Synthesis and Biological Evaluation of Chalcones having Heterosubstituent(s)

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Sweety, *et al.*: Chalcones having heterosubstituent(s)

Chalcones and their synthetic analogues appear to have the same binding site of tubuline as phenstatin, combretastatin steganacin and podophylotoxin and are therefore capable to inhibit cancer cell proliferation. The phenyl rings

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with appropriate substitutions maintain a fixed distance between two centers of aryl rings. The two aromatic rings in these molecules are arranged like the two wings of a butterfly having certain dihedral angle between them, therefore a "butterfly model" is proposed an important structural feature responsible for their antitubulin activity. In this sequence a series of chalcones were synthesized and evaluated for *in vitro* cytotoxic activity against a panel of human cancer cell lines. In addition the synthetics reduced MIC of ciprofloxacin upto four fold this indicates their bioavailability enhancing potential.

Key words: Antimicrobial, chalcones, *in vitro* cytotoxicity

Chalcone (*trans*-1,3-diphenyl-2-propen-1-one) is an α,β-unsaturated ketone that has the skeletal makeup of so-called "chalcones". Chalcones are open-chain flavonoids in which two aromatic rings, joined by a three carbon linker, are synthesized by chalcone synthetase from 3-malonyl-CoA and a starter CoA ester such as 4-coumaronyl-CoA in plants[1]. Chalcone synthetase functions as a key enzyme of flavonoid biosynthesis, utilizing the same substrates as stilbene synthetase[2]. Chalcones are also called anthochlor pigments. This term was coined to identify a group of yellow pigments which turn red in the presence of alkali. In some plants, chalcones contribute significantly to the corolla pigmentation. They are also found in naturally occurring compounds, such as plant allelochemicals, and insect hormones and pheromones[3].

Chalcones are prepared by condensing aryl ketones with aromatic aldehydes in the presence of suitable condensing agents. They undergo a variety of chemical reactions and are found useful in synthesis of variety of heterocyclic compounds[4]. α,β-unsaturated ketones, which are structures in which the double bond is adjacent to the carbonyl group, have been used as starting materials for the synthesis of various chemicals, including plastics, resins, pesticides, dyes, and pharmaceuticals[5].

The compounds with the backbone of chalcones have been reported to possess various biological activities such as antimicrobial[6], antiinflammatory[7], analgesic[8], antiplatelet[9], antiulcerative[10], antimalarial[11], anticancer[12], antiviral[13], antileishmanial[14], antioxidant[15], antitubercular[16], antihyperglycemic[17], immunomodulatory[18], inhibition of chemical mediators release[19], inhibition of leukotriene B4[20], inhibition of tyrosinase[21] and inhibition of aldose reductase[22], estrogenic activities[23]. Since anticancer compounds like phenstatin, combretastatin, colchicines, steganacin and certain other synthetic analogues of these compounds have a common structural feature of possessing two rings with appropriate substitutions. We therefore propose a butterfly model with two wings represented by two aromatic rings connected by n number of carbon atoms (n= 1-4).

Due to the rapid development of bacterial resistance to antibacterial agents, it is vital to discover novel scaffold for the design and synthesis of the new antibacterial agents to help in the battle against pathogenic microorganisms[24].

N-(2-acetylphenyl acetamide) (0.5 g, 0.003 M) was dissolved in methanol (10 ml) in a 100 ml conical flask. To the solution substituted aromatic aldehyde (0.003 M) and 10% aq. NaOH solution (2 ml) were added respectively. Reaction mixture was kept in stirred condition. Temperature was maintained below 15° (in any case not more than 20°). The progress of reaction was monitored on TLC (0.2 % methanol in chloroform). After completion of reaction, the reaction mixture was poured in ice cold water and the solid was separated by filtration, washed with cold ice water and crystallized from alcohol (fig. 1 and Table 1). Spots were visualized by spraying the chromatogram with vanillin boric acid spray reagent (methanol:vanillin:boric acid:H_2SO_4::1000 ml, 1 g, 1 g, 20 ml) followed by heating the plate at 120°. From ^1H NMR spectra it was found that the synthetic compounds were pure and with *trans*- configuration (*J*= 15.45-15.51Hz).

