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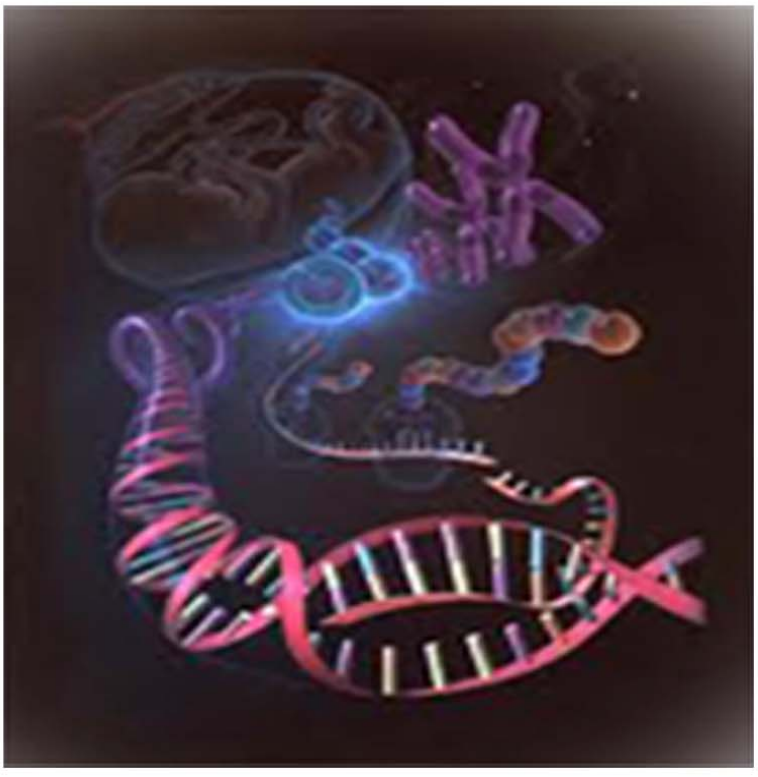

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Research Paper

SYNTHESIS OF 4-(4'-CHLOROPHENYL) -4-HYDROXY PIPERDINE ANALOGUES HAVING PROMISING COMPATIBILITY WITH ALPHA AMYLASE ENZYME

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A new sequence of 4-(4'-Chlorophenyl)-4-hydroxy piperdine derivatives (II-V) were synthesized with different phenacyl halide and these newly synthesized compounds were characterized by analytical and spectroscopic data. The desired therapeutic target of the synthesized moieties can be achieved by providing a good bioavailability. When drug taken orally, first interacts with alpha amylase enzyme. The excellent compatibility of compounds with this digestive enzyme *in vivo* assures the enhanced ADME. Hence the synthesized compounds were screened for their *in vitro* antiamylatic activity by semiquantitative agar plate method and the results showed tremendous interface between them for promising receptor binding.

Keywords: Bioavailability, ADME, *In vivo*, Antiamylatic Activity

INTRODUCTION

Piperidine analogues have been proved for many therapeutic activities after altering its chemical structure, most were found to possess antidiabetic analgesic, hypotensive and anticancer activity due to the conformational flexibility of the molecule (Takai *et al*., 1985; Lawson *et al.,* 1988). Due to the structure activity relationship and good receptor binding piperidine considered as a leading nucleus having potent pharmacological activities (Vijayakumar *et al*., 2004). 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 (Platel and Srinivasan, 1996).

The α-amylase enzyme, the foremost product important for carbohydrate digestion, release from

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the pancreas (about 5-6%) and saliva producing glands (Boivin *et al.,* 1988; Whitcomb and Lowe, 2007). One exclusive behavior of pancreatic alpha-amylase enzyme is found at the site where the carbon atom of the terminal glutamine molecule forms a linkage with the amino group, hence results in the creation of a pyrrolidone subsidiary (Kandra, 2003) and this remarkable structure causes a protection from other digestive enzymes (van der Maarel *et al.,* 2002). In Asia, kalii mirch (*Piper nigrum*, source of piperine) has been a traditional remedy for gastrointestinal disorders as, it controls the secretion and the activity of amylase enzyme. Consequently piperidine being a chief constituent of black pepper and an alkaloid have ever been a positive factor for producing effects on digestive system (Tundis *et al.*, 2010).

