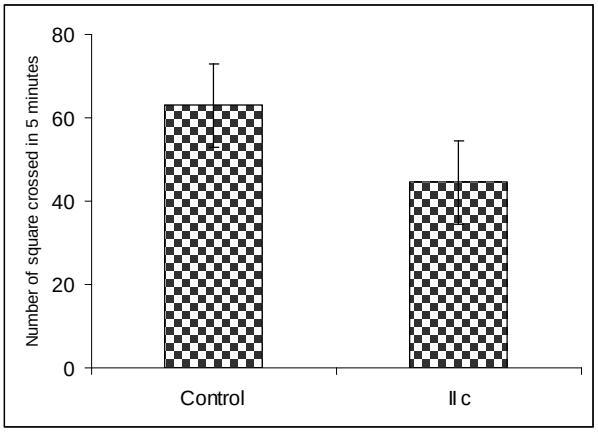

Fig 2c: Showing open field activity of Compound II c. Values are mean ± S. E. M. (n = 10) 30 minutes after injection signification differences by student's t-test *p <0.05 and **p< 0.001.

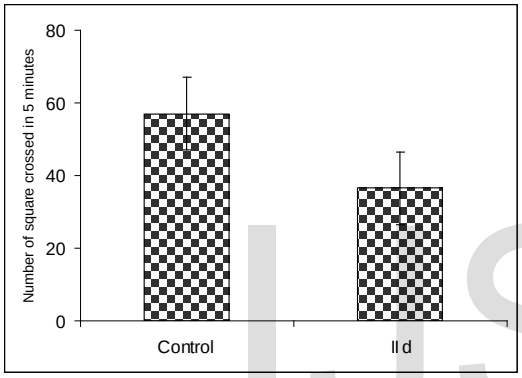

Fig 2d: Showing open field activity of Compound II d. Values are mean ± S. E. M. (n = 10) 30 minutes after injection signification differences by student's t-test * p <0.05 and ** p< 0.001.

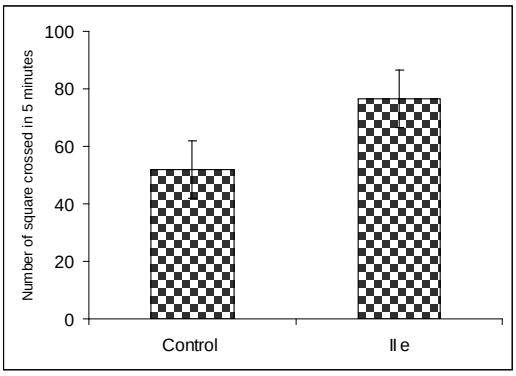

Fig 2e: Showing open field activity of Compound II e. Values are mean ± S. E. M. (n = 10) 30 minutes after injection signification differences by student's t-test *p <0.05 and **p< 0.001.

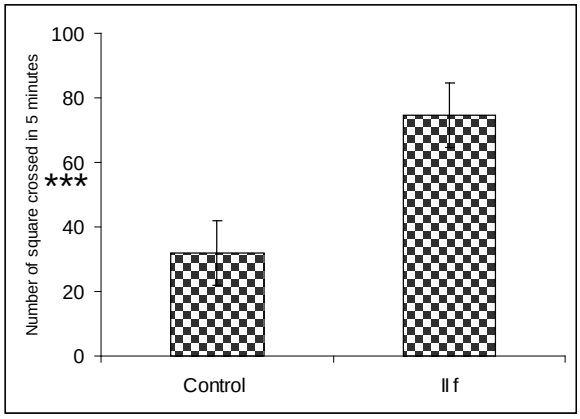

Fig 2f: Showing open field activity of Compound II f. Values are mean ± S. E. M. (n = 10) 30 minutes after injection signification differences by student's t-test *p <0.05 and **p< 0.001.

References

1 Porsolt, R.D., Anton, G., Blavet, N. and Jalfre, M. "Behavioral despair in rats: A new model sensitive to anti-depressant treatment". *Eur. J. Pharmacol*. **47:** 379-391 (1978).

2 Porsolt, R.D., Bertin, A. and Jalfre, M. "Behavioral despair in mice: A primary screening test for antdepressants". *Arch. Int. Pharmacology.* **229:** 327-336 (1977).

3 Porsolt, R.D., Lenegre, A. and Mcarthur, R.A. In: Animal models in Psychopharmacology, advances in Pharmacological Sciences. Birkhauser Verlag Basel, pp: 137-159 (1991)

4. Willner, P. "The Validity of animal models of depression". *Psychopharmacol.* **83:** 1-16 (1984).

5 Shalyapina, V.G., Rakitskaya, V.V. and Rodionov, G.G. "Involvement of dopaminergic processes in the striatum during the effects of corticoliberin on the behavior of active and passive rats". *Neurosci. Behav. Physiol.*, **33** (6): 629 (2003).