N-[2-(3-Phenylacryloyl)-phenyl]-acetamide (SN-1): Yield 63%; m.p. 54-56; ^1H NMR ($CDCl_3$) δ: 2.26 (3H, *s*, $COCH_3$), 7.17 (1H, *dd*, J=7.81 and 7.42 Hz, H-5′), 7.42 (1H, *d*, J=15.51Hz, H-2), 7.65-7.38 (6H, *m*, H= 4′,2″,3″,4″,5″,6″), 7.81 (1H, *d*, J=15.51Hz, H-3), 7.98 (1H, *d,* J= 7.55Hz, H-3′), 8.71 (1H, *d*, J=8.31Hz, H-6′); IR (KBr,cm^{-1})-1680.35 (-C=O), 1650.45 (-$NHCOCH_3$), 1585.72 (aromatic); MS (m/e): 265 M^+ calculated for $C_{17}H_{15}O_2N$; UV- λ_{max}: 321 nm (CH_3OH)

N-{2-[3-(3,4,5-Trimethoxy-phenyl)-acryloyl]-phenyl}-acetamide (SN-2): Yield 72%; m.p. 81-82; ^1H NMR ($CDCl_3$)δ: 2.25 (3H, *s*, -$COCH_3$), 3.93 (9H, *s*, -OCH_3), 6.86(2H, *s*, H-2″,6″), 7.18 (2H, *t*, J=7.54 and 7.91Hz, H-5′), 7.4 (1H, *d*, J=15.51Hz, H-2), 7.58 (1H, *dd*,

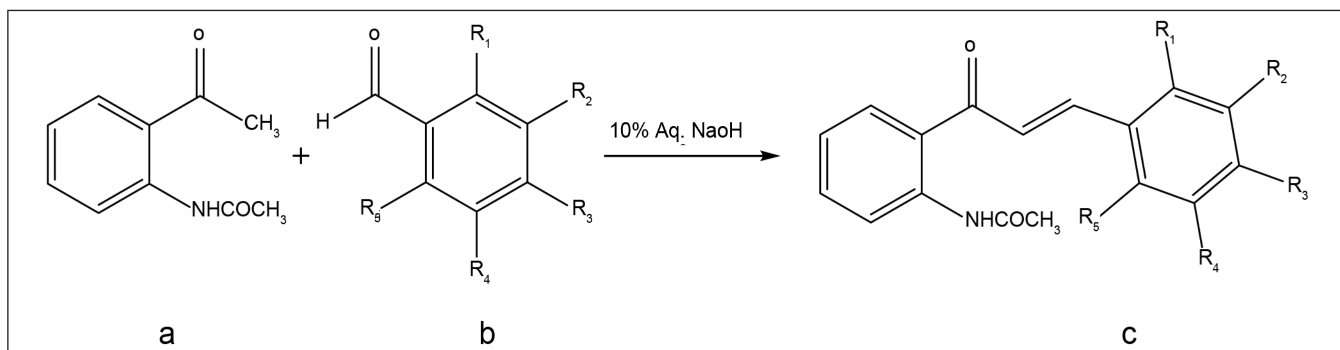

Fig. 1: Synthesis of compounds of N-(2-acetylphenyl acetamide)
(a) N-(2-acetylphenyl acetamide); (b) Substituted aromatic aldehydes; c, Substituted chalcones

TABLE 1: VARIOUS R GROUPS

Code	R_1	R_2	R_3	R_4	R_5
SN-1	H	H	H	H	H
SN-2	H	OCH_3	OCH_3	OCH_3	H
SN-3	OCH_3	OCH_3	OCH_3	H	H
SN-4	H	OCH_3	OCH_3	H	H
SN-5	H	NO_2	H	H	H
SN-6	H	Br	H	H	H
SN-7	H	H	Br	H	H
SN-8	H	OCH_3	OH	H	H
SN-9	OCH_3	H	H	OCH_3	H
SN-10	Cl	Cl	H	H	H

J=7.34 and 7.58Hz, H-4′), 7.72 (1H, *d*, J=15.51Hz, H-3), 7.97 (1H, *d*, J=7.98 Hz, H-3′), 8.70 (1H, *d*, J=8.45Hz, H-6′); IR (KBr, cm^{-1})- 1693.37 (-C=O), 1649.01 (-$NHCOCH_3$), 1585.01 (aromatic); MS (m/e): 355 M^+ calculated for $C_{20}H_{21}O_5N$; UV- λ_{max}: 362.50 nm (CH_3OH)

