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Synthesis and Evaluation of Anticancer Activity using Hela Cell Line for Piperidin-4-One Derivatives

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Abstract: Three series of substituted piperdin-4-ones derivatives were synthesized and their anticancer activities were evaluated *in vitro* against the HeLa cell line. The results indicated that synthesized compounds possessed less anticancer activities and one of them are more potent and discussed.
Keywords: Anticancer Activity,Hela Cell Line, Piperidin-4-One Derivatives.

Introduction

Anticancer therapy usually brings destruction or legal injury to healthy cells and tissues, and thus brings about serious side effects. Drug therapy in cancer patients includes chemotherapy; it has been employed for cancer treatment since 1978. Chemotherapy is used in patients after surgery to reducing their numbers of malignant cells. Heterocyclic compounds bearing piperidin-4-one skeleton are exhibit in wide range in biological properties [1]. Piperidine-4-one nucleus has great interest due to their variety of pharmacological activity. Basically systems having such as antiviral, antitumor, anti-inflammatory, central nervous system, local anesthetic, anticancer and antibacterial activity [2-5].The chemistry have been used in the preparation of many potential drugs and possess a broad spectrum of remarkable diversity in structure may be observed even when only a small change in mannich bases occurs. Specifically, piperidin-4-ones based chemical entities with aryl substituents at carbons 2 and 6 of the piperidine ring have been documented as potent antimicrobial agents [14]. The properties depend on the substituents on the heterocyclic ring [15]. The numerous pharmacological activities of substituted piperidin-4-ones prompted as to study the invitro anticancer activity of synthesized substituted piperdin-4-ones compounds using various MTT assays.

Materials and Methods

Chemistry

Synthesis of piperidin-4-ones has been described by Noller and Balasubramanian [16]. The reagent grade chemicals and reagents were purchased from Merck and Sigma Aldrich and purified by either distillation or recrystallisation before use. The purity of the synthesized compounds was checked by thin layer chromatography (TLC) with silica gel plates.

Instruments

The melting points determined in open capillary are reported as such using Barnstead 9001 electro thermal melting point apparatus with the rate of heating 2.5 deg min^{-1}.Electron impact mass spectra (EI-MS) were measured on a Jeol GC mate instrument at 70 eV. Infrared (IR) spectra (ν,cm^{-1}) was carried out on a Shimadzu FT/IR-330E, Fourier Transform Infrared Spectrometer (4000-400 cm^{-1}) in KBr pellets. Proton nuclear magnetic resonance (^{1}H-NMR) spectra and Carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra were recorded with Jeol GSX 4OONB NMR spectrometer operating at 500.13MHz and 125.76MHz using

dimethylsulphoxide as solvent and tetramethylsilane as an internal standard.Elemental analysis was performed in Elementar Vario EL 111-Germany (C,H and N analyzer).

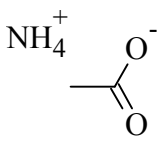
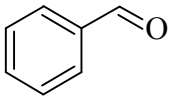
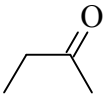
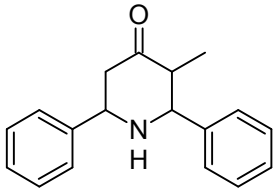
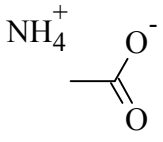
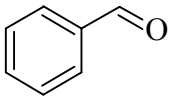
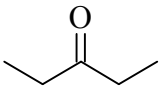
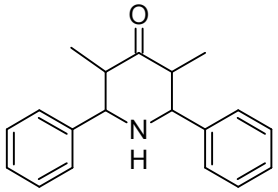
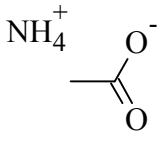
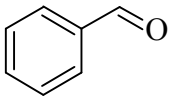
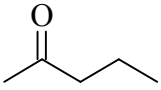
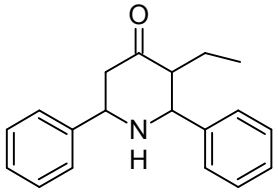
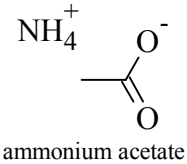
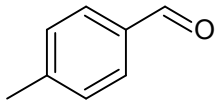
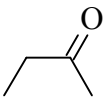
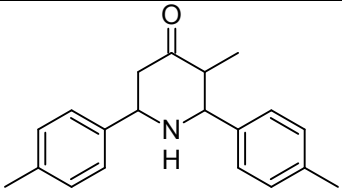
Biology