6 Klejbor, I., Luczynska, A., *et al*. "The development pattern of c-fos expression in the rat thalamus following open-field stress stimulation". *Pol. T Vet. Sci*., **6** (3): 201 (2003).

7 Dere, E., De Souza-Silva, *et al*., "Connexin-30-deficient mice show increased emotionality and decreased rearing activity in the open-field along with neurochemical changes". *Eur. J. Neurosci*., **18** (3): 629 (2003)

8 Mogilnicka, E. "Increase in β- and α_1- adreno-receptor binding sites in the rat brain and in the α_1- adrenoreceptor functional sensitivity after the DSP-4-induced noradrenergic denervation". *Pharmacol Biochem Behav*., **25** (4): 743 (1986).

9 Hall, C.S. (1934). "Emotional behavior in the rat". *J. Comp. Psychol*., **18:** 385.

10 Archer I (1973) "Tests for emotionality in rats and mice: a review". *Anim. Behav*., **21:** 205.

11 Saify, Z.S., Atia Zia and Ahmed Viqaruddin. (1996). "Synthesis and Analgesic Activity of 4-Piperidinol Derivatives". *Pak. J. Pharm*. **9:**1, 37-42.

12 Saeed, M., Saify, Z.S., Iqbal, Z. and Nazrul-Islam. (1997). "Studies on the effects of piperidine derivatives on blood pressure and smooth muscle contractions". *Arch. Pharma. Res.* **20**(4) 338-341

13 Saify, Z.S., Hanifa Shahnaz, Shamim Akhtar, Moazzam Haider and D.J. Haleem. (1999). "A Study on the Effects of Some New Derivatives of Piperidine on Neurotransmitters". Pak. *J. Pharm. Sci*., **12**(1) 43-47.

14 Saify, Z.S., Hanifa Shehnaz, Shamim Akhtar and Kamran A. Chishti. (2001). "Cytotoxic Study of Some New Synthetic Derivatives of Piperidine". *Pak. J. Pharmacology*. **18**(1), 13-16.

15 Saify, Z.S., Shamim Akhtar, M. Arif, Hanifa Shehnaz and Darakshan J. Haleem. (2005). "Neuropharmacological Estimation of Some New Morphine-Like Quaternary Phenacyl Bromo Piperidinium Compounds". *Pak. J. Pharm.Sci*. **18**(2): 52-54.

16 Bailey PD, Millwood PA and Smith PD (1998) "Asymmetric routes to substituted piperidines". *J. Chem. Soc., Chem. Commun*., 633-640.

17 Buffat MGP (2004) "Synthesis of piperidines". *Tetrahedron*, **60**:1701-1729.

18 Hammann, P. (1995). In*:* Organic Synthesis Highlights II; Waldmann, H., Ed., VCH: New York, page No.323.

19 Laschat, S. and Dickner, T. (2000). "Stereoselective Synthesis of Piperidines". *Synthesis*, 1781–1813.

20 Jahan, *et al*., 2012 Sarwat Jahan**,** Shamim Akhtar, Arfa Kamil, Zafar Saied Saify, Nousheen Mushtaq and M.Arif. (2012). "Antibacterial, Antifungal and Antioxidant Activities of Derivatives of Alkyl Piperidine". *FUUAST. J. BIOL*., **2**(1): 29-35.

21 Sarwat Jahan, Shamim Akhtar, Zafar Saied Saify, Nousheen Mushtaq, Ali Akbar Sial, Arfa Kamil and Muhammed Arif. (2013). "Synthesis and cytotoxic activity of some derivatives of alkyl Piperidine". *Pak. J. Pharm. Sci*., Vol.**26**, No.3, pp.517-523.

22 Tang G, *et al*., 2010) Tang G, Kertsez DJ, Yang M, Lin X, Wang Z, Li W, Qiu Z, Chen J, Mei J, Chen L, Mirzadegan T, Harris SF, Villasenor AG, Fretland J, Fith WL, Hang JQ, Heilek G and Klumpp K. (2010). "Exploration of piperidine-4-yl-aminopyrimidine as HIV-1 reverse transcriptase inhibitors. N-phenyl derivatives with broad potency against resistant mutant viruses. *Bioorg. Med. Chem. Lett*. **20**(20): 6020-3.

23 Weintraub, P.M., Sabol, J.S., Kane, J.M. and Borcherding, D.R. (2003). "Recent advances in the synthesis of piperidines and piperidines". *Tetrahedron* **59**, 2953–2989.

24 Yadav, J.S., Subba, B.V., Reddy, D.N., Chaya, G.G.K.S. Narayana Kumar, Naresh, P. and Jagadeesh, B. (2009). "Heteropoly acid-catalyzed aza-Prins-cyclization: an expeditious synthesis of 4-hydrooxypiperidines". *Tetrahedron Lett*., **50**, 1799-1802.

25 Yadav, J.S., Kumar, N.N., Reddy, M.S. and Prasad, A.R. (2007). "Stereoselective synthesis

of tarchonanthuslactone *via* the Prins Cyclization". *Tetrahedron* **63**, pp. 2689–2694.

