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Research Article

SYNTHESIS, SPECTROSCOPIC CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF 2,6 DI-SUBSTITUTED PIPERIDINE-4-ONE DERIVATIVES

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ABSTRACT

Objective; To synthesize the series of 2, 6 disubstituted piperidine-4-one derivatives, characterization by IR, ^{1}H-NMR and Mass spectroscopy and evaluated for antimicrobial activity at two concentrations by disc diffusion method.

Method; Mannich reaction (condensation method).

Results; The compound 1(DALI) possessed highly potent antibacterial activity against gram positive bacteria and all the compounds possessed antifungal activity against *aspergillus niger*.

Conclusion; A new series of 2,6 disubstituted piperidine 4 one derivatives were synthesized, characterized and exhibited promising antibacterial and antifungal activity at both concentrations.

Keywords: Piperidine derivatives, Mannich reaction, Mass spectroscopy, Antimicrobial activity.

INTRODUCTION

The main objectives of organic and medicinal chemistry is the synthesis, characterization and pharmacological evaluation of molecules having highly therapeutic and efficacy in nature. Now a days increasing the resistance of many microorganisms and we have to synthesis the new molecules for active against the resistant microbes, particularly bacteria, virus and fungus is the major area in the antimicrobial research. The aim of this research is to synthesize and characterize the biological active molecules for resistant bacteria and fungus. More than 9000 piperidine compounds mentioned in clinical and preclinical studies(1). The substituted piperidine derivatives were reported for the various pharmacological activity including antimicrobials(2), anticonvulsants(3), antihypertensives(4), antidepressants(5), anti-inflammatory agents(6). The present study, a series of 2,6 di-substituted piperidine 4-one derivative were synthesized by condensation of aliphatic aldehydes and ammonium acetate with ketone or keto ester or keto acid by mannich reation(7).

MATERIALS AND METHODS

Chemicals and solvents

Isobutyraldehyde, Levulinic acid were received from Sigma Aldrich. Ethanol-HPLC were received from Brampton(Canada), Diethyl ether, Con-HCL, Acetaldehyde, were received from Nice chemicals. Acetone-AR were received from Spectro chem. Ethylacetoacetate were received from LOBA CHEMIE. Ammonium acetate were received from Fischer.

Instruments

Melting points were recorded on BMQR 796 series digital melting point apparatus. The IR spectra were recorded on PERKIN ELMER spectrometer. The ^{1}H-NMR spectra were recorded on BRUKER-NMR spectrometer at 500 MHZ using TMS as an internal standard and $CDCL_3$ as solvent. Mass spectra were recorded on TRUE ANALYTICA of LCMS-MS.

Procedure for synthesis

Synthesis of DALI

11.34ml (0.2mole) of Isobutyraldehyde, 13.87 ml (0.1mole) of ethyl aceto acetate and 7.7 gm (0.1mole) of ammonium acetate were added with ethanol 100ml placed in a conical flask fitted with reflux condenser. The reaction mixture was refluxed for 50 min by using hot plate at 75^0 C. The reaction mixture kept in a room temperature for overnight. Recrystalized the precipitated salt by using ethanol.

Synthesis of DALII

Recrystalized salt was dissolved in diethyl ether (200ml) and Con-HCL was added upto get the precipitate. The precipitated salt was separated by filtration, dried and washed with diethyl ether. Finally recrystalized (white color) by using ethanol.

Synthesis of $DALIIA_1$

11.34ml (0.2mole) of Isobutyraldehyde, 4.59 ml (0.1mole) of acetone and 7.7 gm (0.1mole) of ammonium acetate were added with ethanol 100ml placed in a conical flask fitted with reflux condenser. The reaction mixture was refluxed for 50 min by using hot plate at 75^0 C. The reaction mixture kept in a room temperature for overnight. After removed the solvent, the reaction mixture was dissolved in diethyl ether (200ml) and Con-HCL was added upto get the precipitate. The precipitated salt was separated by filtration, dried and washed with diethyl ether. Finally recrystalized (white color) by using ethanol.

