Pakistan Journal of Pharmaceutical Sciences
Vo111(1), January 1998, pp.15-21

SYNTHESIS AND ANTI-MICROBIAL SCREENING OF SOME PIPERIDINE DERIVATIVES

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ABSTRACT

Synthesis of piperidine derivatives viz, 1-(4'-methoxy phenacyl)-N-Methyl piperidinium bromide, 1-(4'-nitrophenacyl)-N-methyl piperidininm bromide, and 1-(3', 4'-dihydroxy phenacyl)-N-methyl piperidinium chloride was carried out by quarternizing N-methyl piperidine with corresponding phenacyl halides. The structures of these compounds were elucidated by spectroscopic techniques such as UV, IR, El mass and ^{1}H-NMR. The compounds were subjected to antimicrobial screening in quantities of 10 mg/ml dissolved in DMSO. Disc diffusion method was used to observe the significance of these compounds against seven bacterial and one fungal cultures.

INTRODUCTION

Amines containing nitrogen as part of a complex ring system are quiet common in nature. Compounds that have one or more atoms in the ring other than carbon are called heterocyclic compounds. One example of a simple nitrogen heterocyclic compound is piperidine which is a secondary amine (Ouellette, 1984). Various derivatives of piperidine molecule are used as antimicrobial agents (Cross and Dawe, 1979; Rusinov et al., 1990; Takatani et al., 1992).

Adrenaline and tyrosine being biogenic amines constitute one of the important parameters in transforming the essential neuronal messages to the required site of action. It would be specifically important if such system is attached with piperidine or pyridine like compounds.

Kronhnke (1934) observed that certain I-substituted pyridinim compounds possess both pressor and ergot like activity and antibacterial activity. An investigation on various derivatives of 1-phenacyl pyridinium salts was carried out to assess the anti- tubercular activity. The synthetic compounds showed anti- tubercular activity, particularly 4-alkyl-l-(2-hydroxy-2-phenyl ethyl)-piperidines. Compounds 4-(I-hexyl)-1-(2-hydroxy-2-phenyl ethyl) piperidine hydrochloride was found to be only 0.6%as active as chlotomycetin against Salmonella, whereas compound 4- (1-octyl)-1-(2-hydmxv-2-phenylethyl) piperidine hydro-chloride exhibited amoebicidal activity at 1:5000 and inactive at 1:50,000 dilution. However, 4-alkyl-l-(2-hydroxy-2-phenyl ethyl) piperidines were active at 10 mg% in the presence of serum (Tmilt and Middleton, 1951; Trail(and Bryant, 1952; Tmilt et al., 1952).

During literature search it was also observed that certain compounds that possess a piperidine group in their structural chain behave as good antibacterial agents. One example of such

compound is reported by Kanda et al. (1994). This compound showed a minimum inhibitory concentration of 0.18 mg/ml against Candida albicans and Pseudomonas aemginosa.

In view of these studies it was proposed to prepare new derivatives of piperidine so that their potential as growth inhibitors of microorganisms could be investigated. Thus, three compounds were synthesized by re0uxing N-methyl piperidine with three different phenacyl halides and were subjected to anti-bacterial and anti-fungal testing.

EXPERIMENTAL

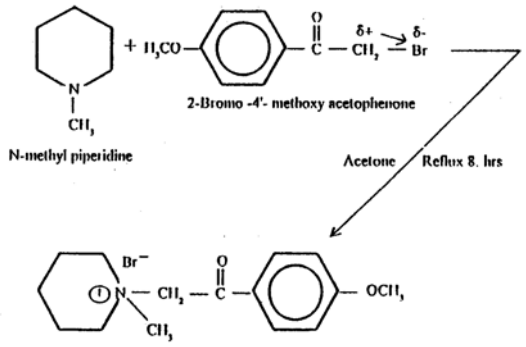
Material, Reagents and Instruments:

N-methyl piperidine. 2bromo-4-methyl acetophenone. 2-bromo-4'- nitroacetophenone, and 2chloro-3', 4'-dihydroxv acetophenone were obtained from Aldrich (England). Extra pure acetone, methanol and chloroform were from Merck (Germany). Silica gel TLC plates were used to check to purity of samples. Melting points were determined on Electrothermal GALLEN KAMP (England) melting point apparatus. The spectroscopic instruments used were: PYE-UNICAM SP-800 spectrophotometer, JASCO A-302 infrared spectrophotometer, FINNIGAN MAT-312 VARIAN MAT-200 EI Mass spectrometer and BRUKER AM-300 Proton NMR spectrometer. KBr discs were used for IR and TMS was used as internal standard for NMR determinations.

