ANTIBACTERIAL, ANTIFUNGAL AND ANTIOXIDANT ACTIVITTES OF DERIVATIVES OF ALKYL PIPERIDINE

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Abstract

Antibacterial, antifungal and antioxidant activities of alkyl piperidine derivatives have been reported. Derivatives of compound I (Ia- If) and compound II (IIa-IIf) were screened for antibacterial activity against several gram negative and gram-positive bacteria. The criterion of activity was the inhibition of growth of microorganisms i.e. zone of inhibition and it is measured in millimeter. It was found that all the derivatives displayed moderate to good antibacterial activity.

Introduction

Piperidine is one of the basic constituents in pepper, *Piper nigrum L.* (Johnstone. 1888; Spath and Englaender, 1935) and tobacco (Spath and Zajic. 1936) in low yield, abundantly in *Psilocaulon absimile* (Remington and African. 1934) and in *Petrasimonia monandra* (Yurashevskii and Stepanov. 1939). It is a liquid with characteristic odor and highly toxic upon inhalation and skin absorption (Cahours. 1852). However, it is the member of a series of bases that have attracted the attention of alkaloid chemists (Manske. 1959) since several decades.

Therapeutically active agents having piperidine ring as essential part are well known for their anti-microbial activities (Thomas. 1988). Molecular modifications of these compounds led to new opiates or narcotic analgesic (Janseen. *et al.*, 1986; Brown. *et al*., 1995; Saukara. *et al.*, 2001; Sabine. *et al.*, 2003; Dianging. *et al*., 2009; Girisha. *et al*., 2009 and Vinaya. *et al*., 2010).

Piperidine derivatives have also been evaluated for their antibacterial and antifungal activities by a number of workers both *in vivo* and *in vitro.* (Mukhopadhyay. *et al.*, 2003). Antiviral and antifungal activities of certain piperidine analogs have also been discovered. Among them, 1-amidino-3-amino-2-C-methyl-D-glucitols had shown pronounced antiviral activity (McKenzie. *et al*., 1949).

The interest in piperidine derivatives is due to their pharmacological activity as radical scavengers (Cotelle. *et al.*, 1996). The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples (Lee. *et al.,* 2003). It was found that the radical scavenging activities could be increased with increasing concentration (Kamran. *et al*., 2009).

The structures of compound Ia and Id shows that due to the presence of nitro group at *meta* position in compound Ia it exhibited 22% inhibition while in the structure of Id nitro group is present at *para* position which was devoid of antioxidant activity. It is concluded that changes in the position of functional group also affects the antioxidant activity. It was clearly shown that *meta* nitro derivative was successful in getting antioxidant activity (Ia) which was lost in the *para* nitro derivative. Introduction of another nitro group at *meta* position i.e. 3′, 5′, Dinitro derivative (IIf) was also not successful to induce antioxidant activity.

In compound Ib, bromo group was present at *para* position which showed 5% inhibition and in compound Ic fluoro group is present at *para* position which was found highly active exhibiting 31% inhibition. In compound Ie methoxy group was present at *para* position, which is devoid of antioxidant activity whereas in compound If chloro group is present at *para* position and it showed 16% inhibition. Derivatives of compound II i.e., IIa, IIb, IIc, IId, IIe and IIf were totally devoid of antioxidant activity.

Among all the derivatives having different functional groups and among the halogenated derivatives, compound Ic having the flouro group at *para* position was found the most active one in this series.

The following newly synthesized (synthesis have been submitted for publication in Pakistan Journal of Pharmaceutical Sciences) derivatives of alkyl piperidine were employed for antibacterial, antifungal and antioxidant activities.

(I) Piperidine-2-methanol (parent compound).
(Ia) 2-hydroxymethyl-1-[(3-nitro-phenyl)-2-oxoethyl]-piperidinium bromide.
(Ib) 2-hydroxymethyl-1-[(4-bromo-phenyl)-2-oxoethyl]-piperidinium bromide.
(Ic) 2-hydroxymethyl-1-[(4-fluoro-phenyl)-2-oxoethyl]-piperidinium bromide.
(Id) 2-hydroxymethyl-1-[(4-nitro-phenyl)-2-oxoethyl]-piperidinium bromide.

(Ie) 2-hydroxymethyl-1-[(4-methoxy-phenyl)-2-oxoethyl]-piperidinium bromide.
(If) 2-hydroxymethyl-1-[(4-chloro-phenyl)-2-oxoethyl]-piperidinium bromide.
(II) Piperidine-2-ethanol (parent compound).
(IIa) 2-hydroxy-ethyl-1-[(4-bromo-phenyl)-2-oxoethyl]-piperidinium bromide.
(IIb) 2-hydroxy-ethyl-1-[(4-fluoro phenyl)-2-oxoethyl]-piperidinium bromide.
(IIc) 2-hydroxy-ethyl-1-[(3-nitro- phenyl)-2-oxoethyl]-piperidinium bromide.
(IId)2-hydroxy-ethyl-1-[(4-nitro-phenyl)-2-oxoethyl]-piperidinium bromide.
(IIe) 2-hydroxy-ethyl-1-[2-nitro-phenyl)-2-oxoethyl]-piperidinium bromide.
(IIf) 2-hydroxy-ethyl-1-[3',5',dinitro-phenyl)-2-oxoethyl]-piperidinium bromide.