As, the synthesized compounds have piperidine ring and this class of compounds has been reported as an excellent remedy for treating diabetes (Brugge and Rosenfeld, 1987; Clissord and Edwards, 1988) whereas interaction with alpha amylase enzyme of drug molecule, plays an important role for producing the hypoglycemic effect (Trescot *et al*., 2008).

Hence, the interaction of digestive enzyme specially alpha amylase was investigated because as the drug when taken orally, first come across the digestion and absorption prior to produce desired response; ADME (Brayer *et al.,* 1995; Brogard *et al.,* 1989; Scheppach *et al.,* 1989).

MATERIALS AND METHODS

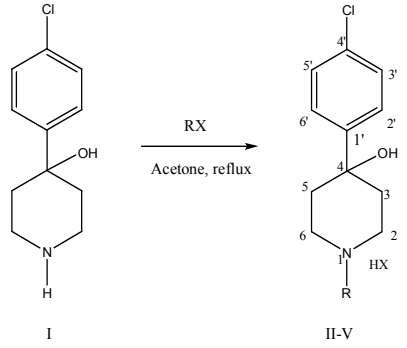
All the reagents of Sigma Aldrich Chemical Company and solvents of analytical grade were used. Melting point of the reactants and products was noted on Gallenkamp melting point equipment. In methanol-d, UV spectra was taken on 1601 spectrophotometer. IR spectra were noted on Jasco 302 Fourier transform FTIR spectrophotometer. Mass spectras were determined on electron impact (EI) condition by Varian Massen spectrometer MAT 312, MAT 113DMASPEC system.

The instrument used for HNMR spectra Bruker AM 300 spectrophotometer and the solvent was DMSO-d6/MeOD. The ^{13}C spectra were scanned by the same instrument as for HNMR.

General Method of Synthesis

For synthesizing the novel compounds, 4-(4'-Chlorophenyl hydroxy piperdine was selected as parent moiety and the reactions were conducted according to the designed scheme (Figure 1 and Table 1). During the reaction the parent compound was allowed to react with corresponding phenacyl halides in equimoler quantities (0.01 moles). Both the reactants were dissolved in 15-20 ml acetone separately and mixed. The reaction mixture was stirred on hot plate magnetic stirred for 24-48 h at temperature 60°C-80°C till the completion of reaction. The solid product was filtered and recrystallized. Melting point of the product was

Figure 1: Reaction Method Of 4-(4'-chlorophenyl)-4-hydroxy Piperdine Derivatives



I II-V

Table 1: Substituents of 4-(4'-Chlorophenyl) -4-hydroxy Piperdine

Compound No	R	X
II	$OC_{12}H_{17}$	Br
III	$O_2C_5H_5N_2$	Cl
VI	OC_9H_{11}	Br
V	C_9H_{11}	Br

noted and the compound further was confirmed through spectral information.

Reaction Scheme

STRUCTURAL ELUCIDATION OF SYNTHESIZED DERIVATIVES

Compound II; 1-(1"-Adamantan acyl)-4-(4'-chlorophenyl)-4-hydroxy piperidinium Hydrobromide,

White amorphous powder, 74% , mp 210 °C, UV λ_{max} (MeOH) nm: 222, IR n_{max} ($CHCl_3$) cm^{-1}: 3306,2912,1707,1598,1380, 980,825

