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SYNTHESIS OF SOME NOVEL ANALOGUES OF 4-(1-PYRROLIDINYL) PIPERIDINE AND THEIR EFFECT ON PLASMA GLUCOSE LEVEL

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ABSTRACT

In the present study some compounds of 4-(1-Pyrrolidinyl) Piperidine (I) have been synthesized. Structures of compounds were confirmed by using HNMR, IR, Mass and UV spectrophotometer techniques. All the derivatives (II, III, IV and V) and the parent compound (I) at the dose of 100 mg/kg were evaluated for their effect on plasma glucose level. Compound (II) was the only derivative which showed effect on plasma glucose level.

Keywords: Piperidine (I), plasma glucose level, derivatives.

INTRODUCTION

Diabetes is characterized by an impaired glucose metabolism manifesting itself among other things by an elevated blood glucose level in the diabetic patients (Lundgren *et al.,* 1999). It is affecting million of people worldwide and may lead to mortality if not treated properly. The treatment of diabetes has attracted global attention because of growing complications like cataract formation, retinopathy, neuropathy and nephropathy (Campfield *et al.* 1995; Mitani *et al.,* 2002; Coconi *et al.* 2003; Wiedeman and Trevillyan, 2003; Misra *et al.,* 2003).

In number of attempts piperidine and pyrrolidine ring containing compounds were evaluated for their effect on plasma glucose level (Campfield *et al.* 1995; Mitani *et al.,* 2002; Coconi *et al.* 2003; Wiedeman and Trevillyan, 2003; Misra *et al.,* 2003). A novel series of pyrrolidine-constrained phenethylamines were developed as dipeptidyl peptidase IV (DPP4) inhibitors for the treatment of type 2 diabetes (Backes *et al.,* 2007). Dipeptidyl peptidase-4 (DPP-4) inhibitors like sitagliptin and vildagliptin (an oral antidiabetics agent for Type2 diabetes) are promising new medicines for the treatment of type 2 diabetes mellitus. They are supposed to improve metabolic control (as measured by lowering blood glucose) without causing severe hypoglycaemia (low blood sugar levels leading to unconsciousness and other symptoms) (Richter *et al.,* 2008).

O-toluidine method is an established method for the determination of plasma glucose concentration and reported in number of studies (Dochev, *et al.,* 1983; Wolf and Zschiesche, 1986; Morcol and Velandar, 1991; Viner *et al.,* 1993; Reshetilov *et al.,* 1996; Pishak and Iarmol, 1998; Yarat *et al.,* 2001). Considering the potential for antidiabetic activity, four derivatives were synthesized having piperidien and pyrrolidine rings and evaluated for their effect on blood glucose level.

EXPERIMENTAL

General

Reagents were purchased from Aldrich Chemical Company. All solvents were reagents grade. Reactions were monitored by TLC using pre-coated silica gel, GF-254 and were visualized under ultraviolet light at 254 nm and 366 nm on HP-UVIS Desaga (Heidelberg). Iodine vapors were also employed for the detection of spots. All melting points were recorded on Gallenkamp melting point apparatus and are uncorrected. Solid calcium sulphate from E. Merck was used for drying reaction product after workup. Ultraviolet (UV) spectra were recorded in methanol/DMSO on a Hitachi U-3200 spectrophotometer. Infra Red (IR) spectra were measured on a Shimadzu IR 460 spectrophotometer using KBR disc. Mass spectra (MS) were determined on Varian Massen spectrometer MAT 311A spectrometer. Nuclear magnetic resonance (^{1}HNMR) spectra were recorded in DMSO-d_6 /MeOD on Bruker AM-300, 400 and 500 spectrometer operating at 300, 400 and 500 MHz.

Final pure product was confirmed by taking melting point and then instrumental methods were used to confirm the structure of the product. In IR spectra compounds gave peaks at 3400-3700 cm^{-1} (NH), 2900-2500 cm^{-1} (C-H), 1600-1700 cm^{-1} (C=O), 1500-1600 cm^{-1} (C=C), 13-1400 cm^{-1} (CH_2). Mass spectra (MS) were determined on varian Massen spectrometer mat 311a spectrometer. Ultraviolet (UV) spectra were

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recorded in Methanol/DMSO on a Hitachi U-3200 spectrophotometer. ^{1}HNMR was performed in d_6-DMSO and MeOD at 300, 400 and 500 MHZ. Chemical shifts are reported in ppm. Selected data are reported as: chemical shift, multiplicity (s =singlet, d =doublet, dd = doublet of a doublet, t = triplet, m = multiplet), number of protons (1H = one proton, 2H = two proton,……. NH = n proton), coupling constant, *J*=(HZ), and assignment = *H*-n. Signals were at δ 7-9 showing the presence of aromatic hydrogen while a sharp singlet at δ 3-6 confirmed the presence of CH2 of the chain of the phenacyl moiety. Peaks at δ 1-3 showing the presence of remaining aliphatic hydrogens.