Synthesis of $DALIIL_1$

11.34ml (0.2mole) of Isobutyraldehyde, 13.27 ml (0.1mole) of levulinic acid and 7.7 gm (0.1mole) of ammonium acetate were added with ethanol 100ml placed in a conical flask fitted with reflux condenser. The reaction mixture was refluxed for 50 min by using hot plate at 75^0 C. The reaction mixture kept in a room temperature for overnight. After removed the solvent, the reaction mixture was dissolved in diethyl ether (200ml) and Con-HCL was added upto get the precipitate. The precipitated salt was separated by filtration, dried and washed with diethyl ether. Finally recrystalized (white color) by using ethanol.

Synthesis of $DAAI_2$

6.9 ml (0.2mole) of acetaldehyde, 13.87 ml (0.1mole) of ethyl aceto acetate and 7.7 gm (0.1mole) of ammonium acetate were added with ethanol 100ml placed in a conical flask fitted with reflux condenser. The reaction mixture was refluxed for 50 min by using hot plate at 75^0 C. The reaction mixture kept in a room temperature for overnight. The precipitate was formed, filtered and dried. Finally the precipitated salt was recrystalized by using ethanol.

Synthesis of $DAAIIA_1$

6.9 ml (0.2mole) of acetaldehyde, 4.59 ml (0.1mole) of acetone and 7.7 gm (0.1mole) of ammonium acetate were added with ethanol 100ml placed in a conical flask fitted with reflux condenser. The reaction mixture was refluxed for 50 min by using hot plate at 75^0 C. The

reaction mixture kept in a room temperature for overnight. After removed the solvent, the reaction mixture was dissolved in diethyl ether (200ml) and Con-HCL was added upto get the precipitate. The precipitated salt was separated by filtration, dried and washed with diethyl ether. Finally recrystalized (white color) by using ethanol.

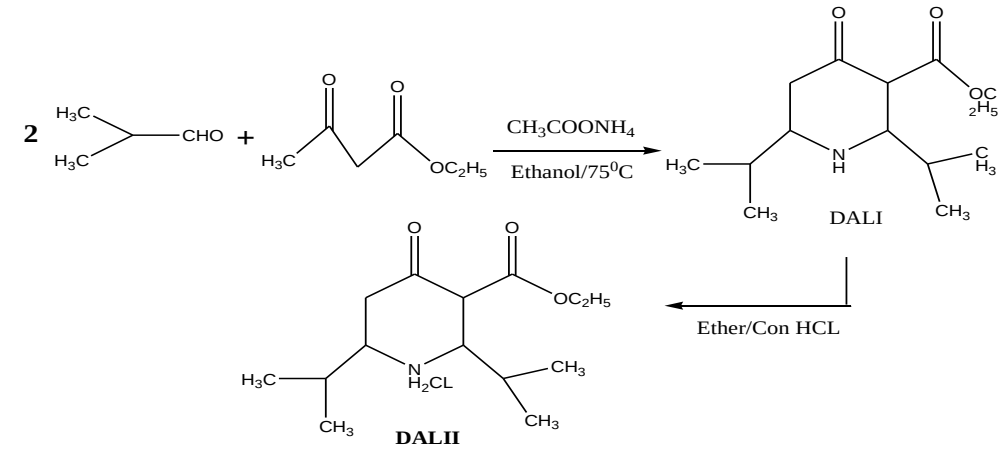
Synthesis of DAAIL$_1$

6.9 ml (0.2 mole) of acetaldehyde, 13.27 ml (0.1mole) of levulinic acid and 7.7 gm (0.1mole) of ammonium acetate were added with ethanol 100ml placed in a conical flask fitted with reflux condenser. The reaction mixture was refluxed for 50 min by using hot plate at 75º C. The reaction mixture kept in a room temperature for overnight. Recrystalized the precipitated salt by using ethanol.