^{1}H NMR (CD_3OD, 300 MHz) δ: 9.22 (1H,s, *N-H*), 7.50 (2H, d, *J*=8.4 Hz, H-22/H-62), 7.37 (2H, d, *J*=8.4 Hz, H-32/H-52), 4.51 (2H, S, H-7), 4.12 (1H, s, *H-4*), 3.52 (4H, m, H-2/H-6), 2.37 (4H,dt, *J*= 15.0, 4.5 Hz, H-3/H-5), 2.06 (3H, m, H-322/ 522/822),1.90 (6H, m, *H-222/ H-622/H-1022*), 1.79 (6H, m, *H -422/H-722/H- 922).* ^{13}C NMR (CD_3OD, 75 MHz): 209.3 (C-8), 147.1 (C-12), 134.3 (C-42), 129.5 (C-32/C-52), 127.4 (C-22/C-62), 72.1 (C-4), 62.5 (C-7), 51.3 (C-2/C-6), 47.1 (C-122), 38.9 (3C-222), 37.2 (3C-422), 36.5 (C-3/C-5), 29.2 (3C-322). Anal calcd. for $C_{23}H_{31}BrClNO_2$ (468.85); C=58.92%, H=6.66%, Br=17.04%, N=2.99%, O=6.82%, EIMS *m/z*: 388(M^+-HBr), 370, 224, 206.

Compound III: 1-(6"-Methyluracil)-4-(4'-chlorophenyl)-4-hydroxy piperidinium Hydrochloride, White amorphous powder, 53%, mp 220 °C, UV λ_{max} (MeOH) nm: 222.

IR n_{max} ($CHCl_3$) cm^{-1}: : 3020, 2933, 1710, 1540, 1416, 974, 821.

^{1}H NMR (CD_3OD, 300 MHz) δ: 8. 54(1H, s, *N-H*),7.49 (2H, d, *J*=8.4 Hz, H-22/H-62), 7.38 (2H, d, *J*=8.4 Hz, *H-32/H-52*), 5.66(2H,s, *H-122/H-322*), 4.73 (1H, s, *H-522*), 4.41 (2H, s , *H-7*), 4.34 (1H, s, *H-4*), 3.48-3.34 (2H, m, *H-2* α */H-6* α), 2.75-2.62 (2H, m, *H-2*β */H-6*β), 2.24-2.12 (2H, m, *H-3*α*/H-5* α), 1.95-1.90 (2H, m, *H-3* β */H-5* ß).

^{13}C NMR (CD_3OD, 75 MHz): 170 (C-522), 157 (C-222/C-422), 150 (C-42), 147.1 (C-12), 129.2 (C-62/C-22), 127.59 (C-52/C-32), 101.3 (C-622), 70.1 (C-4), 50.7 (C-2/C-6), 42 (C-7), 41.26 (C-3/ C-5). Anal calcd. for $C_{16}H_{19}Cl_2N_3O_3$ (372.25); C=51.62%, H=5.14%, Cl =1119.05%, N=11.29%, O=12.89%, EIMS *m/z*: 337 (M^+- HBr), 224, 206.

Compound IV: 1-(1"-Phenoxypropyl)-4-(4'-chlorophenyl)-4-hydroxy piperidinium Hydrobromide,

White amorphous powder, Yield: 74% , m p 210°C UV λ_{max} (MeOH) nm: 230, 270.

IR n_{max} ($CHCl_3$) cm^{-1}: 3327, 2919 , 1598, 1493, 1245,968,761.

^{1}H NMR (CD_3OD, 300 MHz) δ: 8.46 (1H,s, *N-H*) ,7.52 (2H, d, *J*=8.7 Hz, *H-22/H-62*), 7.44 (2H, d, *J*=8.7 Hz, *H-32/H-52*), 7.26 (2H, m, *H-222/H-622*), 6.93 (3H, m, *H-322/H-422, H-522*), 4.43 (1H, s, *H-4*) , 4.13 (2H, t, *J*=6.0 Hz, *H-9*), 3.34-3.50 (4H, m, H_2*-2/*H_2*-6*), 3.31 (2H, m, *H/7*), 2.30 (2H, m, *H/8*), 2.21 (2H, dt, *J*=13.5, 4.8, Hz, *H-3* α */H-5* α), 1.93 (2H, m, *H-3* β */H-5* β).

