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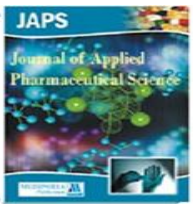

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Synthesis, Cytotoxic and Anti-bacterial studies of some novel derivatives of *N*'-[(2*E*)-3-phenylprop-2-en-1-yl]-2-(quinolin-8-yloxy) acetohydrazide

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ABSTRACT

An efficient method for preparing chalcones, commencing from acetohydrazide and substituted aromatic aldehydes, have been developed. The reaction of acetohydrazide with substituted aldehydes in the presence of ethanol to yield chalcones 4(a-j) and further the compounds thus obtained were identified by IR, ^{1}H NMR and Mass spectral data and have been screened for their anti-cancer and anti-microbial activity. All the compounds shown significant anti-bacterial activity against S. aureus, B. Subtilis, S. typhi and E. coli and potent anti-fungal activity against A. Niger and C. Albicans. Further moderate activity against carcinoma cells.

Key words: *8*-hydroxyquinoline, chalcone, cytotoxic activity, anti-bacterial activity**.**

INTRODUCTION

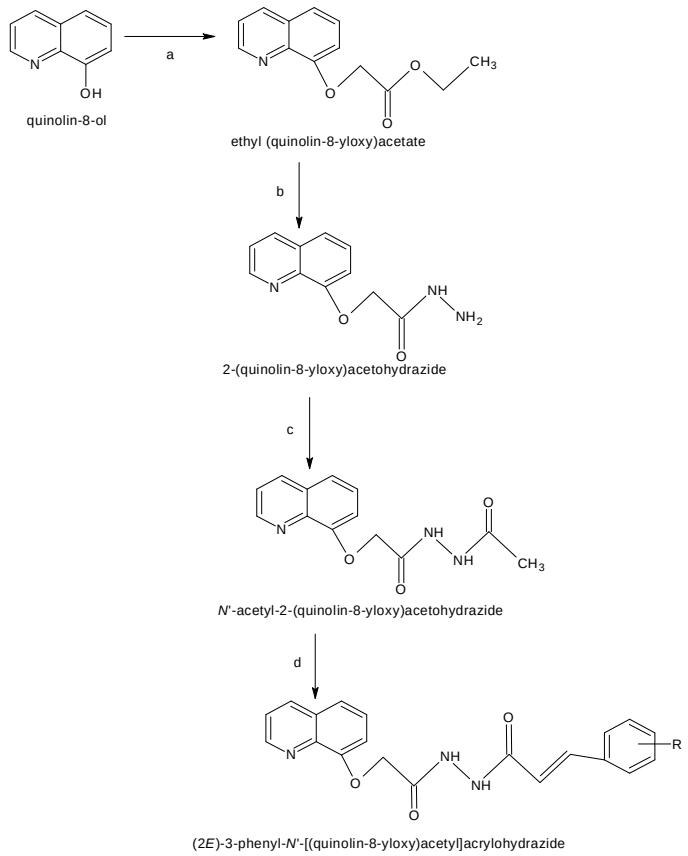
Chalcones constitute an important family of substances belonging to flavonoids, a large group of natural and synthetic products with interesting physicochemical properties, biological activity and structural characteristics. Chalcones are highly reactive substances of varied nature, and they experience chemical and physical transformations of great importance. These are characterized with diverse biological activities, among which anti-inflammatory, anti-malaria, anti-protozoal, anti-bacterial(Vibhute YB, 2003), cytotoxic (Bhat B.A 2005), anti-mitotic (Michael LE,1990), anti-viral (Alka Pande, 1987)and anti-inflammatory,(Hesieh HK,2000), antileishmanial,(Nielsen SF, 1998) and antitubercular(Lin YM, 2002) activity. With these observation in the present work we report synthesis and reactions of *N*'-[(2*E*)-3-phenylprop-2-en-1-yl]-2-(quinolin-8-yloxy) acetohydrazide. The structures were confirmed by IR, ^{1}H NMR and Mass spectral data.