Synthesis of DAAIIL$_1$

After removed the solvent, the reaction mixture was dissolved in diethyl ether (200ml) and Con-HCL was added upto get the precipitate. The precipitated salt was separated by filtration, dried and washed with diethyl ether. Finally recrystalized (white color) by using ethanol.

SCHEME I



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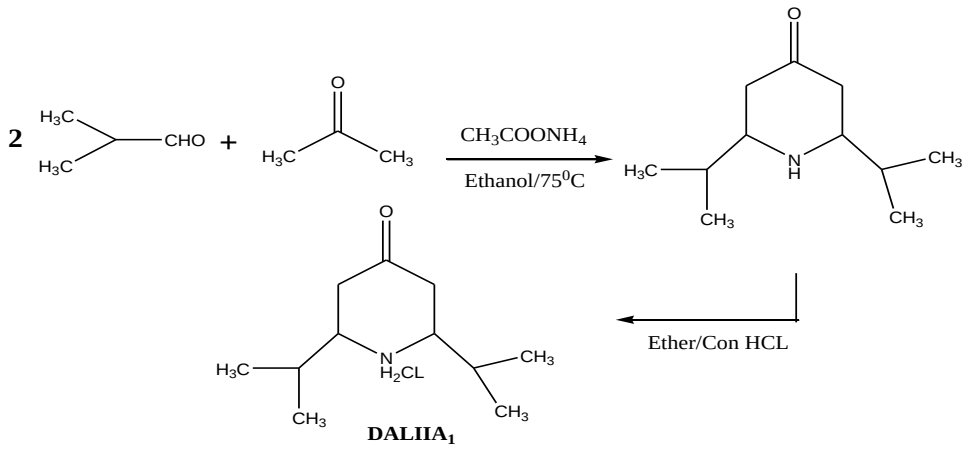

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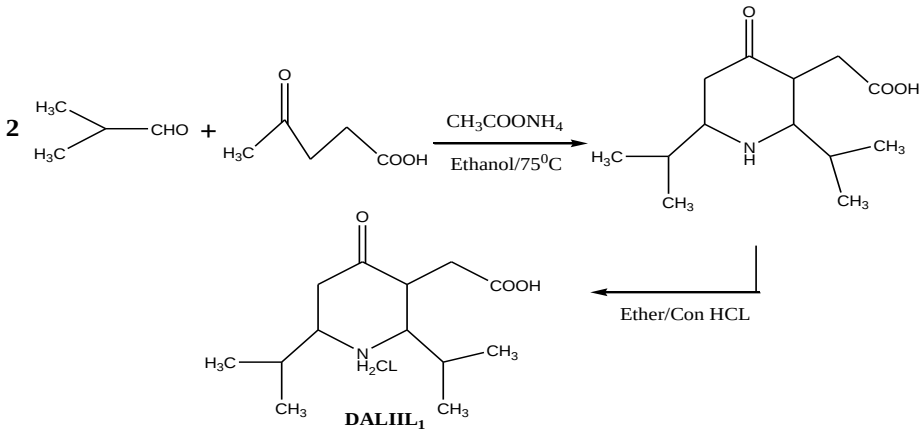