Materials and Methods

Determination of Antibacterial Activity: Among various methods Agar well diffusion method was employed to test the compounds for *in vitro* antibacterial activity (Carron. *et al.,* 1987).

Media Preparation: Three types of media were used

1- Solid medium (Nutrient agar).
2- Liquid medium (Nutrient broth).
3- Semi-solid medium (Soft agar).

Nutrient agar: Dissolved 28 gm nutrient agar in one liter distilled water and autoclaved at 121^{o}C for 15 minutes, cool up to 45^{o}C and pour 40-50 mL media in sterile (14 cm diameter) Petri dishes, and then allow solidifying the media and keep it at room temperature to check the sterility of the prepared media.

Nutrient Broth: 0.8 gm nutrient (soft Agar) broth was dissolved in 100 L distilled water and dispense approximately 3mL nutrient broth in screw capped test tubes and autoclaved at 121^{o}C for 15 minutes and then refrigerated.

Test Sample preparation: 1 mg/mL in DMSO.

1. First day: Single colony of bacterial culture was inoculated in nutrient broth and incubate it at 37^{o}C for 24 hours.
2. Second day: Soft agar tube was melted and cooled up to 45^{o}C then added 10 µL of fresh bacterial culture, shaked well and then poured on to the nutrient agar containing plate. Plate was rotated to make even distribution of the culture and then allowed to solidifying.
3. Make wells by using 6mm-diameter sterile borer.
4. Mark the well with sample code.
5. Added 100 µL of sample in respective agar well plate according to bacterial culture.
6. Other wells supplemented with DMSO and reference antibacterial drug serving as positive and negative control.
7. Incubate the plates at 37^{o}C for 24 hours.
8. Next day note down the result in terms of zone of inhibition in mm.

All these cultures were kept at 4^{o}C prior to testing. They were sub-cultured in liquid nutrient broth and incubate at 37^{o}C for 18-24 hours and then used for the screening (Alves. *et al*., 2000; Stepanovic. *et al*., 2003 and Carron. *et al*., 1987).

Determination of Antifungal Activity: Compounds have been tested for antifungal activity by Agar tube dilution method (Attaur-Rahman. *et al*., 1999).
During the present study, test compounds were screened through the following stages:
First screening (preliminary screening): In the preliminary stage of the antifungal bioassay, the test compounds were screened against the following fungi:
Trichophyton longifusis, Candida albicans, Candida glabarata, Fusarium solani, Microsporum canis, Aspergillus flavus, Aspergillus niger, Rhizopus sp. and *Sachromyces cervaciae* taking miconazole, penicillin and amphotericin B as standard drugs.
If the test compound shows, significant activity that is above 70% growth inhibition then MIC is calculated. If the MIC is equal to or higher than the MIC of the reference drug it is considered as an active compound against the fungi mentioned above.

Agar Tube Dilution Method

1. Test sample was prepared by dissolving 12 mg of pure compound in 1 mL sterile DMSO serving as stock solution.
2. For the growth of fungus, Sabouraud dextrose agar (SDA) was used. Media with acid (pH 5.5-5.6) containing relatively high concentration of glucose or maltose 2% is prepared by mixing 32.5 gm/500 mL distilled water.
3. It is then steamed to dissolve the contents and dispensed as volumes 4mL into screw capped tubes.
4. Autoclaved at 121^{o}C for 15 min.
5. For loading of sample, tubes were allowed to cool to 50^{o}C and non-solidified SDA is loaded with 66.6 µL of compound pipette from the stock solution. This will give the final concentration of 200µg/mL of the media for test compound.
6. Tubes then allowed for solidifying in slanting position at room temperature.
7. Each tube was inoculated with 4 mm diameter piece of fungus removed from a seven-day old culture of fungus.
8. For non-micellial growth, an agar surface streak was employed.
9. Other media supplemented with DMSO and reference antifungal drugs were used as negative and positive controls respectively.
10. The tubes were incubated at 27-29^{o}C for 3-7 days.
11. Cultures were examined twice weekly during incubation.

Growth in the compound amended media was determined by measuring linear growth (mm) and growth inhibition calculated with reference to the negative control.
Calculating % Inhibition of fungal growth:
% Inhibition =100 - linear growth in test (mm)/ linear growth in control (mm) × 100
(Choudhary *et al*., 1995; Janaki and Vijayasekaram.1998).