MATERIAL AND METHODS

Melting points were determined with open capillary and are uncorrected. I.R spectra were recorded on a Shimadzu FTIR model 8010 spectrophotometer, 1H NMR spectra were recorded in CDCl3 on a Bruker supercon FT-NMR instrument using TMS as internal standard. Mass spectra were recorded on Schimadzu LCMS 2010 A Mass spectrometer.

Ethyl (quinolin-8-yloxy) acetate: (1)

An equimolar mixture of 8- hydroxy quinoline ethyl chloroacetate and anhydrous potassium carbonate (0.02mol) in dry acetone (60 ml) was refluxed on a water bath for 24 hr. The inorganic solid was filtered and the excess solvent was removed on a rota vapour. The reddish brown product was obtained, filtered, dried and recrystalized from ethanol. yield 85%, m.p. 78-80^{o}. IR (KBR) γ max: 3553, 3048, 1747, 1578, 1507, 1472, 1372, 1285, 1165, 1093, 973, 817, 741 cm^{-1}. H^1 NMR ($CDCl_3$, 400 MH_z): δ 8.94-7.18 (m, 6H, Phenyl), 4.95 (s, 2H, -OCH_2), 4.21 (m, 2H, CH_2 of ethyl), 1.20 (m, 3H, CH_3 of ethyl).



Where R: 4-Cl, 4-NO_2, 3-OH-4-OCH_3, $N(CH_3)_2$, 2-OH, 3,4,5- (OCH_3), 3-OCH_3, 4-OCH_3, 3-OH-4-OC_2H_5, 4-OH

Reagents and conditions: a. dry acetone, K_2CO_3 reflux 24 h; **b.** NH_2NH_2, abs. EtOH, reflux 15 h; **c**. glacial acetic acid, reflux 5h **d.** Ar-CHO, ethanol, 10%NaOH stir 1 h.

2-(quinolin-8-yloxy) acetohydrazide (2)

To a suspension of (**1)** (0.01 mol) in absolute ethanol (200 ml), hydrazine hydrate (99%, 0.015 mol) was added and the reaction mixture was refluxed for 15hr. The solution was concentrated and allowed to cool overnight. The resulting solid obtained was filtered, washed with cold ethanol, dried and recrystalized from ethanol. The compound was separated as brown crystals. yield 74%, m. p. 110-112 ^{o} C. IR (KBR) γ max: 3326, 3257, 1662, 1610, 1504, 1474, 1382, 1257, 1118, 1079, 819, 751 cm^{-1}. H^1 NMR ($CDCl_3$, 400 MH_z): δ 8.96 (s, 1H, NH), 8. 80- 7. 12 (m, 6H, Ar), 4.90 (s, 2H, -OCH_2), 3.10 (br, s, 2H, NH_2).

***N*'-acetyl-2-(quinolin-8-yloxy) acetohydrazide (3)**

2-(quinolin-8-yloxy) acetohydrazide (0.1 mol) (2) was suspended in glacial acetic acid (15 ml) and reflux for 6hrs. After completion of the reaction mixture was allowed to stay at room temperature for 30min and the pale green solid that separated was collected, washed with cold water. The product was recrystallized by using hot ethanol. The compound was separated as white crystals. Yield: 74% m. p.: 110-112 ^{o} C. IR (KBR) γ max: 3326, 3257, 1662, 1610, 1504, 1474, 1382, 1257, 1118, 1079, 819, 751 $cm^{-1}H^1$ NMR ($CDCl_3$, 400 MH_z): δ 8.96 (s, 1H, NH), 8. 80- 7. 12 (m, 6H, Ar), 4.90 (s, 2H, -OCH_2), 3.10 (br, s, 2H, NH_2).

