World Journal of Pharmaceutical research

Volume 3, Issue 1, 1537-1546. Research Article ISSN 2277 – 7105

THE STRUCTURAL MODIFICATION CAUSES THE ENHANCEMENT OF ANALGESIC ACTIVITY OF 4-HYDROXY 4-PHENYL PIPERIDINE ALONG WITH AN EXCELLENT INTERACTION WITH DIGESTIVE ENZYME, LEADING GOOD ADME

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Article Received on
19 November 2013
Revised on 15 December 2013,
Accepted on 12 January 2014

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ABSTRACT

The marvelous position of piperidine analogues has proven them an important core in the structures of therapeutically active molecules, pharmaceuticals and as synthetic intermediates with attractive biological and pharmacological behaviors. The piperidine ring containing compounds like pethidine and fentanyl have established as potent opiod analgesics. Due to similarity in structure, the present study was aimed to estimate the analgesic activity of synthesized derivatives of 4-Hydroxy-4-Phenyl Piperidine. The study was conducted in animal model, mice by using Pethidine as standard drug by hot plate method and analgesia was observed, furthermore the compounds were also analyzed for their interaction with digestive enzyme, amylase through agar plate method. The result illustrated the more high up response of substituted compounds than the parent one "4-hydroxy-4-Phenyl Piperidine" and by studying the structural activity relationship it was concluded that the modification in structure of the parent molecule is accountable for a better and positive pharmacological activities. Hence the synthesized derivatives will prove as potent analgesics with strong interface with amylase enzyme.

KEY WORDS: Opiod, Analgesic, Piperidine, Amylase.

INTRODUCTION

There is a long series of piperidine derivatives which has proved potent antinociceptive agent. Among them Fentanyl (Figure-1), a synthetic opiod analgesic, that was derivatized from 1-Phenethyl-4-piperidone, was found to exhibit a better profile of activity as compare to morphine [1, 2]. The opioid receptors are responsible for producing nociception and also causes antinociception effects because analgesic activity engages a diverse communication of nervous system from the skin and the muscle tissues to the brain**.**