N-{2-[3-(2,3,4-Trimethoxy-phenyl)-acryloyl]-phenyl}-acetamide (SN-3). Yield 65%; m.p. 82-83; ^{1}H NMR ($CDCl_3$) δ: 2.25 (3H, *s*, -$COCH_3$), 3.93 (9H, *s*, -OCH_3), 6.17 (1H, *d*, J=8.32, H-5″), 6.64 (1H, *d*, J=8.25, H-6″), 7.18 (1H, *dd*, J=7.54 and 7.93Hz, H-5′), 7.4 (1H, *d*, J=15.51Hz, H-2), 7.58 (1H, *t*, J=7.34 and 7.58Hz, H-4′), 7.72 (1H, *d*, J=15.51Hz, H-3), 7.97 (1H, *d*, J=7.98Hz, H-3′), 8.70 (1H, *d*, J=8.45Hz, H-6′); IR (KBr, cm^{-1})- 1682.57(-C=O), 1648.51 (-$NHCOCH_3$), 1585.66(aromatic); MS (m/e): 355 M^+ calculated for $C_{20}H_{21}O_5N$; UV- λ_{max}: 362.50 nm (CH_3OH)

N-{2-[3-(3,4-Dimethoxy-phenyl)-acryloyl]-phenyl}-acetamide (SN-4).Yield 62%; m.p. 79-81; ^{1}H NMR ($CDCl_3$) δ: 2.7 (3H, *s*, -$COCH_3$), 3.9 (6H, *s*, 2-OCH_3), 6.61 (1H, *d*, J=8.62 Hz, H-5″), 6.70 (1H, *s*, H-2″), 6.75 (1H, *d*, J=8.41Hz, H-6″), 6.9 (2H, *m*, H-4′,5′), 7.2 (1H, *d*, J=15.45Hz, H-2), 7.6 (1H, *d*, J=15.45Hz, H-3), 8.7 (1H, *d*, J =7.42Hz, H-6′); IR (KBr,cm^{-1})- 1682.53(-C=O), 1686.4(-NHCO CH_3), 1585.72(aromatic); MS (m/e): 325 M^+ calculated for $C_{19}H_{19}O_4N$; UV- λ_{max}: 342.40 nm ($CHCl_3$)

N-{2-[3-(3-Nitro-phenyl)-acryloyl]-phenyl}-acetamide (SN-5). Yield 65%; m.p. 84-86; ^{1}H NMR ($CDCl_3$) δ: 2.7 (3H, *s*, -$COCH_3$), 6.9 (2H, *m*, H-4′,5′), 7.2 (1H, *d*, J=15.45Hz, H-2), 7.47 (1H, *dd*, J=8.66, 8.71Hz, H-5″), 7.6 (1H, *d*, J=15.45Hz, H-3), 7.69 (1H, *d*, J=8.4Hz, H-6″), 8.07 (1H, *m*, H-4″), 8.23 (1H, *s*, H-2″), 8.7 (1H, *d*, J=7.44Hz, H-6′); IR (KBr,cm^{-1})- 1683.09(-C=O), 1654.48(-$NHCOCH_3$), 1588.65(aromatic); MS (m/e): 310 M^+ calculated for $C_{17}H_{14}O_4N_2$; UV- λ_{max}: 300.60 nm (CH_3OH)

N-{2-[3-(3-Bromo-phenyl)-acryloyl]-phenyl}-acetamide (SN-6). Yield 78%; m.p. 89-91; ^{1}H NMR ($CDCl_3$)δ: 2.7 (3H, *s*, -$COCH_3$), 6.9 (1H, *m*, H-4′,5′), 7.10 (1H, *dd*, J=8.68, 8.71Hz, H-5″), 7.2 (1H, *d*, J=15.45Hz, H-2), 7.24 (1H, *d*, J=8.42Hz, H-6″), 7.31 (1H, *d*, J=8.53Hz, H-4″), 7.47 (1H, *s*, H-2″), 7.6 (1H, *d*, J=15.45Hz, H-3), 8.7 (1H, *d*, J=7.44 Hz, H-6′); IR (KBr, cm^{-1})- 1680.77(-C=O), 1646.31(-$NHCOCH_3$), 1585.15(aromatic); MS (m/e): 344 M^+ calculated for $C_{17}H_{14}O_2NBr$; UV- λ_{max}: 313.80 nm ($CHCl_3$)

