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ORIGINAL ARTICLE **OPEN ACCESS**

Design Synthesis and *In Vitro* Biological Evaluation of Novel Chalcone Derivatives as Antitumor Agents

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ABSTRACT
A series of new, 2′-Methoxy chalcone ,4'methoxy chalcone & 3', 4', 5'-trimethoxy chalcone derivatives were designed, synthesized and evaluated for their Anti-proliferative and Nitric oxide inhibition activities. The results demonstrated that out of four compounds submitted, compound-1exhibited potent anti-proliferative activity with GI50 value of lower than 10µg/ml against Human Hepatoma Cell Line (HEPG2) whereas compound 3 showed moderate anti-proliferation activity & compound 9 & compound 11 from showed low anti-proliferation activities. The nitrous oxide (NO) inhibition efficacy of the representative compound was also evaluated. Out of eight compound submitted, the results showed that compound 8 & compound 10 exhibited potent inhibition of NO production further it exhibited the highest efficacy in Nitric oxide inhibition assay with % inhibition of 75.65% & 74.08 % at the given concentration.. Therefore, these results provided insight into the correlation between some structural properties of 2-methoxychalcones, 4-methoxychalcones and 3', 4', 5'-trimethoxy chalcones and their In Vitro anti-inflammatory and anti-cancer differentiation activity.
Keywords: Synthesis; Methoxy chalcone; antitumor; NO inhibition; HEPG2

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INTRODUCTION

Despite recent advances in our understanding of the biological processes leading to the development of cancer, there is still a need for new and effective agents to help bring this disease under control. Among the currently identified antitumor agents, chalcones represent an important class of molecules that are abundant in edible plants. Chemically, they are open-chained molecules bearing two aromatic rings linked by a three-carbon en-one fragment [1-3]. Chalcone derivatives have received a great deal of attention due to their relatively simple structures, and wide variety of pharmacological activities reported for these compounds include anti-inflammatory [4], antibacterial [5], antifungal [6], and antitumor activities [7-8]. These activities are largely attributed due to the α, β-unsaturated ketone moiety. The introduction of various substituents into the two aryl rings is also a subject of interest because it leads to useful structure–activity relationship (SAR) conclusions and thus helps to synthesize pharmacologically active chalcones [7-8]. In recent years, noteworthy advancement has been made by computational chemistry, which led new challenges to drug discovery. Quantitative structure activity relationship (QSAR) which has become an reputable tool for establishing quantitative relationship between biological activity and physicochemical properties of the compounds in a series using various statistical methods (linear regression and non-linear regression analysis) and it helps to calculate the biological activities of newly designed analogues contributing to the drug discovery process [9-10].
Protein–ligand docking is a powerful tool to study and provide a proper understanding of protein–ligand interactions. Docking is regularly used in different stages of drug design strategies, such as to facilitate design of potentially active leads [11-12]. Detection of the best ligand poses and proper ranking of several ligands' relative docking propensity are of great importance. Molecular docking, in practice, has two essential requirements [13]: structural data, for candidate ligands and the protein target of interest, and a procedure to estimate protein–ligand interaction poses and strengths [14].The RSCB Protein Data Bank (PDB) repository [15, 16] is the main source of protein target structures for docking studies. The number of structures deposited in the PDB repository has been rapidly increasing for many years. Cancer is one of the leading causes of mortality worldwide. Approximately one-third of people are affected by cancer during their lives [17]. A novel series of compounds as EGFR and NO inhibitors were designed and

evaluated for epidermal growth factor receptor inhibitory potential by using various computational tools along with NOS enzyme based cell line studies towards anti-cancer activity [18].

Research into the anti-tumor properties of chalcones has received significant attention over the last few years, particularly with the discovery that these compounds possess a similar mode of action to the structurally related combretastatins. Combretastatin A-4 (CA-4, Fig. 1), a natural cis stilbene product has received major attention owing to its strong inhibition of tubulin polymerization and selective targeting of tumor vascular systems (cancer vascular disrupting), which cuts off the tumor blood flow, leading to haemorrhagic necrosis as well as cancer antiangiogenesis[19]. A reported critical structural requirement for the activity of these compounds is the cis-configuration of the double bond and the 3,4,5-trimethoxyphenyl ring (ring A) [20]. Taking into account of these reports, and an attempt to discover a potent compound that suppresses both the inflammation and cancer was an attractive idea [21]. It is reasonable that the dual inhibitors of both inflammatory mediators such as NO and tumor cell proliferation were more effective than single inhibitors as blocking multiple signaling pathways in cancer therapy and could be beneficial to overcome drug resistance. With the goal of identifying such a new anti-inflammatory and anti-cancer strategy, therefore, we intended to synthesize 3 ,4 ,5 -trimethoxy chalcone analogues, 4 .methoxy chalcone analogues & 2 ,methoxy chalcone analogues with different substituent on B-ring. Similarly We now present the synthesis of 3, 4, 5 –trimethoxy chalcones analogues , 4 .methoxy chalcone analogues & 2, methoxy chalcone analogues using a classical base catalyzed condensation reaction and their inhibitory effects on nitric oxide production and tumor cell proliferation.