Determination of Antioxidant Activity: Antioxidant activity of synthesized compounds was determined by the method reported by Lee *et al.* (1998). In this method, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was prepared in ethanol and all of the samples were prepared in dimethylsulfoxide (DMSO). Reaction mixture containing 10 µL of test samples and 90 µL of DPPH were added in 96- well micro titer plates (final conc. of sample is 200 µg/mL and DPPH is 300µM solution). Plates were incubated at 37^{o}C for 30 minutes. Absorbance was measured at 515 nm using spectrophotometer. Percent inhibition by sample treatment was determined by comparison with DMSO treated control group.

$$\%\ \text{inhibition} = \frac{\text{Absorbance of the control} - \text{Absorbance of test sample}}{\text{Absorbance of the control}} \times 100$$

Ascorbic acid was used as standard control.

The EC_{50} value calculated denotes the concentration (µg/mL) of sample required to scavenge 50% of DPPH (Lee. *et al*., 1998; Casetta. *et al*., 2005; Fang. *et al*., 2002; Joyce. D.A. 1987; Liu and Ng. 2000; Zheng and Wang. 2001).

Results and Discussions

Antibacterial activity: Results of antibacterial activity of compounds I (Piperidine 2-methanol, Ia-If) and II (Piperidine 2-ethanol, IIa-IIf) have been represented in the table 1. From these tables it was found that all the derivatives displayed moderate to good antibacterial activity.

The activity against gram-positive bacteria was good by compound I and Ia as compared to compound Ib. Compound Ic showed good activity against *Bacillus subtillus* and *Bacillus cereus* and moderate activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*. Compounds Ie, If, II and IIa also showed good activity against gram-positive microorganisms. While against gram-negative compound I and Ia showed low activity and compounds Ic, Ie, II and IIa showed moderate activity. Most of the derivatives were found effective as broad spectrum antibacterial agents.

Table 1. Screening of piperidine-2-methanol and piperidine-2-ethanol and their derivatives for antibacterial activity *in vitro* against gram-positive organisms, gram-negative organisms, against fungal species and antioxidant activity.

		Against gram (+ve)				Against gram (-ve)			Against fungal species					Antioxidant
S.No.	**Compounds**	**BS**	**BC**	**SA**	**SE**	**EC**	**PA**	**ST**	**AN**	**AF**	**RS**	**CA**	**SC**	**Inhibition %**
1	Piperidine-2-methanol(I)	10	10	-	-	9	9	10	9	-	-	-	-	-
2	Derivative Ia	10	10	10	9	9	9	10	9	-	-	-	10	22%
3	Derivative Ib	10	9	10	10	-	-	-	9	-	+	-	10	05%
4	Derivative Ic	10	10	12	12	10	9	9	10	-	+	-	-	31%
5	Derivative Id	-	-	-	-	-	-	-	9	10	+	-	-	-
6	Derivative Ie	10	10	10	10	10	10	10	10	+	-	10	10	-
7	Derivative If	10	-	-	-	-	-	-	10	10	-	12	12	16%
8	Piperidine-2-ethanol(II)	10	10	10	10	10	10	10	-	-	-	-	-	-
9	Derivative IIa	10	10	10	10	10	10	10	9	-	-	-	-	-
10	Derivative IIb	12	12	12	12	-	-	-	9	-	+	-	10	-
11	Derivative IIc	9	9	9	9	10	10	10	10	-	+	-	-	-
12	Derivative IId	8	8	8	8	9	9	9	9	10	+	-	-	-
13	Derivative IIe	10	10	10	10	10	10	10	10	+	-	10	10	-
14	Derivative IIf	10	10	10	10	8	8	8	10	10	-	12	12	-
15	Standard Drug	35	35	50	20	35	20	31	12	12	10	10	12	-

Activity Key:
- = No zone of inhibition
12= good activity
10 =Moderate activity
9 = low activity
Std. Drug Imipenum 10 µg / disc

Species*: BS=*Bacillus subtillus*, BC=*Bacillus cereus*, SA=*Staphylococcus aureus*, SE=*Staphylococcus epidermidis*,EC=*Escherichia coli*, PA=*Pseudomonas aeruginosa*, ST=*Salmonella typhii*, AN=*Aspergillus niger*, AF=*Aspergillus flavus*, RS=*Rhizopus sp.* , CS=*Candida albicans*, SC=*Sacharomyces cervaciae*.

For antioxidant activity Ascorbic acid (controlled): 87%
Concentration used: 100µg/mL

It was shown that the parent compounds possessed some amount of antibacterial activity but the derivatives of parent I showed pronounced and significant effects against different strains while the derivatives of parent II were found inactive except compound IIb. This compound 2-[1-Bromo-2-(2-hydroxy-ethyl)-piperidine-1-yl]-1-(4-fluoro phenyl)-ethanone exhibited significant antibacterial activity against all the tested strains of bacteria.