26 Yadav, J.S., Reddy, B.V.S., Maity, T. and Narayana Kumar, G.G.K.S. (2007). "A diastereoselective synthesis of 4-azidotetrahydropyrans *via* the Prins-cyclization". *Tetrahedron Lett.* **48**, pp.7155–7159.

27 Yadav, J.S., Subba, B.V., Reddy, D.N., Chaya, G.G.K.S. Narayana Kumar, Aravind, S., Kunwar, A.C. and Madavi, C.(2008). "Gallium iodide/iodine as a versatile reagent for the aza-Prins cyclization:an expeditious synthesis of 4iodopiperidines".*Tetrahedron Lett.* **49**, pp. 3330-3334.

28 Yadav, J.S., Subba, B.V., Reddy, G.G.K.S., Narayana Kumar and Reddy, M.G. (2007). "$CeCl_3.7H_2O$/AcCl-Catalyzed Prins-Ritter reaction sequence: a novel synthesis of 4-amido tetrahydropyran derivatives". *Tetrahedron Lett.* **48**, pp. 4903–4906.

29 Yadav, J.S., Subba, B.V., Reddy, G.G.K.S., Narayana Kumar and Swamy, T. (2007). "Stereo selective C-O Ring Construction". *Tetrahedron Lett.* **48**, pp. 2205–2208.

30 Gao, M., Wang, M., Hutchins, G.D. and Zheng, Q.H. (2010). "Synthesis of carbon-11-labeled piperidine ring of N-[omega-(6-methoxynaphthalen-1-yl)alkyl]derivatives as new selective PET sigma 1 receptor probes". *Appl. Radiat. Isot.* **68**(3):459-65.

31 Ferhat Batool, Aisha Kamal, Madiha Sattar, Asad Hussain Shah, Syed Dilnawaz Ahmed, Zafar Saied Saify and Darakhshan Jabeen Haleem.(2011) . "Evaluation of antidepressant-like effect of aqeous extract of sea buckthorn (Hippophae rhamnoides l. ssp. turkestanica) fruits in experimental models of depression". *Pakistan journal of Botany*, **43**(3): 1595-1599.

32 Shamim Akhtar, Muhammad Arif, Nousheen, Zafar Saeed Saify, Ahsan Ahmed, Darakhshan Jabeen Haleem and Arfa Akram. (2012). "Behavioral and Neurochemical profile of some novel phenacyl based isonipecotamide derivatives". *Pak. J. Pharm.Sci.* vol. **25**, No.4, 705-713.

33 Nighat Sultana and Zafar Saify. (2012). "Naturally occurring and synthetic agents as potential anti-inflammatory and immunomodulants". Anti- inflammatory Anti- allergy Agents *Med Chem*. **11**(1):3-19.

34 Asghari Ghous and Zafar Saeed Saify(2011). "Neurochemical and Behavioral Effects of Indole Substituted Piperidine Derivatives". *Karachi University Journal of Sciences*, **39**, 25-31.

35 Nousheen Mushtaq, Saify.Z.S. , Shamim Akhtar, Muhammad Arif, Saida Haider and Nazish Saba. (2010). "Synthesis of some novel analogues of 4-(1-pyrrolidinyl) piperidine and their effect on plasma glucose level". *Pak J. Pharm. Sci.* **23**(2): 220-223.

36 Nousheen, M., Saify, Z.S., Khalid, M.K., Perveen, S., Shah, S.T., Abdel-Jalil, R.J., Fecker, M. and Voelter, W. (2005). "5-Synthesis and biological activities of novel 4-(4´-chlorophenyl)-4-hydroxypiperidine derivatives".*Chem. Pharm. Bull. (Tokyo)*.**53**(1): 64-6.

37 Haleem, D.J., Yasmeen, A., Parveen, T. and Zafar A. (1994). "Enhancement of hepatic tryptophan pyrrolase activity and decrease of

open-field locomotion following single and repeated administration of high doses of caffeine in rats". *Life Science*, **54**: 297-304.

38 Schildkraut, J.J. (1978). In: Psychopharmacology: a generation of progress, (Eds) Lipton, M.A., Di Mascio, A. and Killam, K.F. Raven Press, New York, and pp: 1223-1224.

39 Wells, K.B., Stewart, A. and Hays, R.D.(1989). "The Functioning and Well-being of Depressed Patients: Results From the Medical Outcomes Study". *JAMA*. **262**(91): 914-919.

40 Archer I (1973) "Tests for emotionality in rats and mice: a review". *Anim. Behav.*, **21:** 205.

41 Shamim Akhtar, Saify Z.S, Muhammad Arif, Nousheen Mushtaq and Darakhshan J. Haleem. (2005). "Neurochemical estimations of some new quaternary Phenacyl-Bromopiperidinium compounds". *Pak. J .Pharm. Sci.***18** (2):52-4.