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THE STUDY OF ANTI-INFLAMMATORY RESPONSE IN RELATION WITH ANTITRYPTIC ACTIVITY OF 4-HYDROXY-4-PHENYL PIPERIDINE DERIVATIVES

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ABSTRACT

Piperidine ring containing compounds are more potent and diverse pharmacological moieties and focal chemical entity. There are various piperidine ring containing compounds, which exhibit strong anti-inflammatory activity furthermore these compounds were found to have strongly compatible with trypsin enzyme that has been proved as a an excellent tool for controlling the inflammation. Depending on these facts , the present study was done to examine the interaction of 4-Hydroxy-4-phenyl piperidine synthesized derivatives with trypsin enzyme through agar plate method. The outcome of study helped to evaluate the compounds in two ways as the compounds were found in strong interaction with this digestive enzyme that behavior will help to work against inflammation.

Key words: Inflammation, trypsin, piperidine.

INTRODUCTION

Piperidine analogues have been established for many therapeutic activities after altering its chemical structure for treating diabetes, pain and inflammation [1-3]. Piperidine is a constituent of piperine which is present in pepper and found to produce a significant antioxidant enzyme activity and observed protective for gastric intestinal mucosa[4]. Consequently piperidine being a chief constituent of black pepper and an alkaloid have ever been a encouraging factor for producing effects on digestive enzymes including trypsin. Trypsin is a digestive enzyme that causes degradation of biological molecules in intestine[5-

7]. Sometimes under some conditions, the enzyme starts an auto-digestion process, resulting in provokes of mediators having inflammatory activity, when they penetrate into the central circulation cause inflammation and create multi-organ failure. Recently, it has been verified by the experiment that obstruction of pancreatic proteases drastically trims down the inflammation [8]. Hence, the inflammation can be overcome by controlling the activity of trypsin. In this regard trypsin inhibitor that is a protease plays an important role [9]. As the synthesized compounds were piperidine derivatives and the piperidine ring containing compounds already renowned anti-inflammatory agents therefore the *in vitro* antitryptic activity of synthesized derivatives was evaluated in order to investigate their trypsin inhibition in inflammation [10].

METHOD

4-(4'-Chlorophenyl hydroxy piperdine was reacted with corresponding phenacyl halides in equimoler quantities (0.01 moles)according to the designed scheme (Figure 1 and Table 1). The solid product was filtered and recrystallized. Melting point of the product was noted and the compound further was confirmed through spectral information [11].

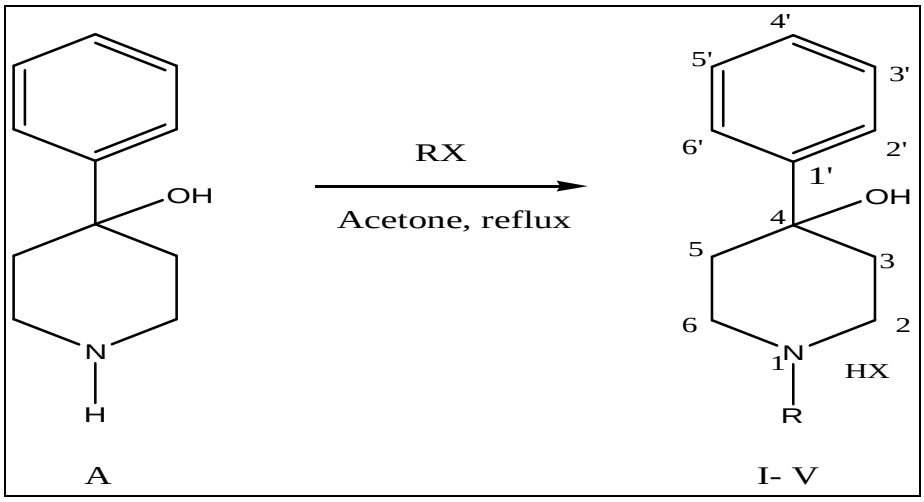

Figure-1: Reaction Method of 4-Hydroxy-4-phenyl piperidine (Parent Compound "A") Derivatives