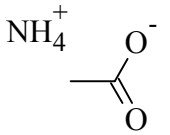
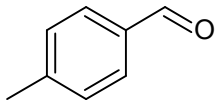
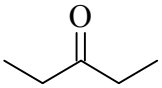
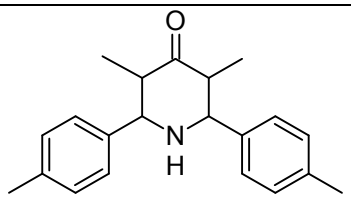
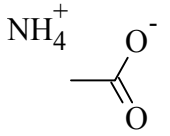
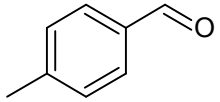
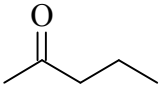
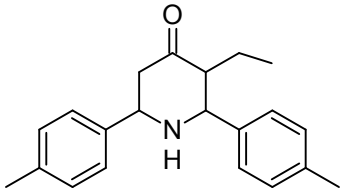
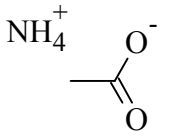
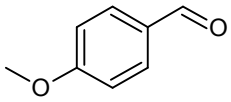
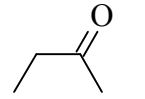
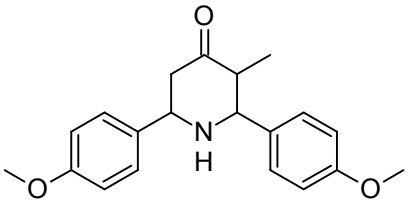
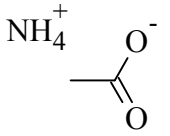
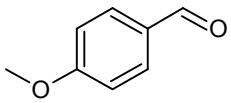
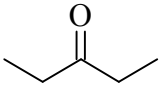
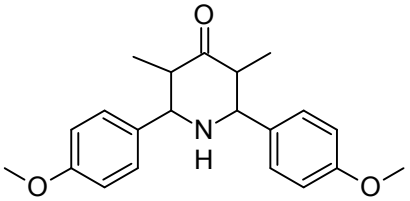
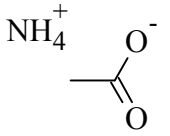
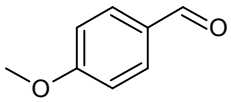
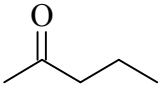
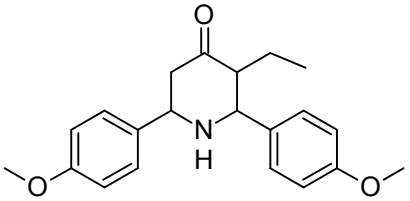
The in vitro anticancer activity of the synthesized compounds was evaluated by human cervical carcinoma cell line (HeLa).

Synthesis of Mannich Bases

The preparation of substituted piperdin-4-ones by mannich condensation reaction between aliphatic carbonyl compounds having active methylene protons with aromatic aldehydes and ammonium acetate (1:2:1) by refluxing in presence of ethanol (30ml) yielded corresponding substituted piperdin-4-ones(L_1-L_9).The reaction mixture was allowed to stand overnight at room temperature followed by addition of concentrated hydrochloric acid (30ml), the precipitated hydrochloride was collected and washed with ethyl alcohol and ether mixture (1:5) and transferred to one liter beaker. The hydrochloride was suspended in acetone and basified with strong ammonia solution. On dilution with excess of water the free base was separated out. The product was filtered, rinsed with water and dried. Crystallization of the product from ethanol results substituted piperdin-4-ones [16] (See Table 1).

Table 1: Synthesis of mannich Bases

Formation of Substituted 2,6-Diphenylpiperdin-4-Ones			
Reactants			**Products**
 ammonium acetate	 Benzaldehyde	 ethyl methyl ketone	 3-methyl-2,6-diphenylpiperidin-4-one(L_1)
 ammonium acetate	 Benzaldehyde	 pentan-3-one	 3,5-dimethyl-2,6-diphenylpiperidin -4-one(L_2)
 ammonium acetate	 Benzaldehyde	 pentan-2-one	 3-ethyl-2,6-diphenylpiperidin-4-one(L_3)
Formation Of Substituted 2,6-Ditolylpiperdin-4-Ones			
Reactants			**Products**
 ammonium acetate	 4-methyl benzaldehyde	 ethyl methyl ketone	 3-methyl-2,6-ditolylpiperidin-4-one(L_4)

 ammonium acetate	 4-methyl benzaldehyde	 pentan-3-one	 3,5-dimethyl-2,6-ditolylpiperidin-4-one(L_5)
 ammonium acetate	 4-methyl benzaldehyde	 pentan-2-one	 3-ethyl-2,6-ditolylpiperidin-4-one(L_6)
Formation Of Substituted 2,6-Dianisylpiperdin-4-Ones			
Reactants			Products
 ammonium acetate	 4-methoxy benzaldehyde	 ethyl methyl ketone	 3-methyl-2,6-dianisylpiperidin-4-one(L_7)
 ammonium acetate	 4-methoxy benzaldehyde	 pentan-3-one	 3,5-dimethyl-2,6-dianisylpiperidin-4-one(L_8)
 ammonium acetate	 4-methoxy benzaldehyde	 pentan-2-one	 3-ethyl-2,6-dianisylpiperidin-4-one(L_9)