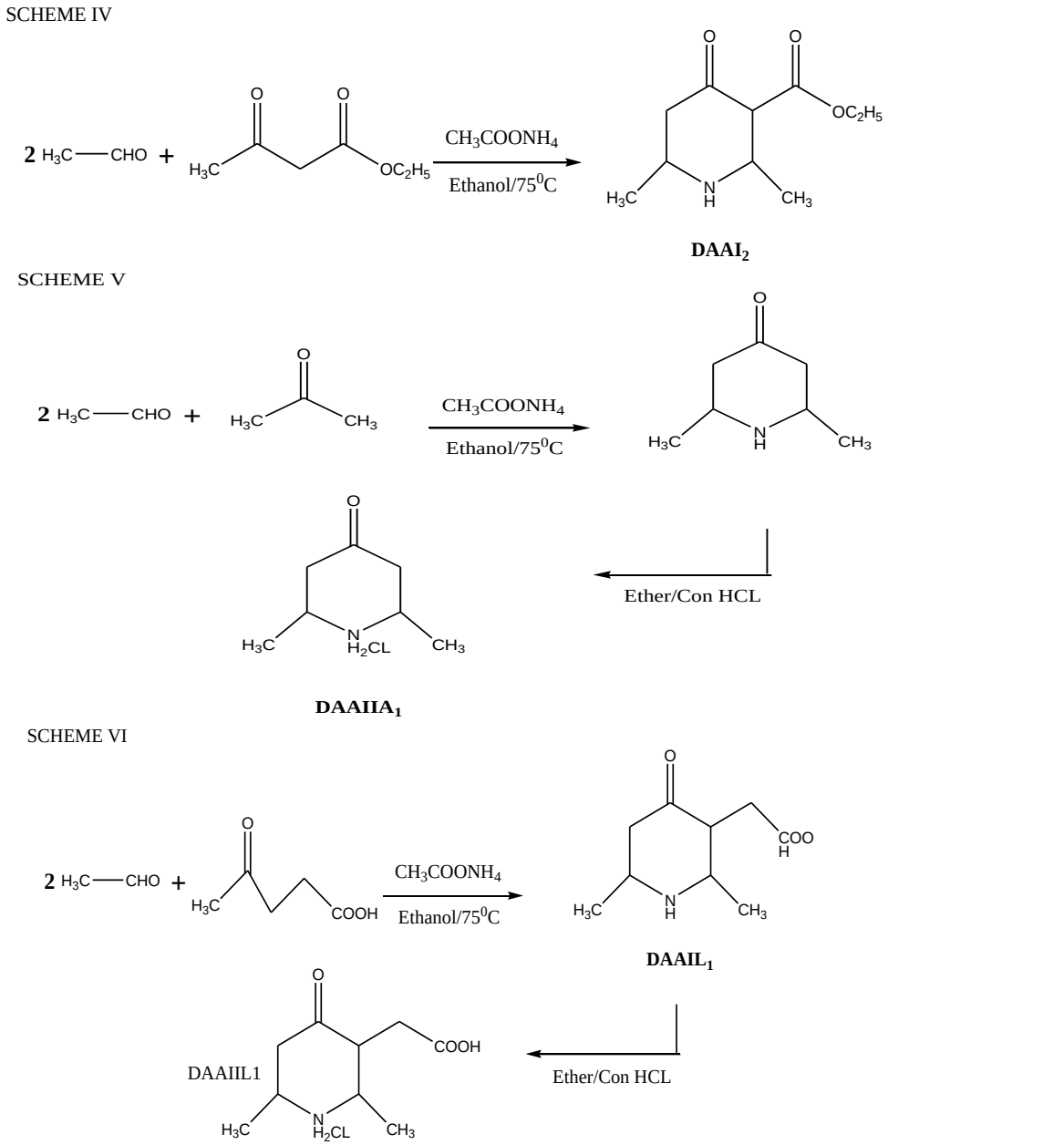

Fig. 1: Schemes of the 2, 6 disubstituted piperidine 4 one derivatives

***In-vitro* antimicrobial activity**

Antibacterial activity (Disc diffusion method**)**

The antibacterial activity of the synthesized compounds was studied systematically against four different strains of bacteria *Staphylococcus aureus, Bacillus subtillis* (Gram-positive), and *Escherichia coli, Pseudomonas aerugenosa* (Gram-negative) by using disc diffusion method. The organisms were sub-cultured on Mueller Hinton Agar medium, incubated at 37°C for 24 h and stored at 4°C in the refrigerator to maintain stock culture. Petri plates were prepared with 20 ml of sterile Mueller Hinton Agar (MHA) (HIMEDIA, Mumbai, India). The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. The tests were conducted at two different concentrations at 100 and 200 µg /ml respectively of the synthetic derivatives. The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Negative control was prepared by using respective solvent(DMSO). Amikacin (50 µg/ml) was used as positive control. The plates were incubated for 24 h at 37°C. The zone of inhibition were recorded in millimeters(8).

