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Synthesis, antimicrobial and antioxidant activity of novel 2,6-dipheny-l-3-alkylpiperidin-4-one-O-[2,4,6-tritertiarybutyl-cyclohexa-2,5-dienon-4-yl]oximes

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ABSTRACT

Synthesis of some novel 2,6-Diphenyl-3-alkylpiperidin-4-one-O-[2,4,6-tritertiarybutylcyclohexa-2,5-dienon-4-yl] oximes by treating 2,4,6-tritertiarybutyl phenol with corresponding piperidin-4-one oximes in the presence of an oxidising agent, MnO_2 under nitrogen atmosphere. The compounds are characterized using IR, GC-MS, Elemental analysis and ^{1}H and ^{13}C NMR spectral analysis. All the synthesized compounds are screened for anti-oxidant and anti-microbial activities.

Keywords: 2,6-dipheny-l-3-alkylpiperidin-4-one-O-[2,4,6-tritertiarybutyl-cyclohexa-2,5-dienon-4-yl] oximes, NMR Spectral analysis, antibacterial activity, antifungal activity, antioxidant activity.

INTRODUCTION

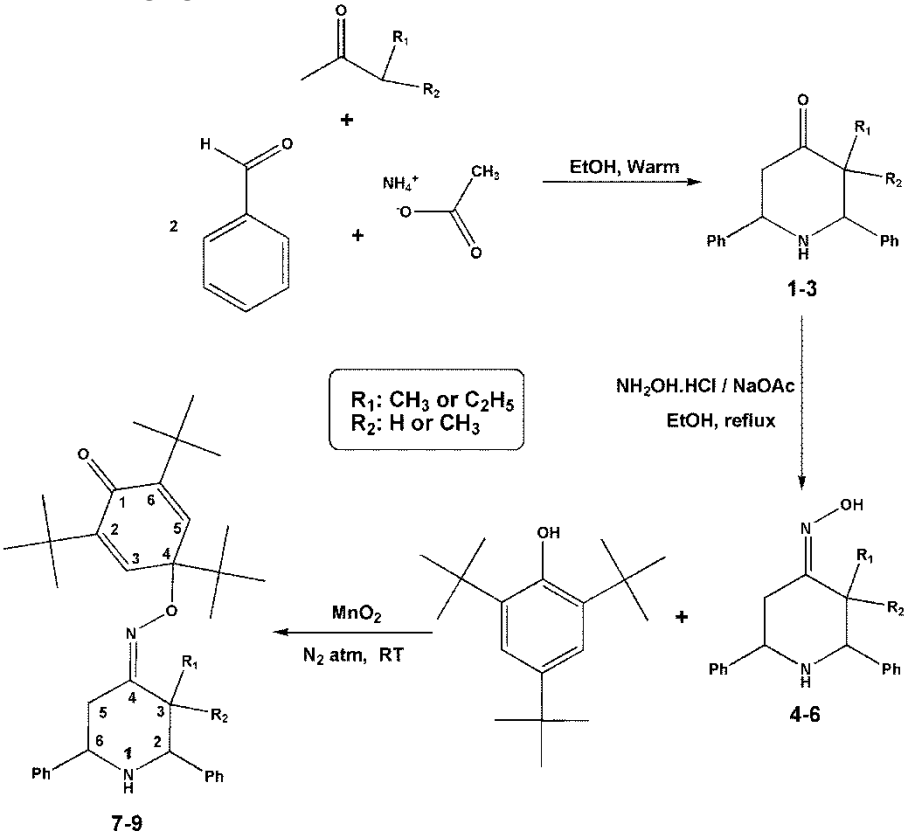
Heterocyclic compounds bearing piperidine skeleton are enchanting targets of organic synthesis owing to their pharmacological activity and their wide existence in nature [1-3]. Basically systems having piperidine-4-one nucleus have aroused great interest in the past and recent years due to the their variety of biological properties such as antiviral [4], antitumor [4-5], antiinflammatory [6], central nervous system [7-12], local anesthetic [8-13], anticancer [14], and antibacterial activity [15] and their derivatives piperidines are also biologically important and act as neurobinin receptor antagonists [16], analgesic and antihypertensive agents [17]. The signifance of piperidin-4-one as intermidate in the synthesis of a collection of compounds of physiologically active has been reviewed by Prostakov and Gaivoronskaya [18]. The extensive studies undertaken in the past on 4-piperidones have their relation to the synthesis of drug [19]. Specifically, piperidine based chemical entities with aryl substituents at carbons 2 and 6 of the piperidine ring have been documented as potent antimicrobial agents [20].The numerous pharmacological activities of piperidin-4-one oximes and cyclohexadienone prompted as to study the antioxidant and antimicrobial activity of newly synthesized compounds using various assays.