3-methyl-2,6-diphenylpiperidin-4-ones (L_1)

Benzaldehyde (0.02mol, 20ml),ethylmethylketone (0.1mol, 9ml) and ammonium acetate (0.1 mol, 7.7g) in ethanol.Recrystallisation by petroleum ether. Yield 75%. Anal. $C_{18}H_{19}NO$, 265.3512, IR (KBr, cm^{-1}): ν(C=O) 1710, ν (N-H) 2928, ν (O-H) 3200-3700, ν (HOH) 1633, ν (Ar-H) 802.

3, 5-dimethyl-2,6-diphenylpiperidin-4-one (L_2)

Benzaldehyde (0.02mol, 20ml), 3-pentanone (0.1mol, 10.5ml) and ammonium acetate (0.1 mol, 7.7g) in ethanol.Recrystallisation by ethanol. Yield 85%.Anal.$C_{19}H_{21}NO$, 279.3779, IR (KBr, cm^{-1}): ν(C=O) 1712, ν (N-H) 2924, ν (O-H) 3200-3700, ν (HOH) 1624, ν (Ar-H) 868.

3-ethyl-2, 6-diphenylpiperidin-4-one (L_3)

Benzaldehyde (0.02mol, 20ml),2-pentanone (0.1mol, 10.5ml) and ammonium acetate (0.1 mol, 7.7g) in ethanol.Recrystallisation by ethanol. Yield 65%.Anal. $C_{19}H_{21}NO$, 279.3779 ,IR(KBr,cm^{-1}): ν(C=O) 1713, ν(N-H) 2924, ν(O-H) 3200-3700, ν(HOH) 1625,ν(Ar-H)867.

3-methyl-2, 6-ditolylpiperidin-4-one (L_4)

Tolylaldehyde(0.02mol, 24ml), ethyl methyl ketone (0.1mol, 9ml) and ammonium acetate (0.1 mol, 7.7g) in ethanol.Recrystallisation by petroleum ether. Yield 65%.Anal.$C_{20}H_{23}NO$,293.4045,IR (KBr,cm^{-1}): ν(C=O) 1697, ν(N-H) 2928, ν(O-H) 3200-3700, ν(HOH) 1631,ν(Ar-H) 810.

3, 5-dimethyl-2, 6-ditolylpiperidin-4-one (L_5)

Tolylaldehyde (0.02mol, 24ml), 3-pentanone (0.1mol, 10.5ml) and ammonium acetate (0.1 mol, 7.7g) in ethanol.Recrystallisation by ethanol. Yield 86%.Anal. $C_{21}H_{25}NO$, 307.4312, IR (KBr, cm^{-1}): ν(C=O) 1712, ν (N-H) 3207, ν (O-H) 3200-3700, ν (HOH) 1620, ν (Ar-H) 817.

3-ethyl-2, 6-ditolylpiperidin-4-one (L_6)

Tolylaldehyde (0.02mol, 24ml), 2-pentanone (0.1mol, 10.5ml) and ammonium acetate (0.1 mol, 7.7g) in ethanol.Recrystallisation by ethanol. Yield 67%.Anal. $C_{21}H_{25}NO$, 307.4312 m, IR (KBr, cm^{-1}): ν(C=O) 1710, ν (N-H) 2928, ν (O-H) 3200-3700, ν (HOH) 1633, ν (Ar-H) 802.

3-methyl-2, 6-di (p-methoxy) piperidin-4-one (L_7)

Anisaldehyde (0.02mol, 24ml), ethylmethyl ketone (0.1mol, 9ml) and ammonium acetate (0.1 mol, 7.7g) in ethanol.Recrystallisation by petroleum ether. Yield 75%.Anal. $C_{20}H_{23}NO_3$, 325.4034, IR (KBr, cm^{-1}): ν(C=O) 1710, ν (N-H) 2928, ν (O-H) 3200-3700, ν (HOH) 1633, ν (Ar-H) 802.

3, 5-dimethyl-2, 6-di (p-methoxy) piperidin-4-one (L_8)

Anisaldehyde (0.02mol, 24ml), 3-pentanone (0.1mol, 10.5ml) and ammonium acetate (0.1 mol, 7.7g) in ethanol.Recrystallisation by ethanol. Yield 85%.Anal. $C_{21}H_{25}NO_3$, 339.4301, IR (KBr, cm^{-1}): ν(C=O) 1701, ν (N-H) 3209, ν (O-H) 3200-3700, ν (HOH) 1612, ν (Ar-H) 804.