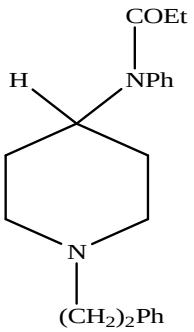


Figure-1: Fentanyl

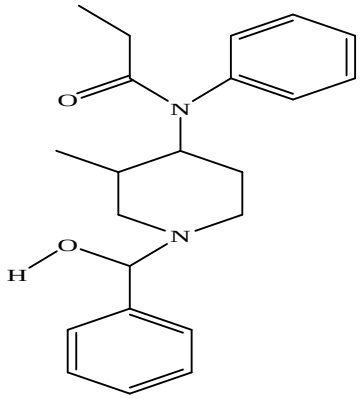


Figure-2: Ohmefentanyl

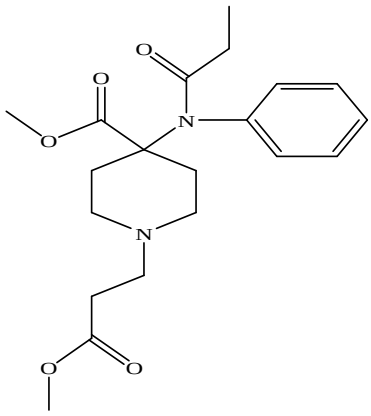


Figure-3: Remifentany

Ohmefentanyl and Remifentanyl (Figure 2 & 3) are extremely potent analgesic agents [3, 4]. Ohmefentanyl has highest receptor affinity and selectivity for the opioid μ receptors. Its eight optically active isomers were possible [5]. Remifentanyl was also found to be an effective anaesthetic agent that is responsible for providing optimal analgesia and sedation [6]. Several p-substituted phenacyl piperazine and piperidine derivatives were prepared and tested for the analgesic activity and different compounds exhibited varying degree of analgesic activity as the carbinol derivatives were found to exhibit the highest potency [7]. Number of other piperidine derivatives having significant analgesic activity were synthesized by different workers, one was proved to be a novel κ-opioid receptor selective ligand. Some 1, 4-substituted piperidine derivatives were experimentally proved strong analgesic agent [8-12]. Hence the essentials reveal that the analgesia and antinoceception are strongly allied with binding of opiod receptor with a suitable psychoactive chemical, an opiate . The recognition of opioid therapy has directed drug experts to recommend opioids [13]. The piperidine ring containing compounds due to having similarity in the structure with morphine belongs to the

same class of drug and cause analgesia by blocking the signaling pathways against pain and considered as leading nucleus used for the treatment pain and inflammation [14]. Piper nigrum, commonly known as black pepper, a widespread remedy for digestion and it was found to increase salivary secretion. It also causes digestive stimulatory action in liver to improve the bile secretion, important for fat metabolism. Piperidine is a constituent of piperine which is present in pepper and found to produce a significant antioxidant enzyme activity, when studied on a group of Wistar rats and also observed protective for gastric intestinal mucosa [15]. Piperine is also responsible for providing prevention from ulcer in experimental animals [16]. Due to having compatable effect with gastric enzymes of piperidine, the study was also done to examine the interaction of synthesized derivatives with amylase as when drug taken orally it has to go through the first pass metabolism and the better bioavailability will responsible to produce the optimum action of drug [17].

METHOD

Analgesic Activity

The analgesic activity of synthesized derivatives of 4-Hydroxy-4-phenyl piperidine was conducted by Eddy's hot plate method [18, 19]. The derivatives were synthesized by reacting with different phenacyl halide [20]. For conducting the analgesic activity albino mice of either sex were taken of 20-30g weight and were kept under standard colony conditions (12 hrs night and 12hrs dark and at a temperature of 30 ± 1°C) on proper diet and water. The parent compound 4-Hydroxy-4-phenyl piperidine and derivatives were tested orally for analgesic effect at the effective dose of 50mg/ Kg weight (as 25mg/ Kg weight was found ineffective and 75mg/ Kg weight was observed lethal. Pethidine (5mg/Kg) and gum tragacanth were used as standard drug and vehicle respectively. The activity of compounds was assessed at a temperature of 55±1^{o}C. The control groups received only the vehicle. Analgesic effects were observed at 30, 60, 90, 120, and180 minutes after administration and six animals were taken in a group. The analgesia was measured by the writhing effect after the administration of drugs (compounds) and the results were summarized in the form of analgesic activity (Tables: 1) that was expressed as analgesia, mean increase in latency after drug administration ± SEM and was calculated. Student's t-test was applied for statistical analysis and $P<0.05$ or $P<0.01$ were considered as significant or highly significant values respectively.

***In Vitro* Antiamylatic Activity**

For conducting the alpha amylase activity of compounds semiquantitative method Agar Plate Activity [21] was adopted and for this purpose the enzyme alpha-amylase (α -1, 4-glucanohydrolase; EC 3.2.1.1) from Sigma Aldrich chemical company was used as standard. In this method 1mg/ml solution of alpha amylase enzyme and compounds were prepared in DMSO. Then the test sample was prepared in1:1 ratio that was 30µl of compound solution was added into 30µl of alpha amylase enzyme solution. The mixture of 1.5 gm Agar and 1.5 gm of soluble starch (of potato) in distilled water, was used to prepare agar plate of standard size thickness of 2mm. Four wells of 7mm in diameter were made with a cork borer in the starch agar gel of each plate. A fixed volume 30µl of α -amylase solution was introduced into one well and in the rest of three wells the sample that is of synthesized compounds to be tested along with α -amylase solution were introduced (30µl) then the plates were covered with a tight fitting glass plate and incubated for a standard time duration of 24 h at 37°C. At the end of incubation, the starch-agar plate was flooded with Lugol solution and the excess of solution was poured off. The presence of amylase activity was indicated by zones around the wells because of lysis of starch and incubation diameter of zone of inhibition was noted by Vernier caliper (Figure 4&5).