(2E)-3-phenyl-N'-[(quinolin-8-yloxy)acetyl]acrylohydrazide (4)

An equimolar mixture of *N*'-acetyl-2-(quinolin-8-yloxy) acetohydrazide **(3)** and aromatic aldehyde (0.01 mol) were dissolved in minimum amount of alcohol. Sodium hydroxide solution (0.02mol) was added slowly and the mixture stirred for 2hr until the entire mixture becomes very cloud. Then the mixture was poured slowly into 400 ml of water with constant stirring and kept in refrigerator for 24 hours. The precipitate obtained was filtered, washed and recrystallized from ethanol. The spectral values were shown in table 1.

Table 1: Spectral Characterization of *N*'-[(2*E*)-3-phenylprop-2-en-1-yl]-2-(quinolin-8-yloxy) acetohydrazide

Sl. No.	*R*	IR (KBr) cm^{-1}	^{1}H NMR (DMSO D_6, 400 MHz) δ ppm	MASS
Va	4-Cl	3568 (NH), 2342, 1718 (C=O), 1637 (CH=CH) , 1560, 1542, 1490, 1458	8.10 (s, 1H, =CH-Ar),7.9- 6.9 (m, 12H Ar), 6.80 (d,1H, -COCH=), 5.3 (s, 2H, O-CH_2), 3.8 (s, 2H, NH).	M+**:** 380, 321, 265, 224,154,136
Vb	4- NO_2	3397 (C-NO_2), 3568(NH), 3011, 2849, 1708 (C=O), 1654 (CH=CH), 1541, 1458, 1345, 1197, 1008, 850, 739.	8.21 (s, 1H, =CH-Ar),8.0- 6.8 (m, 12H Ar), 6.6 (d,1H, -COCH=), 5.42 (s, 2H, O-CH_2), 3.85-3.95 (s, 2H, NH).	M^+ **:** 391, 353,265,224, 154, 136
Vc	3-OH, 4-OCH_3	3590(OH), 3369(NH), 2923 (CH), 2361, 1638 (C=O), 1579, 1508, 1477, 1396, 1087(OCH_3), 779, 721.	8.21 (s, 1H, =CH-Ar),7.5- 6.8 (m, 10H Ar), 6.75 (d,1H, -COCH=), 5.8 (s, 1H, -OH) 5.42 (s, 2H, O-CH_2), 3.85-3.95 (s, 2H, NH), 2.9 (s, 3H, OCH_3).	M^+ **:** 392, 375, 321, 224, 176,154.
Vd	$N(CH_3)_2$	3569 (NH), 2366, 1719(C=O), 1638 (CH=CH) 1561, 1518, 1475, 1109,	8.9 (m,6H, $N(CH_3)$), 8.11 (s, 1H, =CH-Ar),7.5- 6.8 (m, 12H Ar), 6.75 (d,1H, -COCH=), 5.42 (s, 2H, O-CH_2), 3.85-3.95 (s, 2H, NH).	M^+ **:** 389, 353, 307, 242, 224,154,136
Ve	Salicylaldehyde	3855, 3752, 2363, 1718 (C=O), 1655 (CH=CH), 1560, 1542, 1475, 1090 (OCH_3), 670	8.21 (s, 1H, =CH-Ar),7.5- 6.8 (m, 12H Ar), 6.69 (d,1H, -COCH=), 5.8 (s, 1H, -OH) 5.42 (s, 2H, O-CH_2), 3.85-3.95 (s, 2H, NH).	M^+ **:** 390, 321, 224, 154.
V f	3,4,5-(OCH_3)$_3$	3568 (NH), 2342, 1718 (C=O), 1637 (CH=CH) , 1560, 1542, 1490, 1458	9.89 (s, 1H, =CH-Ar),8.89- 7-13 (m, 12H Ar), 6.99 (d,1H, -COCH=), 4.7 (s, 2H,	M+**:** 425, 321, 265, 224,154,136