Table-1: Substituents of 4-Hydroxy-4-phenyl piperidine (Parent Compound A)

Compound No.	R	X
I	OC_9H_{11}	Br
II	$O_2C_5H_5N_2$	Cl
III	$OC_{12}H_{17}$	Br
IV	OC_8H_7	Cl
V	$O_2C_{10}H_8N$	Br

For conducting the antitryptic activity trypsin and protease enzymes were used. Casein and trypsin enzyme were purchased from Sigma Aldrich company and protease enzyme from E. Merck Company. The pancreas was the source of trypsin and that of protease was obtained from A*spergillu s saitoi* (fungal type XIII). All the reagents were of analytical grade. In this technique trypsin and protease enzymes were used as standard. The solution of both enzymes was prepared by taking 1mg/ml in DMSO separately and both were mixed. The 1mg/ml solutions of compounds to be analyzed were prepared also in DMSO. The sample of each synthesized compounds was prepared in 1:1 ratio that was 30µl of sample solution that was added into 30µl of enzyme solution [12]. Agar, 1.5gm, was accurately weighed and dissolved in 50 ml distilled water with constant stirring in a beaker. In another beaker accurately weighed 1mg casein was taken and dissolved in 40 ml double distilled deionized (DDD) water previously alkalized by Sodium carbonate. The agar was melted by heating for 20 to 30 minutes in an autoclave, mixed immediately with the casein solution. Then the mixture was poured into the petri dishes of standard size thickness of 2 mm. The thickness was standardized by pouring a fixed volume into each plate. The plates were allowed to stand until the agar had solidified. As, it was semiquantitative determination of antitryptic activity, for this purpose, four wells of 7mm in diameter were made on the starch agar gel of each plate with a cork borer. A fixed volume 30µlof trypsin and protease solution was introduced into one well and in the rest of three wells the sample that was of synthesized compounds to be tested along with enzyme solution (30µl) were introduced then the plates were covered with a tight fitting glass plate and incubated for a standard time duration of 24hours and at the temperature of 37°C. At the end of incubation plates were observed by zones of inhibition around the wells because of lysis and after incubation diameter of zone of inhibition was noted by Vernier calliper.

RESULTS AND DISCUSSION

Various piperidine analogues have been experimentally proved as significant anti inflammatory agents [13] and some have been established as potent orally anti-inflammatory moieties with little side effects when compared with naproxen and indomethacin . As trypsin inhibition and PAR play important role for the management of inflammation therefore the present study was conducted to evaluate the level of interaction and behavior of synthesized piperidine derivatives with trypsin enzyme, these studies helped to study the antiinflammatory response of synthesized derivatives *in vivo* acting through PAR and trypsin inhibition [14,15]. During the antitryptic studies of synthesized compounds

by agar plate method, exciting results were observed (Table-2, Figure: 2-8). Parent compounds 4-Hydroxy-4-phenyl piperidine (A), exhibited moderate inhibition with trypsin enzyme.

When antitryptic activity of 4-Hydroxy-4-phenyl piperidine derivatives was studied, all the compounds (I-V) with enzyme showed inhibition area almost same as that of enzyme alone. This excellent interaction of derivatives was showing that the synthesized compounds will be responsible for enhancing the activity *in vivo*. It showed that the compound will prove compatable with enzyme *in vivo* for treating the inflammation in an excellent way and would prove a good therapeutic agent in this regard and process will go smoothly. Hence it was concluded that all the derivative4- Hydroxy-4′-phenyl piperidin, showed tremendous interaction with the protease enzyme. These compounds will give good response for controlling the inflammation and sepsis, specifically the derivatives of 4-hydroxy-4-phenyl piperidin were examined highly significant and will be responsible for producing energetic anti-inflammatory response.