Antifungal activity

Fungus culture Candida Albicans and Aspergillus Niger were used for this study. The antifungal activity was performed according to the standard reference method(9-10). The synthetic derivatives were dissolved in 2% DMSO and conducted at two different concentrations at 100 and 200 µg /ml respectively. Each well was inoculated with 50 µL of suspension containing 104 spore/ml of fungi. The anti fungal agent ketoconazole(50 µg/ml) was included in the assay as positive control. The plates were incubated between 24 h to 72 h at 27 °c.

RESULTS

Chemistry

A series of 2,6 disubstituted pperidine 4 one derivatives were synthesized, characterized and screened for *In-vitro* antimicrobial

activity. The physic-chemical parameters of the synthesized compounds is shown in the Table 1. All the comounds were found in good yield. The compounds were characterized by FT-IR, proton NMR and Mass spectroscopy and the data is shown in the Table 2.

Table 1: The physic-chemical parameters of the synthesized compounds

S. No.	Compound code	Molecular formula	Molecular weight	% of yield	Melting point-(°C)
01	DALI	$C_{14}H_{25}NO_3$	255	-	96-98
02	DALII	$C_{14}H_{26}O_3NCl$	291.45	22.8	240-242
03	$DALIIA_1$	$C_{11}H_{22}NOCl$	219.45	45.2	261-263
04	$DALIIL_1$	$C_{13}H_{24}O_3NCl$	277.45	31.9	244-246
05	$DAAI_2$	$C_{10}H_{17}O_3N$	199	81.4	116-118
06	$DAAIIA_1$	$C_7H_{14}NOCl$	163.45	52	243-245
07	$DAAIL_1$	$C_9H_{15}NO_3$	185	-	97-99
08	$DAAIIL_1$	$C_9H_{16}O_3NCl$	221.45	41.6	273-275

Table 2: The spectral data of the synthesized compounds

S. No.	Compound code	Infrared spectroscopy (cm^{-1})	Proton-NMR (ppm)	Molecular weight	M^++1 peak
01	**DALI**	3344.70(N-H), 1692.16(C=O), 1443.57(ethyl-carboxylate), 1369.59 (iso-doublet).	-	225	-
02	**$DAAI_2$**	3340.96(N-H), 2967.03(methyl C-H stretch-asymm),1642.34(C=O), 1376.54(ethyl carboxylate	4.16(m,CH_2), 3.81-3.85(d,CH) 2.26(s,NH), 1.30(t,CH_3), 0.97(d, CH_3)	199	199.20
03	**$DAAIL_1$**	3343.68(N-H), 2963.71(carboxylic-O-H broad-band), 3138.03(carboxylic-O-H broad band), 1670.64(C=O)	-	221.45	221.20

Antibacterial activity

The synthetic compounds were evaluated for antibacterial activity at the concentration of 100, 200 µg/ml by using disc diffusion assay where in amikacin was used as standard (Table 3). All the compounds were possessed active against *S.aureus.* The compound 1(DALI), 4($DAAI_2$) and 5($DAAIL_1$) were possessed significant activity against *S.aureus*. The compound 1(DALI) was emerged highly potent antibacterial activity against *S.aureus and C.albicans* at bothe concentrations. All the compounds showed activity against *E.coli* slightly. None of the compounds activity against *P.aerugenos*.

Antifungal activity

The synthetic compounds were evaluated for antifungal activity at the concentration of 100, 200 µg/ml by using disc diffusion assay where in ketoconazole was used as standard (Table 4). All the compounds were possessed active against *Aspergillus niger, Candida albicans.* The compound 1(DALI), 2($DALIIA_1$), 3($DALIIA_1$**)** and 4($DAAI_2$) were found potent antifungal activity against *Aspergillus niger*. The compound 1(DALI) and 2($DALIIA_1$) were emerged highly potent antifungal activity against *Aspergillus niger* at both concentrations.