			O-CH_2), 3.87-3.86 (s, 2H, NH), 2.08-3.74 (s, 9H,-OCH_3).	
V g	3-OCH_3	3397 (C-NO_2), 3568(NH), 3011, 2849, 1708 (C=O), 1654 (CH=CH), 1541, 1458, 1345, 1197, 1008, 850, 739.	8.87 (s, 1H, =CH-Ar),8.29- 6.61 (m, 12H Ar), 6.18 (d,1H, -COCH=), 5.18 (s, 2H, O-CH_2), 3.35 (s, 2H, NH), 2.5 (s, 3H, OCH_3).	M^+ **:** 365, 353, 265,224, 154, 136
V h	4-OCH_3	3590(OH), 3369(NH), 2923 (CH), 2361, 1638 (C=O), 1579, 1508, 1477, 1396, 1087(OCH_3), 779, 721.	9.88 (s, 1H, =CH-Ar),8.53- 6.73 (m, 10H Ar), 6.69 (d,1H, -COCH=), 5.13 (s, 2H, O-CH_2), 3.65 (s, 2H, NH), 2.51 (s, 3H, OCH_3).	M^+ **:** 365, 375, 321, 224, 176,154.
V i	3-OH, 4-OC_2H_5	3569 (NH), 2366, 1719(C=O), 1638 (CH=CH) 1561, 1518, 1475, 1109,	9.76 (s, 1H, =CH-Ar),8.88- 6.9 (m, 12H Ar), 6.61 (d,1H, -COCH=), 4.83 (s, 1H,-OH), 4.71 (s, 2H, O-CH_2), 3.38 (s, 2H, NH)2.86 (s, 2H, -CH_2), 2.09 (s, 3H, -CH_3) .	M^+ **:** 395, 353, 307, 242, 224,154,136
V j	4-OH	3855, 3752, 2363, 1718 (C=O), 1655 (CH=CH), 1560, 1542, 1475, 1090 (OCH_3), 670	8.87 (s, 1H, =CH-Ar),7.55- 6.97 (m, 12H Ar), 5.51 (d,1H, -COCH=), 5.10 (s, 1H, -OH) 4.61 (s, 2H, O-CH_2), 3.43 (s, 2H, NH).	M^+ **:** 351, 321, 224, 154.

In-vitro Cytotoxic activity in Ehrlich Ascites

Carcinoma (EAC) cells

The EAC cells were collected, counted, and adjusted to 106 cells/mL withnormal saline. The drug dilutions were made with phosphate buffer saline (PBS) and were further adjusted to concentrations ranging from 125-1000 µg/mL. The drug dilutions were then added to the EAC cells and incubated at 37oC for 3 h. At the end of 3 h, the cell viability was determined by tryphan blue exclusion method. Under identical conditions, standard antitumor agent vincristine was evaluated for its *in-vitro* antitumor activity. The percentage cytotoxicity was calculated using the formula. The data for cytotoxic activity is recorded in table-2.

$$\text{Percentage cytotoxicity} = 100 - Tc\text{-}Dc/Tc \times 100,$$

Where, *Tc* = total EAC cells, and *Dc* = dead EAC cells.

In Vitro Evaluation of Antimicrobial Activity of Compounds

The antibacterial and antifungal activity of title compounds was determined by *in vitro* by using cup-plate method. Institute of microbial technology, Chandigarh, India, prepared as per the agar media for each microorganism. The zone of inhibition (ZI) in mm was measured. The concentrations (5-100 µg/ml) of the test compounds were prepared by dissolving the compounds in dimethyl sulphoxide (DMSO). Under identical conditions, ampicillin and clotrimazole were tested as standard drug (25 µg/ml) for bacteria and fungi respectively. The data for anti-bacterial activity is recorded in table-3.

Table-2: Percentage Cytotoxicity shown by *N*'-[(2*E*)-3-phenylprop-2-en-1-yl]-2-(quinolin-8-yloxy) acetohydrazide.