Table:2 Antitryptic Activity of 4-Hydroxy-4-phenyl piperidine Derivatives

Compounds	Inhibition (mm) by Compounds with protease enzyme	Inhibition (mm) by Protease enzyme
4-Hydroxy-4-phenyl piperidine (A)	8.1	12.49
1-(1″-Phenoxypropyl)-4-phenyl-4-hydroxy piperidinium Hydrobromide (I)	12.25	12.38
1-(6″-Methyluracil)-4-phenyl-4-hydroxy piperidinium Hydrochloride (II)	12.21	12.38
1-(1″-Adamantan acyl)- 4-phenyl -4-hydroxy piperidinium Hydrobromide (III)	12.11	12.38
1-(1″-Propiophenone)-4-phenyl-4-hydroxy piperidinium Hydrochloride (VI)	12.15	12.84
1-(1″-Ethyl pthalamide) -4-phenyl-4-hydroxy piperidinium Hydrobromide (V)	12.24	12.84

Activity Key

Below 7mm inhibition = no activity

From 8-10 mm inhibition = moderate activity

Above 10 mm inhibition = significant activity

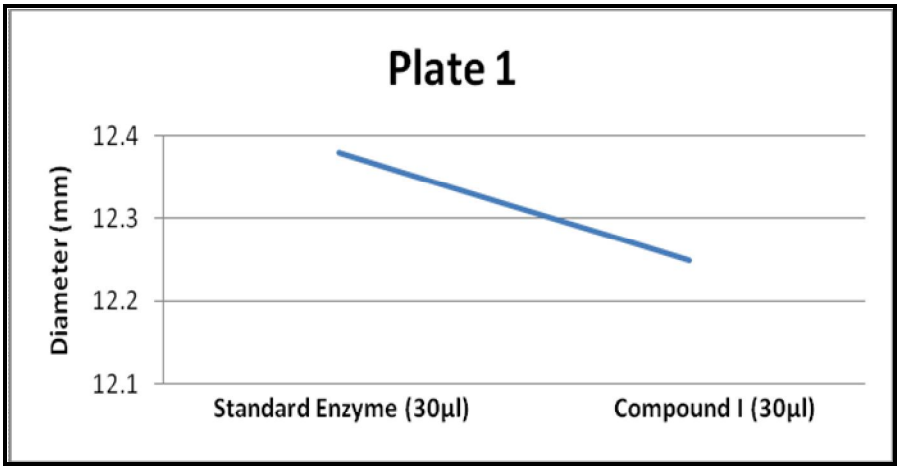

Figure-2: Antitryptic activity of 1-(1′′-Phenoxypropyl)-4-phenyl-4-hydroxy piperidinium Hydrobromide (I)

Figure-3: Antitryptic activity of 1-(6′′-Methyluracil)-4-phenyl 4-hydroxy piperidinium Hydrochloride (II)

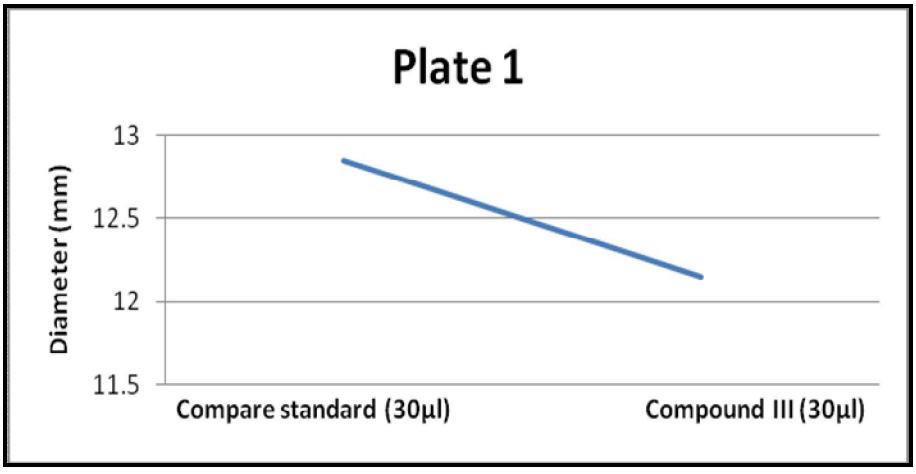

Figure-4: Antitryptic activity of 1-(1′′-Adamantan acyl) -4-phenyl -4-hydroxy piperidinium Hydrobromide (III)