Table 3: The antibacterial activity of synthetic derivatives in different strains

S. No.	Compound code	Con:µg/ml	Zone of inhibition in diameter(mm)			
			S.aureus	*B. subtillis*	*E. coli*	*P.aerugenosa*
1	**DALI**	100 µg/ml	**14**	**15**	-	-
		200 µg/ml	**16**	**17**	02	-
2	**$DALIIA_1$**	100 µg/ml	02	-	02	-
		200 µg/ml	04	-	05	-
3	**$DALIIL_1$**	100 µg/ml	-	-	-	-
		200 µg/ml	02	-	05	-
4	**$DAAI_2$**	100 µg/ml	04	-	02	-
		200 µg/ml	08	-	04	-
5	**$DAAIL_1$**	100 µg/ml	06	-	-	-
		200 µg/ml	**12**	-	-	-
6	**Control**	Nil	Nil	Nil	Nil	Nil
7	**Amikacin (Std)**	**50 µg/ml**	**18**	**19**	**17**	**18**

Table 4: The antifungal activity of synthetic derivatives in different strains

S. No.	Compound code	Con:µg/ml	Zone of inhibition in diameter(mm)	
			Candida albicans	*Aspergillus niger*
1	**DALI**	100 µg/ml	-	**12**
		200 µg/ml	2	**12**
2	**$DALIIA_1$**	100 µg/ml	4	**11**
		200 µg/ml	4	**11**
3	**$DALIIL_1$**	100 µg/ml	-	6
		200 µg/ml	2	4
4	**$DAAI_2$**	100 µg/ml	2	4
		200 µg/ml	2	7
5	**$DAAIL_1$**	100 µg/ml	-	3
		200 µg/ml	-	3
6	**Control**	Nil	Nil	Nil
7	**Ketaconazole (Std)**	**50 µg/ml**	**17**	**16**

DISCUSSION

Chemistry

Series of 2,6 disubstituted piperidine 4 one derivatives were synthesised by the Mannich reaction. The condensation of aliphatic aldehydes and ammonium acetate with ketone or keto ester or keto acid. The compounds were obtained in good yield, particularly the compound $DAAI_2(4)$ were obtained in highest yield of 81.4%. The structure of the compound was confirmed by the comparing the spectral data. The appearance of the strong carbonyl stretch at 1642-1692 cm^1 is a typical of six member cyclic ketone of the synthetic derivatives. The appearance of the secondary amine at 3340-3344 is further evidence of compound 1, 4, 6. The proton NMR of the compound $4(DAAI_2)$ exhibited singlet broad peak at 2.2ppm which were assigned the cyclic N-H proton. The synthetic compounds was confirmed by the molecular ion peak (M+1) in the mass spectroscopy.

Antibacterial activity

The test compounds were found to be active against the *Staphylococcus aureus* and *Bacillus subtillis*, among these the compound 1(DALI) was emerged as highly potent antibacterial activity at both concentrations due to the ethyl carboxylate group present in the second position of the side chain. Whybecause the ethyl carboxylate is a salt of fattyacid. One more reason the isobutyl group present in the second position of the side chain. The compound $5(DAAIL_1)$ was found to be active against S.aureus at the concentrations of 200 µg/ml due to the presence of methyl carboxylic acid(fattyacid) in the third position of the side chain.

Antifungal activity

The test compounds were found to be active against *Aspergillus niger* as compared with *Candida albicans.* Among all(1-5), the compound 1(DALI) and $2(DALIIA_1)$ were emerged as highly potent antifungal activity at both concentrations against *Aspergillus niger*. The main reason is the compound 1(DALI) having ethyl carboxylate(fattyacid salt) group present in the second position and isobutyl group present in the second position of the side chain. The compound $2(DALIIA_1)$ having isobutyl group(more hydro-carbon) present in the second position of the side chain.

CONCLUSIONS

The present study concluded that a new series of 2,6 disubstituted piperidine 4 one derivatives were synthesized, characterized and exhibited promising antibacterial and antifungal activity at both concentrations.

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