Compounds	R	Percentage cytotoxicity of drug at various concentrations on EAC cells (µg/mL			
		1000	**500**	**250**	**125**
Va	4-Cl	**85.7**	**72.5**	**45.0**	32.6
Vb	4-NO_2	69.8	60.4	24.2	16.9
Vc	-$N(CH_3)_2$	65.6	43.8	34.1	23.1
Vd	3-OH, 4-OCH_3	72.3	51.0	31.2	23.8
Ve	2 –OH	52.6	24.3	36.8	31.3
Vf	3,4,5-$(OCH)_3$	42.0	14.8	31.7	28.4
Vg	3-OCH_3	55.3	44.7	36.2	21.7
Vh	4-OCH_3	45.2	34.5	17.2	40.9
Vi	3-OH, 4-OC_2H_5	**90.6**	**75.3**	**55.0**	20.5
Vj	4-OH	68.7	45.8	34.8	13.4
Vincristine		86.7	69.0	46.0	43.7

RESULTS AND DISCUSSION

In the present work, 1-(quinolin-8yloxy) butan-2-one 1 can be synthesized by treating equimolar mixture of 8-hydroxy quinoline, ethylchloracetate and potassium carbonate. The 2-(quinolin-8-yloxy) acetohydrazide 2 can be synthesized by treating with hydrazine hydride in ethanol. These when react with glacial aceticacid to yield *N*'-acetyl-2-(quinolin-8-yloxy) acetohydrazide 3, which later react with various aromatic aldehydes in presence of sodiumhydroxide to yield to *N*'-[(2*E*)-3-phenylprop-2-en-1-yl]-2-(quinolin-8-yloxy) acetohydrazide.4. All the synthesized compounds have been screened for their in-vitro anti-inflammatory and antioxidant activity.

In-vitro Cytotoxic activity in Ehrlich Ascites

In-vitro Cytotoxic activity of *N*'-[(2*E*)-3-phenylprop-2-en-1-yl]-2-(quinolin-8-yloxy) acetohydrazide. The compounds IC-1 & IC-9 shown potent activity in 1000,500 & 250 µg/ml than other substituents.

In-vitro Anti-bacterial activity

In-vitro Anti-bacterial activity of *N*'-[(2*E*)-3-phenylprop-2-en-1-yl]-2-(quinolin-8-yloxy) acetohydrazide, compounds Vf andVh shown potent activity against S. aureus and E.coli and Vd andVg shown moderate anti-bacterial activity compared with standard, compounds Vj, Vd & Vh shown good activity against C.albicans and A. niger. The compounds Vf & Vi showed equal activity with that of standard compound.

CONCLUSION

Compounds with electron withdrawing groups such as methoxy and hydroxyl showed better In-vitro cytotoxic activity than the others not having such groups. Compounds having pharmacophores such as, methoxy , dimethoxy and hydroxy groups

Table 3: Antimicrobial activity of *N'*-[(2*E*)-3-phenylprop-2-en-1-yl]-2-(quinolin-8-yloxy) acetohydrazide

	Minimum inhibitory concentrations (MICs) µg/ml						
Sl. No.	**R**	***S. aureus***	***B. Subtilis***	***E. Coli***	***S. typhi***	***C. Albicans***	***A. niger***
Va	4-Cl	20	15	50	25	30	50
Vb	4-NO_2	35	25	25	50	35	45
Vc	-$N(CH_3)_2$	25	20	15	45	25	45
Vd	3-OH, 4-OCH_3	15	20	15	35	20	25
Ve	Salicylaldehyde	75	35	50	25	75	50
Vf	3,4,5-$(OCH)_3$	10	15	25	50	25	25
Vg	3-OCH_3	15	20	20	25	25	30
Vh	4-OCH_3	25	15	10	20	20	25
Vi	3-OH, 4-OC_2 H_5	30	15	25	30	25	25
Vj	4-OH	20	35	30	25	20	15
	Ampicillin	>12.5	>12.5	>12.5	>12.5	-----	-----
	Clotrimazole	----	----	----	-----	>25	>25

have exhibited potent anti-bacterial activity than the others. These results suggest that the chalcones derivatives have excellent scope for further development as commercial cytotoxic activity and anti-bacterial agents.

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