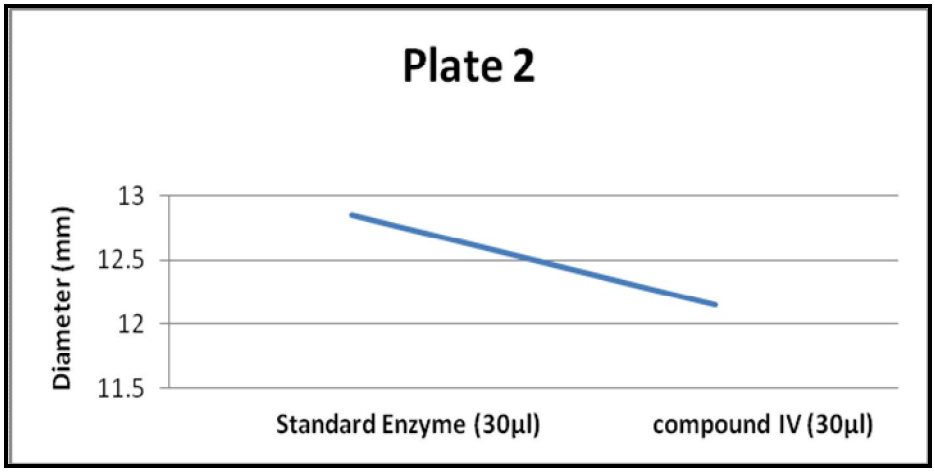

Figure-5: Antitryptic activity of 1-(1′′-Propiophenone)-4-phenyl-4-hydroxy piperidinium Hydrochloride (VI)

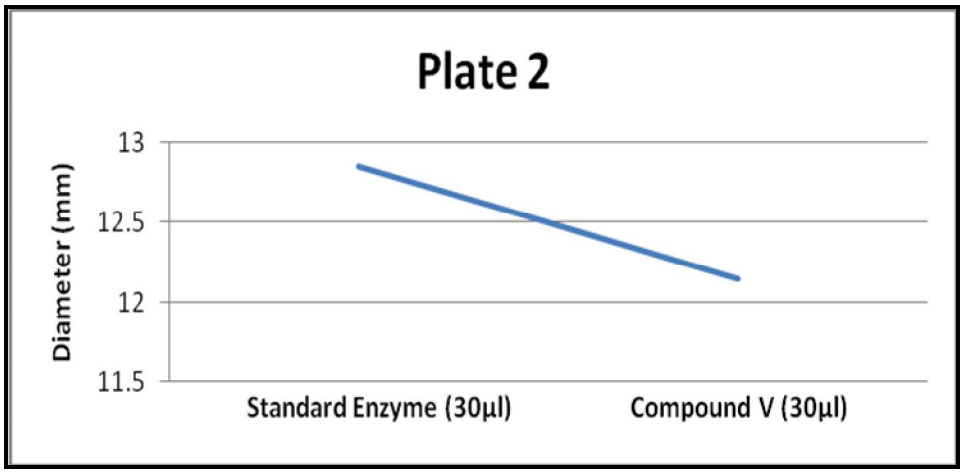

Figure-6: Antitryptic activity of 1-(1′′-Ethyl pthalamide)-4- phenyl-4- hydroxy piperidinium Hydrobromide (V)

