www.sphinxsai.com

PharmTech

International Journal of PharmTech Research
CODEN(USA): IJPRIF ISSN : 0974-4304
Vol.1, No.2, pp 282-287 , April-June 2009

Synthesis, characterization, Biological Evaluation and In silico study of some Novel bis-piperidine derivatives

Hemendra Pratap Singh[1*], sayan D Gupta[2], N.S.H.N. Moorthy[2]

[1]B. N. College of Pharmacy, Udaipur, (Rajasthan), India,
[2]Drug Design and Development Laboratory, School of Pharmaceutical Science, RGPV, Bhopal-462036, Madhya Pradesh, India.

Email: hps_medicinalchemistry@yahoo.co.in

Abstract:A series of piperidine derivatives was synthesized by the reaction between substituted benzeldehyde, acetone and ammonium acetate by refluxing for 7-8 hr in ethanol and the derivatives refluxed with hydrazine hydrate yielded bispiperidine. The structures of the synthesized compounds were confirmed by spectrocopial analysis. These bispiperidine derivatives were also screened for anticonvulsant activity by MES (maximum electric shock) method. Among the compounds synthesized in the series, compound 2b (p-hydroxy substituted bispiperidine) is most active and compound 2a (unsubstituted) and 2e (2-chloro) are least active and substitutions favors activity in 4-OH>2,4-Cl>4-$(Me)_2$N>2-Cl>4-H in respective order. *In silico* metabolism and toxic study of the synthesized compounds were performed by using Pallas ADMET software. Most of the biologically active compounds are free of irritation, sensitivity and immunotoxicity.

Key words: Piperidine, bispiperidine, anticonvulsant, i*n silico* metabolism.

Introduction:

Piperidine and its derivatives has high impact on medical filed due to its wide variety of pharmacological action viz antifungal (*in vitro* antifungal activities against *Candida-6*[1], *A. niger* and *A. flavus)*[2], K+ channel blocker[3], hypoglycemic and hypolipidemic[4], anti-acetyl cholinesterase activity[5], opoid receptor antagonist[6] etc. The 2,5 disubstituted piperidine derivatives are the most important derivatives that are extensively studied because of its potent anticonvulsant activity.

The synthesis of piperidine is easy, economic and less time consuming. The parent molecule is flexible in nature and hence various derivatives can be easily prepared by altering its substituents. Piperidine derivatives formed by the reactions between acetone, ammonium acetate and substituted aromatic aldehyde. Ethanol acts as solvent medium for the reactions[2]. Bispiperidine derivatives, which are formed by the reaction with hydrazine hydrate under reflux. From the literature, it is found out that there is no bispiperidine derivatives reported having anticonvulsant activity.

In the present investigation, we attempt to synthesize some novel biologically active bispiperidine derivatives by conventional method.

Experimental :

Material and Methods

Melting points (M.P.) of the compounds were determined in open capillary method on Jindal melting point apparatus and were uncorrected. The purity of the compounds was routinely checked by thin layer chromatography (TLC) using silica gel G as stationary phase. The instruments used for spectroscopic data are IR: Jasco IR-470 spectrophotometer (KBr) with diffuse reflectance method; MS: MS-JEOL SX102 by using Argon/Xenone (6Kv, 10mA) as the FAB gas and m-nitro benzyl alcohol (NBA) as the matrix. H^1NMR: JEOL GSX-400, 60MHz spectrometer in $CDCl_3$, TMS (tetra methyl saline) as an internal standard. Elemental analysis (C, H, N analysis) were done on a CHN rapid analyser. All the compounds gave satisfactory analysis with in 0.4 of the expected values.

Step I: Synthesis of piperidine derivatives (1a-1e)

Acetone (0.01 mol), substituted benzeldehyde (0.02 mol) (as per Table **1** and ammonium acetate (0.01 mol) were taken in a 500 ml round bottom flask. Further ethanol (25 ml) was added to the flask and mixed well, so as to make a homogenous mixture. Then this mixture was refluxed at 80°C for 7-8 hr. Once the reaction was completed, the mixture was poured over cooled ice. The crude product obtained was filtered and the solid product was collected and washed with cold water. Then which was dried at room temperature and recrystallized with ethanol.