Method of synthesis compounds (II-V)

To a stirred solution of **I** (1.48g, 1mmole) in acetone (15-20ml) was added successively substituted phenacyl halide (2.44g, 1mmole) dissolved separately in 15-20 ml acetone. Reaction mixture stirred for 48 hours at room temperature. The process of reaction was monitored through thin layer chromatography. The crude solid product was filtered and washed with acetone. The product thus obtained was purified through recrystallization by using warm ethanol and ether. The pure compound was dried in desiccator over anhydrous calcium sulphate. Melting point was recorded and spectral data were obtained to confirm the structure of compound.

4-Pyrrolidin-1′-yl-1-[2-(2″-nitro-phenyl)-2-oxo-ethyl]-piperidinium bromide (II) ^{1}HNMR (MeOH, 500 MHz) δ: 7.55 (d, 1H, *J*=7.87Hz, *H*-3″), 7.35 (d, 1H, *J*=8.11HZ, *H*-6″), 7.10-7.06 (m, 1H, *H*-5″), 7.01-6.98 (m, 1H, *H*-4″), 4.86 (s, 2H, *H*-7), 3.83 (d, 1H, *J*=10.82 Hz, *H*-4), 3.01-2.74 (m, 8H, *H*-2′, *H*-5′, *H*-2, *H*-6), 1.81-1.49 (m, 8H, *H*-3′, *H*-4′, *H*-3, *H*-5); EIMS m/z: 317 (M^+-Br, $C_{17}H_{23}N_3O_3$), 154, 124, 110, 98, 80, 72, 57, 53; IR ν_{max} (KBr) cm^{-1}: 3423, 3305, 2950, 1750, 1601, 1593, 1396, 1080, 812, 540; U λ_{max} (MeOH) nm: 295, 201, 198

4-Pyrrolidin-1′-yl-1-[2-(3″-nitro-phenyl)-2-oxo-ethyl]-piperidinium bromide (III) ^{1}HNMR (MeOD, 400 MHz) δ: 8.80 (s, 1H, *H*-2″), 8.47-8.45 (m, 1H, *H*-4″), 8.41-8.38 (m, 1H, *H*-6″), 7.77 (t, 1H, *J*=7.97Hz, *H*-5″), 4.86 (s, 2H, *H*-7), 4.06 (s, 1H, *H*-4), 3.26-3.10 (m, 4H, *H*-2′, *H*-5′), 2.39-2.33 (t, 4H, *J*=10.32Hz, *H*-2, *H*-6), 2.14-2.10 (m, 4H, *H*-3′, *H*-4′), 1.96-1.80 (m, 4H, *H*-3, *H*-5); EIMS m/z: 317 (M^+-Br, $C_{17}H_{23}N_3O_3$), 300, 219, 203, 153, 124, 98, 70, 57; IR ν_{max} (KBr) cm^{-1}: 3433, 3163, 2941, 2572, 1674, 1595, 1396, 1078, 717; UV λ_{max} (MeOH) nm: 228, 204, 201,

4-Pyrrolidin-1′-yl-1-[2-(2″,4″-dimethoxy-phenyl)-2-oxo-ethyl]-piperidinium bromide (IV) ^{1}HNMR (MeOD, 500 MHz) δ: 8.00 (d, 1H, *J*=8.93HZ, *H*-6″), 6.68 (m, 2H, *H*-3″, *H*-5″), 4.84 (s, 6H, O*CH*$_3$-2″, O*CH*$_3$-4″), 4.68(s, 2H, *H*-7), 3.90 (s, 1H, *H*-4), 3.30-3.17(m, 4H, *H*-2′, *H*-5′), 3.14-3.11(m, 4H, *H*-2, *H*-6), 2.45 (d, 4H, *J*=13.3HZ, *H*-3′, *H*-4′), 2.10, 2.02 (m, 4H, *H*-3, *H*-5); EIMS m/z: 332 (M^+-HBr, $C_{19}H_{28}N_2O_3$), 154, 124, 110, 98, 82, 79, 56; IR ν_{max} (KBr) cm^{-1}: 3745, 3444, 2952, 1749, 1601, 1593, 1454, 1080, 812, 538; UV λ_{max} (MeOH) nm: 389, 293, 202.