The bacterial and fungal cultures *viz. Staphvlococcas epidermitidis, Staphylococcus aureus, Staphylococcus citereus, Streptococcus pvogens, Streptococcus lactis, Corynebacterium diphtheriae, Enterobacter aerogenes* and *Candida albicans* were purchased from a local supplier. Culture media such as trvptic Soya Agar, Mueller Hinton Agar and Sabouraud Dextrose Agar were obtained from DIFCO (USA). All glass wares used during the course of study were sterilized before use to avoid any contamination or impurities.

Synthesis of 1-(4'-methoxy phenacyl)-N methylpiperidinium Bromide I:

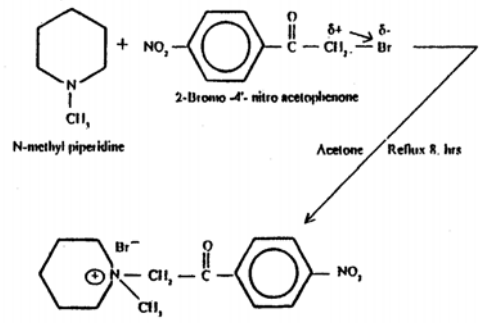
Equimolar quantities of N-methyl piperidine and 2-bromo-4'-methoxy acetophenone were dissolved separately in 30 ml of acetone. The reaction mixture was vigorously sliced at mom temperature for 10 minutes, followed by refluxing till the completion of reaction monitored by TLC in different systems of $CHCl_3$:MeOH. The resulting precipitate was collected by filtration, washed and recrystallized by an appropriate solvent to give white crystals.



1-(4'-methoxyphenuacyl)-N-methyl piperidinium Bromide

Synthesis of 1-(4-Nitro phenacyl)-N-methylpiperidinium Bromide II

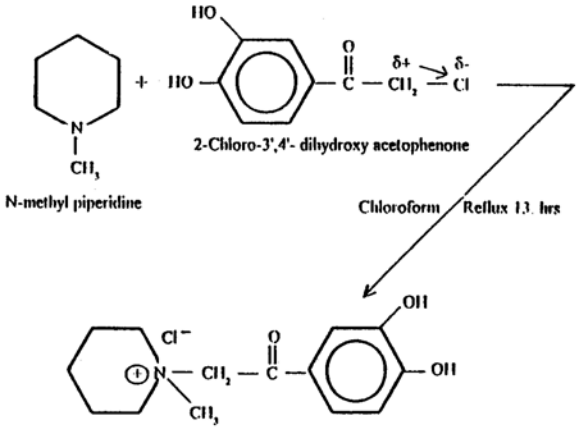
Equimolar quantities of N-methyl piperidine and 2-bromo-4-'-nitro acetophenone were dissolved in 40 ml of acetone separately. The two solutions were thoroughly mixed at room temperature, reflux till the crystals were formed. TLC was carried out in 8:2 system of CH_3Cl:MeOH to confirm the formation of compound. The crystals were filtered, washed, dried and again dissolved in an appropriate solvent to recrystallize into yellow crystals.



1-(4')-nitro phenacyl)-N-methylpiperidinium Bromide

Synthesis of 1-(3', 4'-Dihydroiyphcnacyl)-N-methylpiperidinium Chloride III

Equimolar quantities of N-methyl piperidine and 2-chloro-3', 4'- dihydroxy acetophenone were dissolved in 30 ml of chloroform separately. Both the solutions were mingled together and stirred vigorously at mom temperature for the completion of reaction which was monitored by TLC (system CH_3Cl:MeOH, 82). The resulting precipitate was collected by filtration followed by washing and recrystallization by an appropriate solvent to give damp brown crystals.



1-(3'.4'-dihydroxvphenacyl)-N-methylpiperidinium Chloride

Antimicrobial Screening:

The purity of bacterial and fungal cultures was confirmed by Gram's staining and by performing standard biochemical tests (Goodman, 1967). The maintenance of these bacterial and fungal cultures was carried out using lryptic soya agar and Saboumud dextrose agar respectively. The culture medias were sterilized in an autoclave at 15 psi (121°C) for 15 minutes. Antibacterial and antifungal activity of the newly synthesized compounds i.e., methoxyphenacyl)-N-methylpiperidinium bromide I. 1-(4'-nitrophenacyl)-N-methylpiperidinium bromide II and 1-(3', 4'- dihydroxvphenacyl)-N-methylpiperidinium chloride III, was detected by disc diffusion method which is considered as one of the best method todate (Bauer et al., 1966).

Two different agar plates were prepared one for the detection of antibacterial activity and one for detecting the antifungal activity.

Preparation of agar plate for antibacterial activity:

Agar plate for antibacterial activity was prepared by pouring melted sterile Mueller Hinton agar in sterile 100 mm petri dish and allowed to solidify.