Among the halogenated derivatives *para* flouro derivative of parent II was found highly active against gram +ve organisms. Among the nitro derivatives, compound IIc displayed its activity against some strains of gram –ve while IId was found effective against both gram -ve and gram +ve organisms. *Ortho* nitro (IIe) and *meta* dinitro (IIf) were ineffective.

The activity possessed initially by the compound II (piperidine-2-ethanol) against *Bacillus subtillus* and *Bacillus cereus* was lost completely in some derivatives and or became insignificant in the others.

The derivatives (Ia-If and IIa-IIf) of piperidine-2-methanol (I) and piperidine-2-ethanol (II) tested for antibacterial activity exhibited moderate to good activity against gram +ve and gram –ve organisms. This effect may be due to the fact that the quaternary ammonium compounds attack gram +ve and gram –ve bacteria (Arniker. *et al.,* 1992). Therefore, these derivatives being quaternary compounds may be included in the class of broad spectrum antibiotics.

Antifungal Activity: Compounds I, Ia-If, II and IIa-IIf (table 1) were evaluated for their antifungal activity against six fungal cultures.

Antifungal activity results concluded that compounds I, Ia, Ib and Id showed low activity while compounds Ic, Ie and If showed moderate activity. Compound II showed no activity against any fungal specie and compound IIa showed low activity only against Aspergillus niger.

The parent compound (I) was found active against Aspergillus niger and its derivatives showed low to moderate activity against Aspergillus niger and to a little extent to Aspergillus flavus. They showed no activity against other strains.

Parent II was totally inactive against all the tested fungal strains while it's only one derivative IIa 2-hydroxy-ethyl-1-[(4-bromo-phenyl)-2-oxoethyl]-piperidinium bromide was found active only for aspergillus niger. As a whole antifungal activities were not very encouraging.

The compounds found active as antifungal agents were most effective against two fungal strains *candida albicans* and *sacharomyces cervaciae*. These both are chloro phenacyl (If) and dinitro phenacyl (IIf) derivatives. Therefore, it can be predicted that chloro and dinitro groups contributed to the fungicidal activity.

Antioxidant Activity: Antioxidant activity of compound I, compound II and their derivatives were determined through the method reported by (Lee, e*t al*., 1998). The results of antioxidant activity have been represented in table- 4.

From the table it was evident that the compounds I, II, and their derivatives showed no significant inhibition except only a few.

The parent compounds, I and II were totally devoid of antioxidant activity but some derivatives of parent I (Ia, Ib, Ic and If) displayed some amount of antioxidant activity. As compared to their parent (I), Ia showed 22%, Ib 5%, Ic 31% and If 16% inhibition at the test dose which were not significant. But the derivatives of parent II were not succeeded to attain the antioxidant activity.

Among the series of these two parent molecules, the derivatives of only parent I exhibited some amount of antioxidant activity. Parent I has methanol group at the 2 position while parent II contains ethanol group at the same position. The only difference was in the chain length of the alkyl group i.e., only one $-CH_2$. Therefore, it can be predicted that methanol group in conjunction with halogens is responsible to impart antioxidant behavior.

Here, it is evident that the parent molecules expressed no antioxidant activity while their derivatives displayed some activity at the test dose. As it is explored earlier (Kamran, *et al.,* 2009) that antioxidant activity is concentration dependent, therefore, the significant activity can be obtained by increasing the dose or concentration.

Conclusions

The parent compound (I) was found active against *Aspergillus niger* and its derivatives showed low to moderate activity against *Aspergillus niger* and to a little extent to *Aspergillus flavus*. They showed no activity against *Rhizopus sp*., *Candida albicans* and *Sacharomyces cervaciae*. Parent II was totally inactive against all the tested fungal strains while its only one derivative IIa 2-hydroxy-ethyl-1-[(4-bromo-phenyl)-2-oxoethyl]-piperidinium bromide was found active only for *Aspergillus niger*. Antifungal activities were not found encouraging.

However, the results obtained from antioxidant assay were somewhat encouraging. Among the halogenated derivatives, compound Ic, having the flouro group at *para* position was found the most active one in this series. Among the series of these two parent molecules, the derivatives of only parent I exhibited some amount of

antioxidant activity. Parent I has methanol group at the 2 position while parent II contains ethanol group at the same position. The only difference is in the chain length of the alkyl group i.e., only one -CH_2. Therefore, it can be predicted that methanol group in conjunction with halogens might be responsible to impart antioxidant behavior.

In conclusion it can be suggested that by selecting appropriate functional groups at appropriate positions and by increasing the concentration of the compounds to be evaluated and/or by selecting the appropriate test methods it is possible to achieve significant antioxidant activity. The compounds might become more active.

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