4-Pyrrolidin-1′-yl-1-[2-(3″,4″-dihydroxy-phenyl)-2-oxo-ethyl]-piperidinium bromide (V) ^{1}HNMR (MeOD, 500 MHz) δ: 7.45-7.38 (m, 2H, *H*-2″, *H*-6″), 6.82 (d, 1H, *J*=8.08HZ, *H*-5″), 4.88 (s, 2H, O*H*-3, O*H*-4), 3.90 (s, 1H, *H*-4), 3.87 (s, 2H, *H*-7), 3.14-3.09 (m, 4H, *H*-2′, *H*-5′), 2.32-2.11 (m, 4H, *H*-2, *H*-6), 2.05-1.99 (m, 4H, *H*-3, *H*-4), 1.86-1.81(m, 4H, *H*-3, *H*-4), 1.86-1.81(m, 4H, *H*-3, *H*-5); EIMS m/z: 304 (M^+-HCl, $C_{17}H_{24}N_2O_3$), 167, 153, 124, 96, 70, 57; IR ν_{max} (KBr) cm^{-1}: 3679, 3361, 2933, 1749, 1656, 1512, 1394, 1278, 806, 621; UV λ_{max} (MeOH) nm: 279, 231, 206, 199.

PHARMACOLOGY

Anti-diabetic activity

Animals

Male Sprague-Dawley rats (locally bred) weighing 200-300 g were purchased from Aga Khan University and Hospital, Karachi. Animals were kept individually in plastic cages in the same environmental conditions with free access to water and standard rodent diet for about three days before experimentation, Rats were randomly assigned as control and test groups taking six animals in each group. Analogues

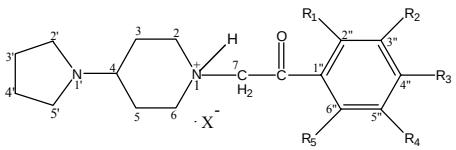
Drugs and Reagents

Test compounds, water for injection, trichloro acetic acid (TCA 3% w/v), O-toludine reagent (0.15g of thiourea, 6ml pure O-toludine and glacial acetic acid to make volume 100ml), stock standard of glucose (10mg/ml, 1g reagent grade glucose was dissolved in 0.2% w/v benzoic acid and diluted up to 100ml), working standard of glucose (1mg/ml, 1ml of stock standard was diluted up to 10ml with deionized water).

Method

Test compounds were given to the rats intraperitonially (i.p), while control received only vehicle (water for injection). Following decapitation 2 hour after the saline or test compound injection, blood for plasma was collected in tubes containing heparin. Samples were centrifuged for 15 minutes and plasma was decanted into eppendorf tubes for storage at -70^{o}C until analysis.

Each plasma sample (0.1ml) was mixed with deionized water (0.1ml) and then TCA (3%, 1.8ml) was added. For calibration curve 0.05, 0.1 and 0.2ml of working standard were taken, volume of each standard was made up to 0.2ml by deionized water while for blank only 0.2ml of deionized water was used TCA (3%, 1.8ml) was added to all standards and blank.



(I)

(II) R_1=NO_2; R_2=H; R_3=H; R_4=H; R_5=H; X=Br
(III) R_1=H; R_2=H; R_3= NO_2; R_4=H; R_5=H; X=Br
(IV) R_1=OCH_3; R_2=H; R_3=OCH_3; R_4=H; R_5=H; X=Br
(V) R_1=H; R_2=OH; R_3=OH; R_4=H; R_5=H; X=Cl

Fig. 1: 4-(1-Pyrrolidinyl)Piperidine (I) and its derivatives (II-V)

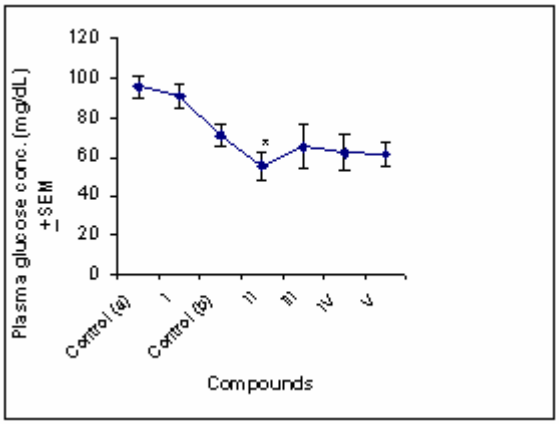


Fig. 2: Effect of 4-(1-Pyrrolidinyl)Piperidine (I) and its derivatives (II, III, IV, V) on plasma glucose concentration in normal rats.