Step II: Synthesis of Bispiperidine derivatives (2a-2e)
2, 6-diphenyl-piperidine-4-one derivatives (0.02 mol) formed in step I and hydrazine hydrate (0.01 mol) was taken in a round bottom flask. Further ethanol (25 ml) was added to the flask and mixed well, so as to make a homogenous mixture. Then this mixture was refluxed at 80-90°C for 7-9 hr. Once the reaction was completed, the mixture was poured over ice. The crude product obtained was filtered and the product was collected and washed with cold water. The product was dried at room temperature and recrystallized with ethanol.

Figure 1: Synthetic schemes for synthesis of bispiperidine derivatives

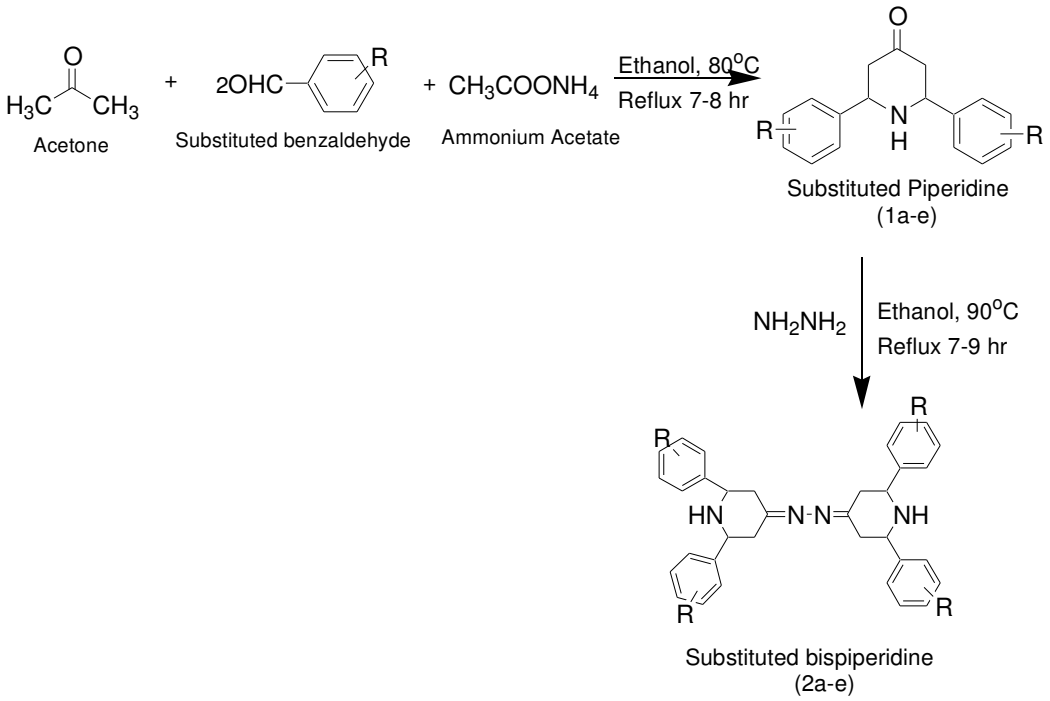
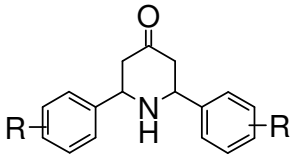
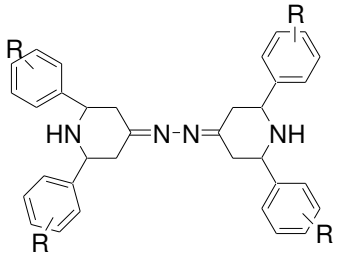