^{13}C NMR (MeOH, 75 MHz): 208.0 (C-8), 159.9 (C-122), 147.4 (C-12), 132.5 (C-22/C-62), 130.5

(C-222/C-622), 127.8 (C-32/C-52),122.3 (C-42), 122.2 (C-422), 115.1 (C-322/C-522), 69.3 (C-4), 66.1 (C-9), 61.6 (C-7) , 51.2 (C-2/C-6), 36.6 (C-3/C-5),

Anal calcd. for $C_{20}H_{25}BrClNO_2$ (426.78), C= 56.29%, H=5.90%, Br=18.72%, Cl=8.31, N=3.28%, O=7.50%, EIMS *m/z*: 346 (M^+ - HBr), 340, 338.

Compound V: 1-(3" -Phenylpropyl)-4-(4'-chlorophenyl)-4-hydroxy piperidinium Hydrobromide,

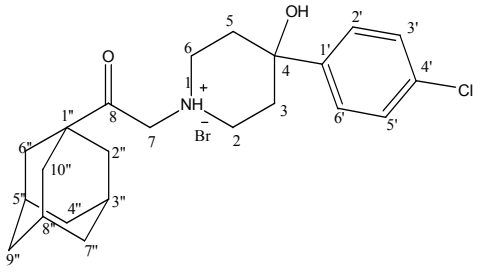
Off white crystalline powder , 55% , mp 230°C, UV λ_{max} (MeOH) nm: 230, 270

IR n_{max} ($CHCl_3$) cm^{-1}: 3323, 2925,1748, 1596,1381,1334

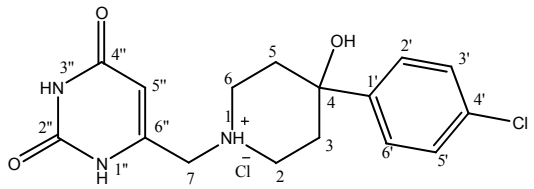
^{1}H NMR (CD_3OD, 300 MHz) δ: 9.01 (1H,s, *N-H*) , 7.52 (2H, d, *J*=8.7 Hz, H-22/H-62), 7.41 (2H, d, *J*=8.7 Hz, H-32/H-52), 7.33-7.18 (5H, m, H-122/ H-222/H-422, H-522/H-622), 4.14 (1H,s,*H-4*), 3.47-3.35 (4H, m, H-2/H-6), 3.17 (2H, m, H/7), 2.73 (2H, d , *J*= 7.5 Hz, H-9), 2.30 (4H, dt, *J*=14.1,4.7, H-3/H-5), 2.11 (2H, m, H/8)

^{13}C NMR (CD_3OD, 75 MHz): 147.4 (C-12), 141.5 (C-122), 132.5 (C-22/C-62), 129.7 (C-422), 129.4 (C-222/C-622), 127.7 (C-32/C-52),127.5 (C-322/C-522), 122.2 (C-42), 69.2 (C-4), 57.1 (C-7), 50.2 (C-2/C-6), 35.7 (C-3/C-5), 33.5 (C-9), 27.2 (C-8).

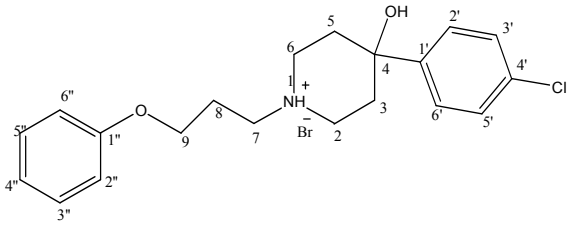
Compound II



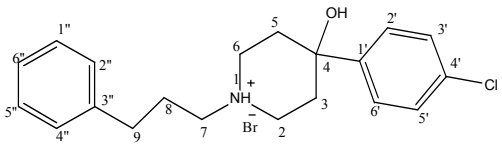
Compound III



Compound IV



Compound V



Anal calcd. for $C_{20}H_{25}BrClNO_2$ (409.08), C= 58.84 %, H=6.13%, Br=19.45%, Cl=8.63, N=3.41%, O=3.89%, EIMS *m/z*: 329 (M^+ - HBr), 325, 324.