Table 1. Spectroscopic determination of newly synthesized Piperidine compounds

Spectroscopic technique	1-(4'-methoxy phenacyl)-N-methyl piperidinium Bromide I	1-(4'-nitro phenacyl)-N-methyl piperidinium Bromide II	1-(3',4'-dihydroxy phenacyl)-N-methyl piperidinium chloride III
UV (λ_{max} nm)	286, 233, 202, 193	261, 201	318, 285, 236, 207
IR (ν cm^{-1})	3010 (CH Aromatic), 2895 (CH Aliphatic), 1600 (C=O Carbonyl, Ketone), 1500, 1485 (C=C Aromatic), 1350 (CH_3), 750, 810 (C=C).	3080 (CH Aromatic), 2980 (CH Aliphatic), 1690 (C=O Carbonyl, Ketone), 1510, 1320 (Aromatic NO_2), 850 (C=C).	3060 (CH Aromatic), 3050 (OH), 2900 (CH Aliphatic), 1680 (C=O Carbonyl, Ketone), 1580, 1490 (C=C Aromatic), 1390 (CH_3), 780, 840 (C=C).
EI MS m/z M^{+1}	249, 231, 151, 137, 100	263, 165, 152, 122, 112, 100	251, 218, 237, 152
NMR (D_2O)	δ 7.98 (2H, d, J=9.03 Hz, H-10, 14) δ 7.11 (2H, d, J=9.03 Hz, H-11, 13) δ 3.91 (3H, S, OCH_3) δ 3.74 (2H, m, H-2α,6α) δ 3.63 (2H, m, H-2β,6β) δ 3.34 (3H, S, N-CH_3) δ 1.95 (4H, m, H-3, H-5) δ 1.70 (2H, m, H-4)	δ 8.34 (2H, d, J=8.64 Hz, H-10, 14) δ 7.11 (2H, d, J=7.84 Hz, H-11, 13) δ 3.78 (2H, m, H-2) δ 3.54 (2H, m, H-6) δ 3.21 (3H, S, N-CH_3) δ 1.99-2.69 (6H, m, H-3, H-4, H-5)	δ 7.48 (1H, dd, J=8.42, 2.26 Hz, H-14) δ 7.42 (1H, d, J=2.22 Hz, H-10, 13) δ 6.94 (1H, d, J=8.41 Hz, H-14) δ 3.73 (2H, m, H-2) δ 3.59 (2H, m, H-6) δ 3.31 (3H, S, N-CH_3) δ 1.47-1.93 (6H, m, H-3, H-4, H-5)

Preparation of agar plate for antifungal activity:

Agar plate for antifungal activity was prepared by pouring melted sterile Saboumud dextrose agar in sterile 100 mm petri dish and allowed to solidify.

Disc Diffusion Method:

The respective bacterial and fungal cultures were streaked on the plate containing sterile media in such a way so as to obtain a confluent lawn. The filter discs (dia 6.0 nm) soaked in solutions of newly synthesized compounds were placed on agar surface inoculated with bacterial and fungal cultures respectively and were incubated at 37°C for 24-48 hours. Antibacterial and antifungal activity was detected by inspecting the presence of zone of inhibition in mm.

Table 2
Antimicrobial Screening of Newly Synthesized piperidine compounds

Organisms	Compounds 10 mg/ml in DMSO		
	I	II	III
Bacterial			
Staphylococcus epidermitidis	-	-	-
Staphylococcus citereus	-	-	-
staphylococcus aureus	-	-	-
Streptococcus pyogens	-	-	-
Streptococcus lactis	-	-	-
Corynebacterium diphtheriae	-	-	-
Enterobacter aerogenes	-	-	-
Fungal			
Candida albicans	-	-	-

Sign (-) = indicating no activity.

RESULTS AND DISCUSSION

Synthesis of compounds I.II and Ill was successfully achieved by simple quaternization reaction taking equimolar quantities of N- methyl piperidine and corresponding phenacyl halide. The structures of these compounds were elucidated by spectroscopic techniques. The respective spectral data is summarized in tablet. It is obvious from these reactions that since N-methyl piperidine nucleus contains a nitrogen atom which is electronegative in cha*rac*ter, it is attacked by

either a bromo or a chloro group of the phenacyl halide resulting in the formation of respective quaternary compound.

The antimicrobial activity of these compounds was detected in terms of zone of inhibition in mm. using disc diffusion method. Samples were prepared individually in a concentration of 10 mg/nil dissolved in DMSO and were put against seven bacterial and one fungal organism. Table 2 presents the antimicrobial potential of these compounds. Results show that they contain no activity. The reason behind this ineffectiveness may be the inability of these compounds to carry out the genetic alternations of microorganisms necessary to exhibit bacteiostatic or bactericidal activity. Thus, it is proposed that some minor changes should be made in the structures of these newly synthesized compounds so that they can alter the genetic make up of microorganisms. This would formulate a new parameter for these type of compounds having both analgesic properties as well as antimicrobial profiles.

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