N-{2-[3-(4-Bromo-phenyl)-acryloyl]-phenyl}-acetamide (SN-7). Yield 76%; m.p. 91-94; ^{1}H NMR ($CDCl_3$)

δ: 2.7 (3H, *s*, -$COCH_3$), 6.9 (1H, *m*, H-4′,5′), 7.19 (2H, *d*, J=7.54Hz, H-2″,6″), 7.2 (1H, *d*, J=15.45Hz, H-2), 7.47 (2H, *dd*, J=8.43Hz, H-3″,5″), 7.6 (1H, *d*, J=15.45Hz, H-3), 8.7 (1H, *d*, J=7.41Hz, H-6′); IR (KBr,cm^{-1})- 1686.07(-C=O), 1646.31(-$NHCOCH_3$), 1589.44(aromatic); MS (m/e): 344 M^+ calculated for $C_{17}H_{14}O_2NBr$; UV- λ_{max}: 326.40 nm ($CHCl_3$)

N-{2-[3-(4-Hydroxy-3-methoxy-phenyl)-acryloyl]-phenyl}-acetamide (SN-8). Yield 65%; m.p. 128-130; ^{1}H NMR ($CDCl_3$) δ: 2.7 (3H, *s*, -$COCH_3$), 3.9 (3H, *s*, -OCH_3), 6.57 (1H, *d*, J=8.44, H-5″), 6.64 (1H, *d*, J=15.45Hz, H-2), 6.9 (2H, *m*, H-4′,5′), 7.19 (2H, *s*, H-2″), 7.6 (1H, *d*, J=15.45Hz, H-3), 8.7(1H, *d*, J=7.45 Hz, H-6′); IR (KBr,cm^{-1})- 1687.17(-C=O), 1654.48(-$NHCOCH_3$), 1588.56 (aromatic); MS (m/e): 311 M^+ calculated for $C_{18}H_{17}O_4N$; UV- λ_{max}: 330.20 nm ($CHCl_3$)

N-{2-[3-(2,5-Dimethoxy-phenyl)-acryloyl]-phenyl}-acetamide (SN-9). Yield 65%; m.p. 77-79; ^{1}H NMR ($CDCl_3$) δ: 2.7 (3H, *s*, -$COCH_3$), 3.9 (6H, *s*, 2, -OCH_3), 6.54(1H, *d*, J=8.52Hz, H-4″), 6.70 (1H, *s*, H-6″), 6.61 (1H, *d*, J=8.72Hz,H-3″), 6.9 (2H, *m*, Hz, H-4′,5′), 7.2 (1H, *d*, J=15.45Hz, H-2), 7.6 (1H, *d*, J=15.45Hz, H-3), 8.7 (1H, *d*, J=7.43Hz, H-6′); IR (KBr,cm^{-1})- 1682.35(-C=O), 1648.45 (-$NHCOCH_3$), 1588.72 (aromatic); MS (m/e): 325 M^+ calculated for $C_{19}H_{19}O_4N$; UV- λ_{max}: 340.20 nm ($CHCl_3$)

N-{2-[3-(2,3-Chloro-phenyl)-acryloyl]-phenyl}-acetamide (SN-10). Yield 76%; m.p. 82-84; ^{1}H NMR ($CDCl_3$) δ: 2.7 (3H, *s*, -$COCH_3$), 6.7 (1H, *m*, H-5″), 6.9 (2H, *m*, H-4′,5′), 7.09 (1H, *d*, J=8.23Hz, H-4″), 7.12 (1H, *d*, J=8.45Hz, H-6″), 7.2 (1H, *d*, J=15.45Hz, H-2), 7.6 (1H, *d*, J=15.45 Hz, H-3), 8.7 (1H, *d*, J=7.42 Hz, H-6′); IR(KBr,cm^{-1})-1681.35 (-C=O), 1648.45 (-$NHCOCH_3$), 1585.72 (aromatic); MS (m/e): 265 M^+ calculated for $C_{17}H_{13}O_2NCl_2$; UV- λ_{max}: 345.00 nm ($CHCl_3$)

The synthetic compounds (SN-1 to SN-10) were assayed for *in vitro* cytotoxicity against A-549 (lung), IGR-OV-1 (ovary), PC-3 (prostate), SF-295 (CNS) cell lines using sulforhodamine B. All compounds showed significant response against A-549, IGR-OV-1, PC-3, SF-295 cell lines. The results of cytotoxic activity against human cancer cell lines are given in (Table 2).