3-ethyl-2, 6-di (p-methoxy) piperidin-4-one (L_9)

Anisaldehyde (0.02mol, 24ml), 2-pentanone (0.1mol, 10.5ml) and ammonium acetate (0.1 mol, 7.7g) in ethanol.Recrystallisation by ethanol. Yield 65%.Anal. $C_{21}H_{25}NO_3$, 339.4301, IR (KBr, cm^{-1}): ν(C=O) 1707, ν (N-H) 3089, ν (O-H) 3200-3700, ν(HOH) 1612,ν(Ar-H) 831.

Results

The structure of the piperdin-4-one compounds, were elucidated by elemental analysis, mass spectra which is consistent with the proposed molecular structure of the compound (Table 2) and in vitro anticancer activity. The physical data of the synthesized piperdin-4-one compounds and structural assignments of the compounds were already investigated and discussed for IR spectral analysis, ^1H and ^{13}C NMR spectral analysis [17].

Table 2: Analytical data for piperdin-4-one compounds

Ligand	Molecular formulae	Molecular weight	Yield %	Elemental Analysis (%)					
				calculated			found		
				C	H	N	C	H	N
L_1	$C_{18}H_{19}NO$	265.34956	75	81.47	7.22	5.28	81.45	7.25	5.26
L_2	$C_{19}H_{21}NO$	279.37614	85	81.68	7.58	5.01	81.66	7.59	5.00
L_3	$C_{19}H_{21}NO$	279.37614	65	81.68	7.58	5.01	81.66	7.59	5.00
L_4	$C_{20}H_{23}NO$	293.40272	65	81.87	7.90	4.77	81.85	7.90	4.76
L_5	$C_{21}H_{25}NO$	307.42930	86	82.04	8.20	4.56	82.01	8.21	4.54
L_6	$C_{21}H_{25}NO$	307.42930	67	82.04	8.20	4.56	82.01	8.21	4.54
L_7	$C_{20}H_{23}NO_3$	325.40152	75	73.82	7.12	4.30	73.79	7.14	4.31
L_8	$C_{21}H_{25}NO_3$	339.4281	85	74.31	7.42	4.13	74.29	7.44	4.12
L_9	$C_{21}H_{25}NO_3$	339.4281	65	74.31	7.42	4.13	74.29	7.44	4.12

Discussion

All the ligands(L_1-L_9) were tested for in vitro against anticancer activity with HeLa cell line and evaluating with MTT colorimetric assay examine versus controls treat by means of check agent used for every compound [18,19]. The inhibitory concentration (IC_{50}) was determined and the concentration of compound to facilitate because 50% inhibition of cell growth is reported (Table 3). The obtained results revealed that all the ligands shows small action expect L_5 and L_7. The results highlight the relationship between substitutents at 2 and 6 positions of tolyl groups of piperdin-4-ones has high potent in anticancer activity. Consequently every single ligand as an effect of resulting in varying the molecular shape and direction [20-22]. Among these positions, substitution of methyl group at 3 and 5-position greatly influences their potency of anticancer activity when compare with ethyl group at 3-position substituted ditolyl piperdin-4-ones.

Table 3: In Vitro Anticancer Activity for HeLa Cell Line

Concentration (μM)	% Growth Inhibition								
	L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8	L_9
6.25	16.67	9.92	5.74	7.70	7.93	10.11	20.16	-0.74	3.73
12.5	15.26	17.30	10.22	13.47	20.15	12.24	20.40	1.03	9.42
25	21.81	23.86	10.00	19.92	30.48	15.22	17.83	4.65	5.28
50	21.37	32.67	12.47	13.28	58.32	22.64	22.53	6.83	19.08
100	20.11	41.83	24.13	39.45	88.34	65.17	22.44	14.48	23.16
IC 50%	>100	>100	>100	>100	37.44	78.8 uM	>100	>100	>100
R^2	-	-	-	-	0.9782	0.8779	-	-	-

Conclusions

In conclusion we have accomplished the synthesized molecules were estimated for their anticancer activities. The convenient protocol for the synthesis of piperdin-4-one by in vitro anticancer activity evaluation by MTT assay concludes that piperidin-4-one exhibit less active anticancer activity by electron donating mechanism (i.e.) it donate unshared pair of electrons on carbonyl oxygen atom. Presence of electron releasing substituent particularly methyl group at 3 and 5-position of 2,6-ditolylpiperidin-4-one ring (L_5) found to be leading for potent anticancer activity. It is remarkable that 3,5-dimethyl-2,6-ditolylpiperidin-4one possessed foremost anticancer activity than 3-ethyl-2,6-ditolylpiperidin-4one because both having same molecular formula and molecular weight.

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