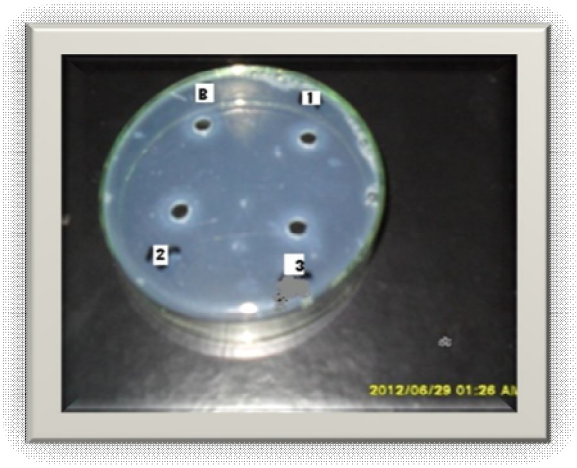

Figure- 7: Image of Agar Plate 1

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

Figure- 8: Image of Agar Plate 2

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

REFERENCES

1. Takai, H., Obase, H., Nakamizo, N., Teranishi, M., Kubo, K., Shuto, K. and Hashimoto, T. (1985). Synthesis and pharmacological evaluation of piperidine derivatives with various heterocyclic rings at the 4-position. *Chem. Pharm. Bull.*, 33(3): 1104-1115.
2. Lawson, J., A., Cheng, A., DeGraw, J., Frenking, G., Uyeno, E., Toll, L. and Loew, G., H. (1988). Effects of addition of 2-methyl group to ethyl nipecotates (beta-meperidines) on receptor affinities and opiate agonist/antagonist activities. *Journal of Medinal Chemistry* , 31(10) :2015-2021.
3. Vijayakumar, R., S., Surya, D. and Nalini, N. (2004). Antioxidant efficiency of blackpepper and piperine in rats with high fat diet induced oxidative stress. *Redox Report* , 9(2): 105-110.
4. Kiran Rafiq, Zafar Saied Saify, Asghari Ghous, Shazia Haider, Nighat sultana, 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 WJPR 3(1), 1537-1546
5. 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.
6. 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.

7. 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.
8. Fitzal, F., Delano, F., Young, C., Rosario, H., Junger, W. and Schmid-Schönbein, G. (2003). Pancreatic enzymes sustain systemic inflammation after an initial endotoxin callenge. *Surgery*, 134(3): 446-456.
9. Miike, T., Mcwilliam, A., S. and Kita, H. (2001). Trypsin induces activation and inflammatory mediator release from human eosinophils through Protease-Activated Receptor-2. *J. Immunol.*, 167(11): 6615-6622.
10. Fitzal, F., Delano, F., Young, C. and Schmid-Schönbein, G. (2004). Improvement in early symptoms of shock by delayed intestinal protease inhibition. *Arch. Surg.*, 139(3): 1008-1016.
11. Kiran Rafiq, Zafar Saied Saify, Faiyaz Vaid, Farzana Navaid, Arfa Kamil, Rana Kausar and Asghari Ghous, synthesis of 4-(4'-chlorophenyl) -4-hydroxy piperdine analogues having promising compatibility with alpha amylase enzyme, IJLBPR,3(2) ,459-465
12. Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities protein utilizing the principal of protein-dye binding. *Journal of Analytical biochemistry*, 72: 248–254.
13. Khanum, S., A., Girish, V., Suparshwa, S., S. and Khanum, N., F. (2009). Benzophenone-N-ethyl piperidine ether analogues-synthesis and efficacy as anti-inflammatory agent. *Bioorganic Medicinal Chemistry Letters*, 19:1887-1891.
14. Schmid-Schönbein, G., Hugli, T., Kistler, E., Sofianos, A. and Mitsuoka, H. (2001). Pancreatic enzymes and microvascular cell activation in multiorgan failure. *Microcirculation*, 8(1): 5-14.
15. Viegas, C., J., Alexandre-Moreira, M., Fraga, C., Barreiro, E., Vda, S., B. and Miranda, A., D. (2008). Antinociceptive profile of 2,3,6-trisubstituted Piperidine alkaloids:3-o-Acetyl-spectaline and semi-synthetic derivatives of (-)-Spectaline. *Chemical Pharmaceutical Bulliten (Tokyo)*, 56(4): 407-412.