Table 1: Effect of 4-(1-Pyrrolidinyl)Piperidine (I) and its derivatives (II, III, IV, V) on plasma glucose concentration in normal rats.

Compound	Plasma glucose concentration mg/dL ±SEM
Control *a* (for parent)	95.67±5.22
4-(1-Pyrrolidinyl)Piperidine **(I)**	91.3±6.4
Control *b* (for derivatives)	70.67±5.7
4-Pyrrolidin-1′-yl-1-[2-(2″-nitro-phenyl)-2-oxo-ethyl]-piperidinium bromide **(II)**	55.67*****±7.3
4-Pyrrolidin-1′-yl-1-[2-(3″-nitro-phenyl)-2-oxo-ethyl]-piperidinium bromide **(III)**	65±11.34
4-Pyrrolidin-1′-yl-1-[2-(2″,4″-dimethoxy-phenyl)-2-oxo-ethyl]-piperidinium bromide **(IV)**	62.16±9.2
4-Pyrrolidin-1′-yl-1-[2-(3″,4″-dihydroxy-phenyl)-2-oxo-ethyl]-piperidinium bromide **(V)**	61.33±6.42

n / group = 6
Significant differences by student 't' test: *p<0.05, **p<0.01 as compared to control.

All the samples, standards and blank were allowed to stand for 5 minutes and then centrifuged for 20 minutes. 1ml of aliquotes was taken out from standard and blank tubes and 5ml of O-toludine reagent was added to all. All tubes were heated in a boiling water bath for 10 minutes the extinction was read at 630 nm.

CALCULATION

Statistical analysis was performed using student t-test and values were considered significant or highly significant when P<0.05 or P<0.01 respectively.

RESULTS AND DISCUSSION

In the present course of study Compound 4-(1-Pyrrolidinyl)piperidine **(I)** fig. 1 and its derivatives **(II, III, IV and V)** were evaluated for their effect on plasma glucose concentration by O-toluidine method in normal rats represented by table 1 and fig. 2.

The inhibitory activity is expressed as mg/dL of glucose concentration inhibited by compound I and its four derivatives at the dose of 100mg/kg of body weight. Normal blood glucose level in rat is 50 to 135 mg/dL.

Parent compound I did not produce any effect on glucose concentration, while among its derivatives (II, III, IV and V) only compound II was able to show the effects on plasma glucose level.

Derivatives evaluated have nitro, methoxy and hydroxy substitution in the structure.

4-Pyrrolidin-1-yl-1-[2-(2-nitro-phenyl)-2-oxo-ethyl]-piperidinium bromide (II) showing marked change in the level of glucose has nitro substitution at 2-C of phenyl ring. This compound significantly decreased the level of glucose in normal rats comparing to control.

4-Pyrrolidin-1-yl-1-[2-(3-nitro-phenyl)-2-oxo-ethyl]-piperidinium bromide (III) which was found without any effect on glucose concentration also has nitro group attached at C-3 of phenyl ring. The only difference of position of nitro in compounds II and III revealing that the position of nitro group making the compound active or inactive for hypoglycemic activity in normal rats.

4-Pyrrolidin-1-yl-1-[2-(2,4-dimethoxy-phenyl)-2-oxo-ethyl]-piperidinium bromide (IV) and 4-Pyrrolidin-1-yl-1-[2-(3,4-dihydroxy-phenyl)-2-oxo-ethyl]-piperi-dinium bromide (V) having methoxy substitution at 2 and 4-C and hydroxy substitution at 3 and 4-C respectively failed to produce any significant effect on the concentration of glucose.

Among all the nitro, methoxy and hydroxy derivatives of compound I only nitro derivative II demonstrated effect on glucose and comparing two nitro derivatives only *ortho* nitro derivatives showed ability of effecting glucose concentration.

CONCLUSION

Considering these results it can be seen that not only the functional group but the position of functional group is playing important role for hypoglycemic activity in normal rats.

Presently the effect of compound on enzyme inhibition is under investigation to see the effect of compound at enzymatic level. There is every possibility in future a desired chemical skeleton can be designed having regioselective nature of receptor.

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