These synthetic compounds were also evaluated for antimicrobial activity and SN-6 having bromo substituent at meta position of ring B showed four fold reduction in MIC of ciprofloxacin when tested against *S. aureus*. The study revealed that the synthetics being reported potentiate antimicrobial activity of ciproflaxacin (Table 3).

A comparative assessment of the activity revealed that unsubstituted compound (SN-1) showed appreciable *in*

TABLE 2: INHIBITION OF VARIOUS HUMAN CANCER CELL LINES BY COMPOUNDS

Code	Conc.(M)	%Growth Inhibition			
		Lung	Ovary	Prostate	CNS
		A-549	IGR-OV-1	PC-3	SF-295
SN-1	1×10^{-5}	92	59	96	95
	1×10^{-4}	95	87	98	98
SN-2	1×10^{-5}	94	63	97	86
	1×10^{-4}	95	68	100	95
SN-3	1×10^{-5}	87	60	72	75
	1×10^{-4}	89	73	74	83
SN-4	1×10^{-5}	57	12	35	12
	1×10^{-4}	60	34	37	20
SN-5	1×10^{-5}	91	45	68	76
	1×10^{-4}	93	48	70	79
SN-6	1×10^{-5}	94	93	99	97
	1×10^{-4}	96	97	100	98
SN-7	1×10^{-5}	91	49	97	74
	1×10^{-4}	93	51	100	85
SN-8	1×10^{-5}	50	30	32	37
	1×10^{-4}	71	64	67	62
Paclitaxel	1×10^{-5}	59	62	50	
Mitomycin	1×10^{-5}			65	
Adriamycin	1×10^{-6}				72

TABLE 3: RESULTS OF ANTIMICROBIAL ACTIVITY OF SYNTHESIZED COMPOUNDS AGAINST *S. AUREUS*

Code	MIC alone (µg/ml) ciprofloxacin	MIC of ciprofloxacin with the synthetics concentration (mg/ml)						
		50	25	12.5	6.25	3.12	1.5	0.75
SN-1		2	2	4	8	8	8	8
SN-2	8	2	2	4	8	8	8	8
SN-3	8	2	2	4	8	8	8	8
SN-4	8	2	2	4	4	8	8	8
SN-5	8	4	4	8	8	8	8	8
SN-6	8	2	2	2	4	4	8	8
SN-7	8	2	2	4	4	8	8	8
SN-8	8	8	8	8	8	8	8	8

vitro cytotoxic activity against all cell lines. However SN-6 and SN-7 having bromo substituent at meta and para positions respectively in ring B are more potent than SN-1

Protocol for anticancer activity: Human cancer cell lines were grown in complete growth medium (RPMI-1640) in a carbon dioxide incubator (37°, 5% CO_2, 90% RH). Stock solutions (2×10^{-2} M) of compounds were prepared in DMSO. *In vitro* cytotoxicity against six human cancer cell lines was determined using 96-well tissue culture plate. The cell growth was determined by substracting mean OD value of respective blank from mean OD value of experimental set. Percent growth in presence of test material was calculated considering the growth in absence of any test material.

Protocol for antimicrobial activity: The combination studies were performed by a broth checkboard method[25]. The experiment was performed in a 96-well microtitre plate. Ciprofloxacin was used as control. The bacterial inocula of the test bacteria *S. aureus* 1199B were prepared from the overnight grown culture by adjusting the bacterial turbidity to 0.1 OD at 625 nm using a spectrophotometer in sterile normal saline. The inoculum was added to the wells of columns 1-12 (leaving 4 wells of column 12 as negative control). The plates were incubated at 37° for 24 h, read visually and the lowest concentration well in each row showing no turbidity was recorded.

In summary, we have synthesized a number of novel chalcone derivatives by condensation of N-(2-acetyl phenyl acetamide) with different substituted aromatic aldehydes. The compounds were purified by repeated crystallization from appropriate solvent. The yields of synthetic ranged between 62-78 %w/w. The structure of synthetics was established by spectral data (IR, NMR, MASS). NMR analysis of all the chalcones obtained showed that the *trans* isomers were formed. Screening of synthetics for cytotoxic activity revealed that all compounds showed significant response against A-549, IGR-OV-1, PC-3, SF-295 cell lines. Screening of synthetics for antimicrobial activity revealed that SN-6 showed four fold reduction in MIC of ciprofloxacin when tested against *S. aureus*.

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