In Vitro Antiamylatic Activity

For conducting the alpha amylase activity of compounds semiquantitative method Agar Plate Activity (Bernfeld, P., 1955) was adopted and for this purpose the enzyme α-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 in 1:1 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

Table 2: Antiamylatic Activity of 4-(4'-Chlorophenyl)-4-hydroxypiperdine Derivatives (II-V)

Compounds	Inhibition (mm) by Compounds with Amylase Enzyme	Inhibition (mm) by Amylase Enzyme
4-(4'-Chlorophenyl)-4-hydroxy piperdine (I)	15.4	23.3
1-(1"-Adamantan acyl)-4-(4' chlorophenyl)-4-hydroxy piperidinium Hydrobromide (II)	21.86	23.4
1-(6"-Methyluracil)-4-(4'-chlorophenyl)-4-hydroxy piperidinium Hydrochloride (III)	15.85	23.3
1-(1"-Phenoxypropyl)-4-(4'-chloroophenyl)-4-hydroxy piperidinium Hydrobromide (IV)	21.47	23.4
1-(3"-Phenylpropyl)-4-(4'-chlorophenyl)-4-hydroxy piperidinium Hydrobromide (V)	20.47	23.3

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 calliper (Figure 2).

ACTIVITY KEY

Below 10mm inhibition = no activity

From 11-20 mm inhibition = moderate activity

Above 20 mm inhibition = significant activity

Note: The size of lysis area was indicating the anti amylatic activity of compounds, as the better compatibility of the synthesized compounds with amylase enzyme facilitates the carbohydrate digestion. To observe the response of compounds with enzyme the above criteria was followed as activity key.

RESULTS AND DISCUSSION

The antiamylatic activity of parent and synthesized

Figure 2: Images of Agar Plate (Antiamylatic Activity) of Synthesized Compounds

compounds were presented in. Activity results were summarized in Table 2.

During the antiamylatic studies of synthesized compounds by agar plate method, parent compounds 4-(4'-Chlorophenyl)-4-hydroxy piperdine showed moderate inhibition but its derivative II, IV and V showed good quality antiamylatic activity as the zone of inhibition was almost similar as that of the standard enzyme. This kind of exhibition indicated that the substitution at nitrogen atom of piperidine ring was accountable for the enhancement of activity. As the alpha amylase enzyme controls the starch digestion and plays important role in metabolism, hence the compounds will not interact or hang the activity *in vivo*. But the derivative1-(6"-Methyluracil)-4-(4'-chlorophenyl)-4-hydroxy piperidinium hydrochloride (II) and alpha amylase is 15.85mm and that of alone enzyme is 23.1mm. It showed that this compound may interfere the activity and response of enzyme will cause a slight decline in response, here the presence of uracil in structure prevented the binding ability of enzyme with compound.

CONCLUSION

Among all the derivative greater part showed an excellent interface with α- amylase enzyme, while very few responded slightly non compatibility. The derivatives that were showing excellent antiamylatic activity, *in vivo* as drug will interact with α- amylase enzyme positively and pleasantly, hence superbly available to the target site. When taken orally, will not be affected by first pass metabolism. As the α- amylase in intestine from where the absorption of drug occurs and results shows that the compound will not agitate by α-enzyme and no presystemic elimination will be experienced unlike the morphine as it reaches very little to the site of action but in case of the synthesized compounds the maximum plasma level will achieved.

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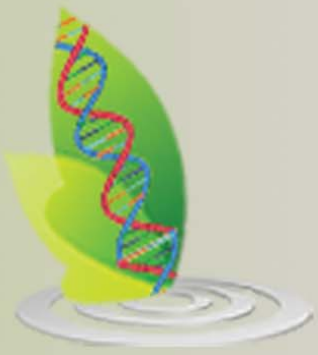

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