RESULTS AND DISCUSSIONS

Multidimensional research has been done for the substitutions of piperidine to get better and improved moiety for the management of pain. [22]. During the present study the synthsized molecules were analysed for the analgesic behaviour (Table-3, Graph-1) and 4-Hydroxy-4-phenyl piperidine (A) was observed strong and significant analgesic at the dose of 50mg/kg body weight (Table -1).

As the parent compound “A” showed strong analgesic response, similarly all its derivatives were observed to exhibit the excellent analgesic effects. Compound. 1-(1″-Phenoxypropyl)-4-phenyl-4-hydroxy piperidinium Hydrobromide (II) and 1-(6″-Methyluracil)-4-phenyl-4-hydroxy piperidinium Hydrochloride (III) displayed highly significant analgesic response ($p<0.01$) just after 30 minutes of drug administration. The strong response was observed throughout the duration and showed again highly significant ($p<0.01$) after two hours that was constant till 180 minutes. 1-(1″-Adamantan acyl)-4-phenyl-4-hydroxy piperidinium Hydrobromide (IV) exhibited quick analgesic response after 30 minutes of administration and exhibited the effect of highly significant level ($p<0.01$) till 180minutes.Whereas

compound 1-(1″-Propiophenone)-4-phenyl-4-hydroxy piperidinium Hydrochloride (V) and 1-(1″-Ethylpthalamide)-4-phenyl-4-hydroxy piperidinium Hydrobromide (VI) showed more or less similar activity as of compound III , as it also showed analgesic response after 30 minutes of administration and that was continued all over the duration till 180 minutes of highly significant level ($p<0.01$).

The results were showing all the phenacyl derivatives of compound 4-Hydroxy-4-phenyl piperidine highly significant and potent analgesics. The substitution of methyl group at nitrogen atom in piperidine ring was responsible for attributing the enhancement of analgesia of parent compound. The investigations showed that significant change in the antinoceceptive activity of piperidine due to the the substitution of methyl group at the phenacyl nitrogen. All the synthesized derivatives of 4- Hydroxy -4-phenyl piperidine were opioid analgesics. Structure-activity-relationship (SAR) studies reveal that the methyl group enhanced the analgesic potency but the duration of analgesic effect did not depend on the methyl group substitution,. SAR studies with refrence to analgesia suggested that the opiod receptors were responsible for antinociceptive, hence the nature of receptor binding with the structure of whole molecule describes analgesic response and may be influenced only by the steric effect. During the antiamylatic studies of synthesized compounds by agar plate method, interesting results were observed. Parent compounds 4-Hydroxy-4-phenyl piperidine (A), exhibited average compatibility with alpha amylase enzyme and hence moderate inhibition was observed . When the derivatives of 4-Hydroxy-4-phenyl piperidine, were studied all the compounds (I-V) showed the inhibition area more than 20mm that was indication of excellent interaction of the synthesized compound and enzyme, hence the compounds were not found to disrupt the enzymatic effect, rather they increased the activity. Specifically compound 1-(1″-Ethyl pthalamide)-4-phenyl-4-hydroxy piperidinium Hydrobromide (V) was showing almost same area of inhibition as that of alpha amylase enzyme. The study showed that the compound will be more similar in temperament with enzyme and will not interrupt its pharmacological behavior.Though all the synthesized piperidine were encouraging analgesics and were found in good dealings with alpha amylase digestive enzyme And hence can be selected for further studies regarding antinoceciptive activity with an excellent ADME with higher bioavailability.