Table 1 : Physicochemical data of piperidine derivatives



Com No	R	Molecular formula	Mol Wt	MP (ºC)	Reaction Time (hr)	Reaction Temp (ºC)	Yield (%)
1a	4-H	$C_{17}H_{17}NO$	**251**	**115-117**	**8**	**90**	**66**
1b	4-OH	$C_{17}H_{17}NO_3$	**283**	**180-184**	**7**	**90**	**55**
1c	4-$(CH_3)_2$N	$C_{21}H_{27}N_3O$	**337**	**125-128**	**8**	**80**	**59**
1d	2,4-Cl	$C_{17}H_{13}NCl_4$	**389**	**90-93**	**7**	**80**	**63**
1e	2-Cl	$C_{17}H_{15}NCl_2$	**320**	**110-113**	**7**	**80**	**70**

Table 2 : Physicochemical data of bispiperidine derivatives



Comp No	R	Molecular formula	Mol Wt	MP (°C)	Reaction Time (hrs)	Reaction Temp (°C)	Colour and nature	Yield (%)
2a	4-H	$C_{34}H_{34}N_4$	**498**	**145-147**	**9**	**70**	**Deep yellow amorphous**	**64**
2b	4-OH	$C_{34}H_{34}N_4O_4$	**562**	**240-242**	**9**	**90**	**Brown amorphous**	**58**
2c	4-$(CH_3)_2N$	$C_{42}H_{54}N_8$	**670**	**290-293**	**8**	**80**	**Brown amorphous**	**45**
2d	2,4-Cl	$C_{34}H_{26}N_4Cl_8$	**774**	**87-90**	**8**	**90**	**Yellow crystalline**	**57**
2e	2-Cl	$C_{34}H_{30}N_4Cl_4$	**636**	**95-98**	**9**	**90**	**Yellow crystalline**	**68**

Figure 2: *In silico* metabolism of the bispiperidine derivatives

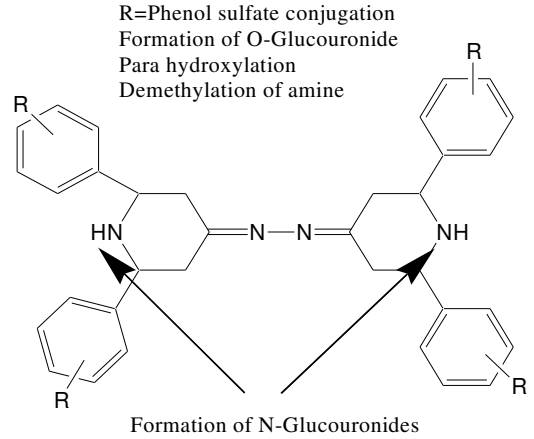


Table 3 : Spectroscopial data of bispiperidine derivatives

Compound No	Spectroscopy data
2a	**IR (KBr/cm^{-1})**= 3163.65, 3055.66(ArC-H), 1673.63(ArC=C), 1542.77(N-N), 1289.74,1247.31(C-N); **C^{13} NMR δ/ppm in CDCl$_3$**= 164.2(CN), 128.1(CH), 51.1(CH), 35.8(CH)
2b	**IR(KBr/cm^{-1})**=3649.62(O-H), 3150.52(ArC-H), 1604.48(ArC=C), 1516.74(N-N), 1257.36(C-N); **Mass(m/e) M$^+$**= 561, **Elemental analysis** for $C_{34}H_{34}N_4O_4$ **Calculated** C 72.58; H 6.09; N 9.96 **found** C 72.35; H 5.78; N 9.62
2c	**IR(KBr/cm^{-1})**=3376.45(N-H), 3130.56(ArC-H),2889.81,2802.00(MeC-H), 1604.48 (ArC=C),1522.52 (N-N), 1241.53(C-N); **H^1 NMR δ/ppm in CDCl$_3$**= 6.52(d,CH), 6.94(q,CH), 3.9(m,CH), 2.85(s,CH_3), 2.0(q,NH); **Elemental analysis** for $C_{42}H_{54}N_8$ **calculated** C 75.19; H 8.11; N 16.7 **found** C 75.67; H 7.98; N 16.3
2d	**IR(KBr/cm^{-1})**=3163.65(ArC-H), 1681.34(ArC=C), 1568.53(N-N),1267(C-N) 866.84, 822.49(C-Cl); **C^{13} NMR δ/ppm in CDCl$_3$**=164.6(CN), 133.5(CCl), 126.8(CH), 42.0(CH), 35.5(CH)
2e	**IR(KBr/cm^{-1})**=3163.65(ArC-H),1681.34 (ArC=C), 1568.53(N-N),1246(C-N), 866.84, 822.49(C-Cl); **C^{13} NMR δ/ppm in CDCl$_3$**= 128.2(CH), 164.6(CN), 35.4(CH), 42.0(CH)