MATERIALS AND METHODS

Synthetic procedure for the target compounds: In the present work, three steps synthetic strategies are adapted for the preparation of expected oximes **(7-9)**. The representation describing the routes of synthesis is depicted in the Scheme-1. The basic compound 2,6-diphenyl-3-alkylpiperidin-4-one **(1-3)** was synthesized by condensation reaction (Mannich reaction) of benzaldehyde, different ketone and ammonium acetate in 2:1:1 respectively. In the next step, compounds **(1-3)** were converted to corresponding oxime that is 2,6-diphenyl3-alkylpiperdin-4-one oximes **(4-6)** by treating with hydroxylamine hydrchloride ($NH_2OH.HCl$) in the presence of sodium acetate in absolute alcohol. Further, key intermediate oximes **(4-6)** oxidised with 2,4,6- tritertiarybutyl phenol by using oxidizing agent, MnO_2 under nirogen atmosphere **Scheme-1**. In order to find the optimum reaction condition, solvent 1,4-dioxane were employed. It is noted that the reaction in 1,4-dioxane afforded the target compounds **(7-9)** in excellent yields.

Chemistry: IR spectra are recorded on a ThermoNicolet-Avatar-330 FT-IR spectrophotometer ^1H and ^{13}CNMR spectra are recorded at 500 and 125MHz respectively on BrukerAvanceIII 500MHz FT-NMR spectrometer. The ESI+ve Mass spectra are recorded on a BrukerDaltonics LC-MS spectrometer. Satisfactory microanalysis data are obtained on Carlo Erba 1106 CHN analyzer.

Microbiology: All the clinically isolated bacterial strains namely *Staphylococcus aureus*, β-Haemolytic streptococcus, *Micrococcus luteus*, *Bacillus subtilis*, *Salmonella typhii*, *Shigellafelxneri*, *Vibreocholerae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and fungal strains namely *Aspergillusflavus*, *Aspergillusniger*, *Mucorindicus*, *Rhizopusarrhizus*and *Microsporumgypseum*are obtained from Faculty of Medicine, Annamalai University, Annamalainagar-608 002, Tamil Nadu, India. Antibacterial, antifungal activity is done by disc diffusion method [21] and *in vitro* antioxidant activities done by free radical scavenging method [22-25].



Scheme 1 Synthetic route for the target compounds **(7-9)**

RESULTS AND DISCUSSION

The structure of the target compounds, were elucidated by elemental analysis and IR spectral analysis. The physical data of the synthesized compounds are given in t**able 1**. Further, the structural assignments of the title compounds were made by using mass, ^{1}H and ^{13}C NMR spectral analysis. In order to investigate the spectral assignments of the target compounds (**7-9)**, 2,6-dipheny l-3-alkylpiperidin-4-one-O- [2,4,6-tritertiarybutyl-cyclohexa-2,5-dienon-4-yl]oximes compound **7** is taken as the representative compound.

Table 1 Analytical data for compound **(7-9)**

Entry	Molecular formulae	R_1	R_2	Yield (%)	Mp (°C)	Elemental Analysis (%)					
						Calculated			Found		
						C	H	N	C	H	N
7	$C_{36}H_{48}N_2O_2$	CH_3	H	90	88	79.96	8.95	5.18	79.93	8.91	5.16
8	$C_{37}H_{50}N_2O_2$	C_2H_5	H	84	84	80.10	9.08	5.05	80.07	9.01	5.02
9	$C_{37}H_{50}N_2O_2$	CH_3	CH_3	82	82	80.10	9.08	5.05	80.06	9.01	4.09

FT-IR analysis of compound 7: The IR spectrum of compound **7** shows an absorption band at 3308cm^{-1} which is assigned as N-H stretching frequency. Aromatic C-H stretching vibrations are observed in the range of 3065-3030cm^{-1} and aliphatic C-H stretching vibrations are observed in the range of 2959-2871cm^{-1}. FT-IR data for the all synthesized compounds **(7-9)** are given in **table-2.**