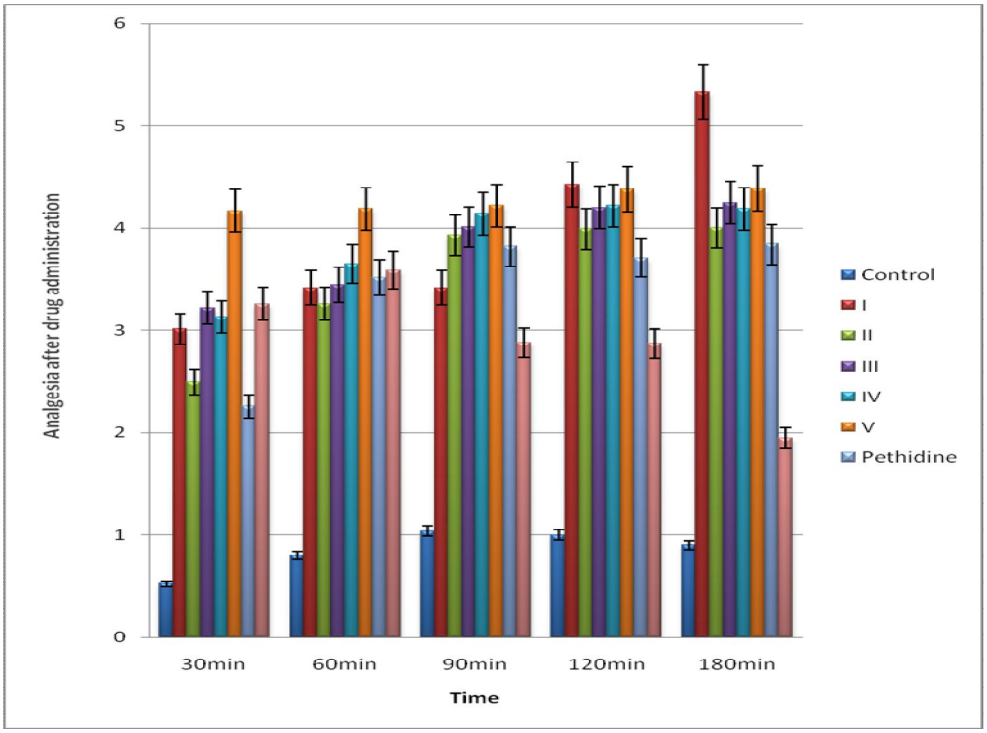
Table-1: Analgesic Effects of 4-Hydroxy-4- phenyl piperdine Derivatives

Dose(Oral) 50mg/Kg	Analgesia (mean increase in latency after drug administration ± SEM)				
Time	30min	60min	90min	120min	180min
Control	0.53±0.53	0.8±0.55	1.04±0.53	1±0.53	0.9±0.53
Parent (A)	3.26**±0.78	3.59**±0.48	2.88**± 1.45	2.87**±0.21	1.95**±0.6
I	3.01**±3.87	3.42**±4.26	3.42**±4.67	4.43**±3.90	5.33**±6.66
II	2.51**±3.82	3.96**±3.20	4.01**±1.96	4.02**±2.54	4.05**±1.60
III	3.22**±2.58	3.45**±3.38	4.01**±1.96	4.2**±2.54	4.25**±3.82
IV	3.13**±4.65	3.65**±1.63	4.14**±3.97	4.22**±2.99	4.19**±3.28
V	4.17**±3.20	4.19**±5.91	4.22**±6.15	4.38**±4.50	4.39**±6.16
Pethidine HCl	2.26±0.63	3.52±0.46	2.82±0.13	2.57±0.23	1.57±0.2