Anticonvulsant activity

All the synthesized compounds were screened for their anticonvulsant activity by MES method[7-10]. The electroshock assay in mice is used primarily as an indication for compounds which are effective in grand mal epilepsy. Tonic hind limb extensions are evoked by electric stimuli which are suppressed by anti-epileptics. The behavioral and electrographic seizures generated in this model are consistent with the human disorder[10]. Balb/C mice of either sex weighing 20–25 g were housed in groups of 6 and acclimatized to their environment for at least two days before the experiments. The animals were allowed to free access to tap water before being tested and to standard commercial mice pellets. The test compounds were dissolved in DMSO as a vehicle and were administered intra peritoneal. Control animals were injected with vehicle only. The test is started 30 min after i.p. injection. An apparatus with corneal or ear electrodes is used to deliver the stimuli. The intensity of stimulus is 50 mA, 50 Hz for 0.2 s have been used. Under these conditions all vehicle treated mice show the characteristic extensor tonus. The animals are observed closely for 2 min. Disappearance of the hind leg extensor tonic convulsion is used as positive criterion. Percent of inhibition of seizures relative to controls is calculated. After one hour the administration of either vehicle or test compounds, mice were injected with Phenytoin (100 mg/kg/day, i.p.). This dose was determined on the basis of a pilot experiment in which it produced tonic-clonic convulsions without being fatal. Abolition of the hind limb tonic extensor component indicates the test compound's ability to inhibit MES-induced seizure spread[11].

Table 4: Anticonvulsant activity of synthesized compound

Comp No	Dose (mg/kg)	Mean ±SEM	Protection (%)	potency
Nomal control	-	**8.983±0.0600**	-	-
Phenytoin	25	**2.0±0.1183*** **	77.7	100
2a	30	**8.967±0.0494**	0.17	0.23
2b	30	**3.167±0.0122*** **	64.7	83.2
2c	30	**4.367±0.0199*** **	51.38	66.1
2d	30	**3.50±0.1844*** **	61	78.5
2e	30	**5.417±0.1493*** **	39.7	51

Table 5: *In silico* predicted toxicity of the synthesized compounds

Com No	Toxicity	Mutagenicity	Teratogenicity	Irritation	Sensitivity	Immuno toxicity	Neuro toxicity
2a	Not probable	0	0	0	0	0	0
2b	Probable	29	0	53	0	0	29
2c	Not probable	0	0	0	0	0	0
2d	Not probable	42	38	0	0	29	40
2e	Not probable	0	18	0	0	0	0

***In silico* metabolites and toxicity prediction**

Metabolites and toxicity of the compounds were predicted by computational method using Pallas version 3.1.1.2. ADME-Tox prediction software[12,13].

Results and Discussion:

Piperidine and its bispiperidine derivatives were synthesized by reaction between substituted benzaldehyde, acetone and ammonium acetate and hydrazine hydrate respectively (Fig 1). The physicochemical properties of the synthesized compounds were determined and are given in Table 1 and 2. N,N' bis-(2,6-diphenyl-piperidin-ylidine)-hydrazine derivatives showed the specific peaks in NMR and IR spectra of compound confirms envisaged structure has formed (Table 3).