Table 2 FT-IR (cm^{-1}) and ^{1}H (ppm) data for the all synthesized compounds **(7-9)**

Entry	**FT-IR stretching frequencies (Cm^{-1})**			**Piperidone ring**						**Cyclohexa dienone ring**		
	N-H	**Aromatic**	**Aliphatic**	**H2**	**H3**	**H5$_{(a,e)}$**	**H6**	**Others**	**Aromatic protons**	**6CH_3 at C-2 & C-6**	**H3 & H5**	**3CH_3 at C-4**
7	3308	3065-3030	2959-2871	3.73	2.58	2.16, 3.69	3.91	CH_3 0.91	7.20-7.39	1.45	5.03	1.30
8	3269	3072-3041	2959-2870	3.71	2.46	2.05, 3.67	3.93	CH_2 1.25-1.30 CH_3 0.87	7.23-7.50	1.48	5.06	1.33
9	3310	3068-3020	2960-2870	3.79	-	2.23, 3.56	3.91	2CH_3 1.05, 1.01	7.28-7.56	1.48	5.06	1.33

Mass spectral analysis compound 7 : Mass spectrum of 2,6-Diphenyl-3-methylpiperidin-4-one-O-[2,4,6-tritertiarybutyl-cyclohexa-2,5-dienon-4-yl] oxime shows Molecular ion at m/z = 540 which is consistent with the proposed molecular structure of the compound.

^{1}H NMR spectral analysis of compound 7: Piperidone ring: The H2and H6 protons are appeared at 3.73, 3.91ppm respectively. The doublet observed at 2.58ppm is due to the H3 proton and the H5 (axial, equatorial) protons are appeared at 2.16 and 3.69ppm. The methyl protons at C3 carbon observed as a singlet at 0.91ppm. Aromatic protons are appeared in the range of 7.20-7.39ppm. **Cyclohexadienone ring:** The six methyl protons attached to the C2 and C6 carbons are appeared at 1.45ppm and the three methyl protons attached to the C4 carbon appeared at 1.30ppm. The proton signal observed at 5.03ppm is

corresponds to the H3 and H5 protons. ^{1}H NMR data for the all synthesized compounds **(7-9)** are given in **table 2**.

^{13}C NMR spectral analysis of compound 7: Piperidone ring: In the piperidone ring the C2 and C6 carbons are observed at 69.2, 60.8ppm respectively. The carbon resonate at 43.5, 33.7ppm are corresponds to the C3 and C5 carbon of the piperidone ring. The C4 carbon resonates at 160.9ppm. The ipso carbons of the phenyl ring appeared at 142.5 and 143.6ppm and the aromatic protons are observed in the range of 121.8-134.9ppm.

Table 3 ^{13}C data for the all synthesized compounds **(7-9)** in ppm

Entry	Piperidone ring								Cyclohexadienone ring							
	C2	C3	C4	C5	C6	Ipso carbons	Aromatic carbons	Others	C1	C2 & C6	C3 & C5	C4	At C2 & C6		At C4	
													C-Me_3	CH_3	C-Me_3	CH_3
7	69.2	43.5	160.9	33.7	60.8	142.5 143.6	121.8- 134.9	CH_3 11.7	161.5	151.3	141.4	84.1	39.3	31.7	34.5	29.6
8	67.5	50.0	159.3	34.5	60.8	142.4 142.5	121.8- 134.9	CH_2 18.9 CH_3 11.9	160.3	151.3	142.4	82.6	37.5	30.4	31.7	26.1
9	70.6	41.5	162.7	31.7	60.9	141.4 144.0	121.8- 134.9	$2CH_3$ 22.1, 20.7	161.2	151.3	140.2	83.7	41.5	30.4	34.5	29.5

Cyclohexadienone ring: The C1 and C4 carbons are resonate at 161.5 and 84.1ppm. The carbon signals at 151.3 and 141.4ppm are due to the C2, C6 and C3, C5 carbons respectively. The tertiary methyl groups attached carbons at C2, C6 and C4 carbons are observed at 39.3 and 34.5ppm. The remaining methyl protons are resonates at 29.6 and 31.7ppm. ^{13}C NMR data for the all synthesized compounds **(7-9)** are given in **table-3.**

APPLICATIONS

Antimicrobial activity: The *in vitro* antibacterial and antifungal activities of the newly synthesized title compounds **(7-9)** was ascertained by disc diffusion method. The potentiality of the synthesized compounds as antimicrobial was assessed for their antibacterial studies against different gram postive bacterial strains of namely staphylococcus aureus, β-Heamolyic streptococcus, Bacillus subtilis and gram negative bacterial strains namely Vibreo cholerae, Shigalla felxneri and Salmonella typhii. Antifungal studies against strains such as Aspergillus flavus, Aspergillus niger and Candida albicans.