Activity Key

Significant= *

Highly significant= **



Graph-1: Analgesic Effects of 4-Hydroxy-4- phenyl piperdine Derivatives

Table: 2 Antiamylati Effects of 4-Hydroxy-4- Phenyl Piperdine Derivatives

Compounds		Inhibition (mm) by Compounds	Inhibition (mm) by Amylase Enzyme
A	4-Hydroxy-4-phenyl piperidine(I)	15.8	23.3
I	1-(1″-Phenoxypropyl)- 4-phenyl-4-hydroxy piperidine(II)	21.58	23.4
II	1-(6″-Methyluracil)- 4-phenyl- 4-hydroxy piperidine(III)	22.6	23.4
III	1-(1″-Adamantan acyl)- 4-phenyl -4-hydroxy piperidine(IV)	20.78	23.4
IV	1-(1″-Propiophenone) - 4-phenyl- 4-hydroxy piperidine(V)	21.28	23.4
V	1-(1″-Ethyl pthalamide) - 4- phenyl-4-hydroxy piperidine(VI)	23.4	23.4

Activity Key:

Below 10mm inhibition = no activity

From 11-20 mm inhibition = moderate activity

Above 20 mm inhibition = significant activity

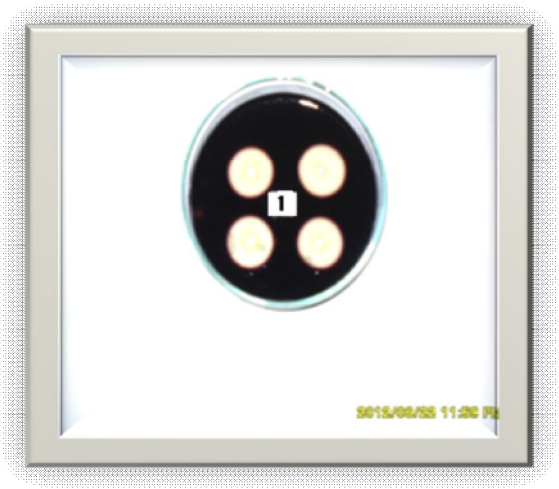


Figure- 4: Image of Agar Plate 1

Antiamylatic Activity of 4-Hydroxy-4-phenyl piperidine Derivatives (I, II, III)

Figure- 5: Image of Agar Plate 2

Antiamylatic Activity of 4-Hydroxy-4-phenyl piperidine Derivatives (IV, V)

REFERENCES

1. Jandhyala R, Fullarton JR and Bennett M I, efficacy of rapid-onset oral Fentanyl formulations versus oral Morphine for cancer-related breakthrough pain: A meta-analysis of comparative trials. J. Pain Symptom Manage 2013, 2013 Oct;46(4):573-80. doi: 10.1016/j.jpainsymman.2012.09.009. Epub 2013 Feb 4.
2. Sakhteman A, Sharifzadeh M, Moradi A, Nadri H, Tabrizian K, Amanlou M, Asadipour A, Divsalar K, Shafiee A and Foroumadi A, antinociceptive activity of some 1,4-substituted piperidine derivatives using tail flick method in mice. African Journal of Pharmacy and Pharmacology , 2011, 5(3): 352 - 357.
3. Gardocki J and Yelnosky J, a study of some of the pharmacological actions of Fentanyl citrate. Toxicol. Appl. Pharmacol., 1964, 6: 48-62.
4. Vuckovic S, Prostran M, Ivanovic M, Dosen-Micovic Lj, Todorovic Z, Nesic Z, Stojanovic R, Divac N and Mikovic Z, fentanyl analogs: structure- activity-relationship study. Current Medicinal Chemistry , 2009, 16 (19): 2468-2474.
5. Garnier M, Zaratin P, Ficalora G, Valente M, Fontanella L, Rhee M, H, Bulmer K, J, Scheideler M A, up-regulation of regulator of G-protein signalling 4 expression in a model of neuropathic pain and insensitivity to morphine . J. Pharmacol. Exp. Ther., 2003, 305,1299-1306.
6. Gardocki, J. and Yelnosky, J. (1964). A study of some of the pharmacological actions of Fentanyl citrate. *Toxicol. Appl. Pharmacol.*, 6: 48-62.