The anticonvulsant activities of the synthesized bispiperidine derivatives were determined by MES (maximum electric shock) and the activity was compared with phenytoin as a reference drug. Table 4 shows that the compounds 2b was effective in delaying the onset of the first myoclonic twitches, but was not protective against MES-induced generalized convulsions. Compounds, 2b and 2d showed maximum potency (83 and 78%, respectively) and the substitution of chlorine (2e) and unsubstitution (2a) on bispiperidine have least protection. From the study it is showing that the chloro substitution on 2^{nd} position, in addition to 4^{th} position and hydroxy substitution on the 4^{th} position in the benzene ring have significant activity. While unsubstitution, having unfavorable effect on anticonvulsant activity in bispiperidine nucleus.

In silico ADMET study on the bispiperidine derivatives shows that the compounds, 2b and 2d have considerable toxicity than other compounds (Table 5). The highly active compounds viz, 2a, 2c and 2e have negligible toxicity like mutagenicity, teratogenicity, irritation, sensitivity, immunotoxicity and nurotoxicity. The hydroxyl derivatives undergo sulfate and O-glucouronide conjugates, it may be the cause of the toxicity (Fig. 2).

From the study it is concluded that the bispiperidine derivatives like 2b, 2d and 2c may be considered promising for the development of new anticonvulsant agents. These derivatives are free from toxicity and are metabolically stable. These bispiperidine derivatives may have variety of other biological activities viz. antitubercular, lishmanicidal, anticancer activity, etc. and it may be a pavement for synthesis of and characterization of some new bispiperidine derivatives.

Acknowledgements:

The author is thankful to the Vice Chancellor, Rajiv Gandhi Technological University, Bhopal, India for providing necessary laboratory facilities. One of the Authors is thankful to All India Council for Technical Education, New Delhi, India for providing grant through Career Award for Young Teachers.

References:

1. Ramalingan, C., Balasubramanian, S., Kabilan, S., Vasudevan, M., **2004**, *Eur. J. Med. Chem.*, 39, 527–533.
2. Balasubramanian, S., Ramalingan, C., Aridoss, G., Cavilan, S., **2005**, *Eur. J. Med. Chem.*, 40**,** 694–700.
3. Daniel, T., Smith, Riyi, S., Richard, B. B., Jennifer, M. M., Kevin, J., Stephen, R. B., **2005**, *Eur. J. Med. Chem.*, 40**,** 908–917.
4. HongWoo, L., BokYoung, K., JoongBok, A., Sung, K. K., Jung, H. L., Jae, S. S., Soon, K. A., Sang, J. L., Seung, S. Y., **2005**, *Eur. J. Med. Chem.*, 40**,** 862–874.
5. Hachiro, S., Shin, A., Yoshiharu, Y., Kiyomi, Y., **1990**, *J. Med. Chem.*, 33**,** 1880-1881.
6. James, B. T., Robert, N. A., Richard, B. R., Scott, E. F. S., Wayne, M., Ariane, A. V., Heng, X., Christina, M. D., Lu, Y. F., Buddy, E. C., Dennis, M. Z., Ivy, C. F., **2001**, *J. Med. Chem.,* 44, 2687-2690.
7. Ellison, G., Eison, M. S., Huberman, H. S., Daniel, **1978**, *Science*, 201**,** 276-278.
8. Rastogi, S. A., Ticku, M. K., **1996**, *Pharmacol. Biochem. Behav.*, 222, 141-146.
9. Sohn, Y. J., Levitt, B., Raines, A., **1970**, *Arch. Int. Pharmacodyn.*, 188**,** 284-289.
10. Swinyard, E. A., Brown, W. C., Goodman, L. S., *J,* **1952**, *Pharmacol. Exp. Ther.,* 106**,** 319-330.

11. Toman, J. E. P., Nodin, J. H., Siegler, P. E., **1964**, *Animal and Clinical Techniques in Drug Evaluation. Year Book Med Publ.*, 1**,** 348-352.
12. Pallas 3.1.1.2, ADME-Tox software, **2001**, compudrug international Inc, USA.
13. Moorthy, N. S. H. N., Rahul, S., Hemendra, P. S., Gupta, S. D., **2006**, *Chem. Pharm. Bull.,* 54(10), 1384-1390

*****