The results acquired as zone of inhibition (mm) are presented in **table 4** and **table 5** respectively. It is more attractive to hypothesize the observation that the result of the antimicrobial activity of the different derivatives of oximes appeared to be related to the nature of substituents on the piperidin-4-one moiety. All synthesized compounds showed varying degree of inhibition against tested microorganisms. Compound **8** possessing ethyl group at position 3 in piperidin moiety exhibited almost equipotent efficacy compared to standard drug Ciprofloxacin against β-H.streptococcus, V.cholerae and S. typhii. Compound **9** possessing two methyl groups exhibited slightly less Antibacterial activity than standard. Compound bearing methyl group such as **(7)** were demonstrated strongly active against three tested bacterial strains.The investigation of antifungal activity **(table 5)** informed that compound **8** possessing ethyl group in third position of piperidine moiety appeared as active antifungal agent against A.flavus compared with standard fluconazole. Modification of ethyl group by two methyl groups led to compound **9** was the next

effectual antifungal agent compound **7** bearing one methyl group exhibited moderate growth inhibitory activities.

Table 4 *in vitro* antibacterial and antifungal activities of compounds **(7-9)**

Microorganisms	Compound 7			Compound 8			Compound 9		
	100 ppm	200 ppm	500 ppm	100 ppm	200 ppm	500 ppm	100 ppm	200 ppm	500 ppm
Staphylococcus aureus	-	+	++	-	++	+++	+	++	+++
β-Heamolytic streptococcus	-	+	+++	-	++	++++	+	++	++++
Bacillus subtilis	-	++	++	+	++	+++	+	++	+++
Vibreo cholerae	+	++	+++	+	+++	++++	-	+	+++
Shigalla felxneri	+	++	+++	+	++	+++	-	++	+++
Salmonella typhii	+	++	+++	+	++	++++	+	++	+++
Aspergillus flavus	+	++	+++	+	+++	++++	+	++	+++
Aspergillus niger	-	+	++	+	++	+++	-	++	++++
Candida albicans	-	++	++	+	++	+++	-	+	++

(-) = inactive, (+) = weakly active(12-16mm), (++) = moderately active(17-21mm), (+++) = strongly active(22-29mm), (++++) = highly active(30-33mm)

Antioxidant activity: All the synthesized compounds **(7-9)** are found to be efficient scavengers of free radicals such as DPPH, ABTS, Hydroxyl, Super oxide and Nitric oxide in dose dependence manner. The various antioxidant activities of the compounds were compared to the standard ascorbic acid. All the compounds are having more antioxidant activity when compared to standard ascorbic acid. Compound **8** exerts more antioxidant activity when compared to other compounds. The IC_{50} values of the compounds are shown in the **table 5**.

Table 5. Antioxidant activity of compounds **(7-9)**

Radicals	Ascorbate (µg mL^{-1})	Compounds (µg mL^{-1})		
		7	8	9
DPPH	4.94	3.12	2.56	4.23
ABTS	5.32	4.38	3.85	4.92
Hydroxyl	4.23	3.81	3.13	3.99
Super oxide	5.35	4.19	3.90	4.19
Nitric oxide	4.50	3.97	2.19	4.21

CONCLUSIONS

In conclusion we have accomplished a convenient protocol for the synthesis of piperdin-4-one oxime **(7-9)**. The synthesized molecules were estimated for their antioxidant and antimicrobial capacities. The antimicrobial study was initiate to evaluate their inhibitory activities on the growth of pathogenic bacteria and fungi. Presence of electron releasing substituent particularly ethyl group at position-3 of piperidone ring (compound **8)** found to be leading for potent antimicrobial activity. It is remarkable that compound **8** possessed foremost antioxidant capacity than standard because of having highest alkyl content. Thus in future, this kind of oxime derivatives may be used to generate better drugs with improved antioxidant, antibacterial and antifungal activities.

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