7. McQuay H, opioids in pain management. Lancet 1999, 353:2229-2232
8. Saify, Z., S., Rasheed, H., Mushtaq, N., Nisa, M., Haider, S., Naz, A., Azhar, K., F.and Miana, G., A. (2012). Investigation of piperidine derivatives in *ex vivo* models of pain and platelet aggregation. *Archives of Pharmacal Research*, 35(11): 1953-1959.
9. Saify, Z., S., Nazar-Ul-Islam and Iqbal, Z. (1997). Synthesis of 4-Hydroxypiperidine derivatives and their screening for analgesic activity. *Archives of Pharmacal Research,* 20(4): 338-341.
10. Saify, Z., S., Nisa, M., Azhar, K., F. and Kamran, M., A. Rasheed, N., Mushtaq, N., Arain, M., A., Haider, S., Khanum, M., Ahmed, W., (2011). Charecterization of *plasmodium falciparum* as partic protease inhibition by piperidine derivatives. *Journal of Natural Product Research*, 25(20): 1965-1968.
11. Saify, Z., S., Mushtaq, N., Khan, K., M., Perveen, S., Akhtar, S., Shah, S., T., A., Jalil, R., J., A., Fecker, M. and Voelter, W. (2005). Synthesis and pharmacological activity of 4-(4-Chlorophenyl)-4-hydroxypiperidine derivative. *Chemical and Pharmaceutical Bulliten*, 53(1): 64-66.
12. Saify, Z., S., Shahnaz, H., Akhtar, S., Haider, M., and Haleem, D., J. (1999). A study on the effects of some new derivatives of piperidine on neurotransmitters. *Pakistan Journal Of Pharmaceutical Sciences*, 12(1): 43-47.
13. Ratka A, Wittwer E, Baker L and Kern S, pharmacokinetics of morphine, morphine-3-glucuronide and morphine-6-glucuronide in ealthy older men and women. American Journal of Pain Management, 2004, 14, 45-55.
14. Mercer S, Cunningham C, Eddington N and Coop A, opioids and efflux transporters. Part 3: P-glycoprotein substrate activity of 3-hydroxyl addition to meperidine analogs. Bioorganic Medecinal Chemistry Letters, 2008, 18(12) 3638-3640.
15. Srinivasan, K. (2007). Black pepper and its pungent principle-piperine. A review of diverse physiologicaleffects. *Critical Reviews in Food Science and Nutrition* , 47(8): 735-748.
16. Platel, K. and Srinivasan, K. (1996). Influence of dietary spices or their active principles on digestive enzymes of small intestinal mucosa in rats. *International Journal of Food Science Nutrition* , 47(1) : 55-59.
17. Bai, Y., F. and Xu, H. (2000). Protective action of piperine against experimental gastric ulcer. *Acta. Pharmacol. Sin.*, 21: 357–359.
18. Eddy N and Leimbach D, synthetic analgesics ii dithienylbutenyl and dithienylbutylamines. J. Pharmacol. Exp. Ther., 1953, 107: 385–393.

19. Niket rai1, Savita Vyas, Pradeep Phadnis, to evaluate the analgesic activity of ficus racemosa leaf extract in albino mice using eddy's hot plate method. Asian Journal of Pharmaceutical and Clinical Research , 2013, 6 (5); 19-21.
20. Kiran Rafiq, Zafar Saied Saify, Faiyaz Vaid, Sikandar Khan, Farzana Akhtarand Rana Kausar(2013) Synthesis with Antibacterial and Antifungal Screening of 4- Hydroxy-4-Phenyl Piperidine Derivatives , 1(10:64_72
21. Bernfeld, P. (1955). Amylases α and ß, Methods in Enzymology. *Mini. Rev. Med. Chem*., 11: 149-158.
22. Bielenica A, Kossakowski J, Struga M, Dybała I, Loddo R, Ibba C, Colla L P. Synthesis and biological evaluation of new thirteen 3-Phenyl-1-[(4-arylpiperazin-1-yl)alkyl]-piperidine-2,6-diones. Scientia Pharmceutica. 2011; 79: 225-238.