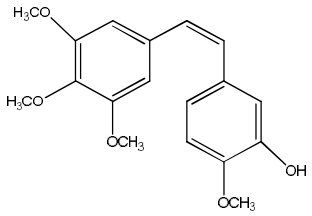


Fig.1: CAMBRETASTATIN A-4 (CA-4)

SYNTHESIS OF DIFFERENT METHOXY SUBSTITUTED CHALCONE ANALOGUE

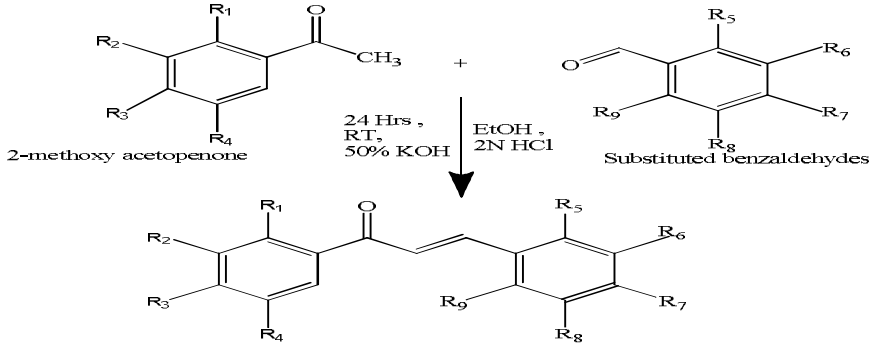


Fig. 2: SYNTHESIS OF DIFFERENT METHOXY SUBSTITUTED CHALCONE ANALOGUE

MATERIAL AND METHODS

GENERAL

The melting point was determine by open capillary method and are uncounted. The purity and homogeneity of compounds was determined by thin layer chromatography (TLC). Silica gel-G was used as stationary phase on glass plates. Iodine vapors were used for detection. The solvent system used was cyclohexane:ethyl acetate (4.5:0.5). . NMR spectra were measured on Bruker Avance 400 MHz spectrometer, using TMS as an internal standard. Abbreviations for signal coupling are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Coupling constants J are re- ported (Hz).

For *In Vitro* tumor cell proliferation SRB assay performed using 10% fetal bovine serum and 2 mM L-glutamine Using 10 % TCA & Sulforhodamine B (SRB) solution at (ACDSF), Advanced Centre for Treatment, Research & Education in Cancer, (ACTREC), Tata Memorial Centre. Kharghar, Navi Mumbai-410210. NO inhibition assay was performed using Sodium nitroprusside in aqueous solution at physiological pH which spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions.

GENERAL PROCEDURES FOR THE PREPARATION OF THE ALDOL CONDENSATION PRODUCTS OF 2′-METHOXY CHALCONE, 4'METHOXY CHALCONE & 3', 4', 5'-TRIMETHOXY CHALCONE ANALOGUES WITH ALDEHYDE [1-11]

To a clean dry R.B.F was added 500 mg (0.003329 mmole) of substituted acetophenone & 503 mg (0.003329 mmole) of substituted aldehyde was added followed by 35 ml of ethanol & 18 ml of 60% KOH drop wise was added and reaction mixture was stirred at RT for 16 Hours (overnight). Water was added to the reaction mixture and the solution was neutralized with 1 M HCl. The product was filtered and washed with cold water till the washing was neutral to litmus. Finally, the product was washed with ice cold ethanol. The crude product (chalcone) was dried in air. It was recrystallized from hot ethanol to produce the pure substituted 2′-Methoxy chalcone, 4'methoxy chalcone & 3', 4', 5'-trimethoxy chalcone analogues (1–11). See the Supplementary data and Table 1.

1-(2-methoxyphenyl)-3- (4-nitophenyl) prop-2-ene-1-one (1)

Yellow solid, mp 138-140°C; 1H NMR (400 MHz, $CDCl_3$): 8.26 (d, J=6.9, 2H), 7.73 (d, J=6.9, 2H), 7.67 (d, J=6.9, 2H), 7.53 (d, J=6.9, 2H), 7.08 (s, J=6.9, 1H), 7.03 (s, J=6.9, 1H), 3.94 (s, J=6.9, 3H), 115.0, 253.8, 283.7, 284.6 MS (ESI): 298.32 (M*+1).

. 1-(2-methoxyphenyl)-3- (3, 4, 5, trimethoxyphenyl) prop-2-ene-1-one (2)

Brown solid, mp 82-84°C; 1H NMR (400 MHz, $CDCl_3$): 7.57 (d, J=9.33, 2H), 7.04 (m, J=15.73, 4H), 4.58 (s, J=6.9, 9H), 4.23 (s, J=6.9, 3H), 3.48 (d, J=0.89, 1H), 3.47 (d, J=6.9, 1H), 134.0 , 328.7, 329.7, MS (ESI): 328.36 (M*+1).

. 1-(2-methoxyphenyl)-3- (4-chlorophenyl) prop-2-ene-1-one (3)

Light brown solid, mp 126-130°C; 1H NMR (400 MHz, $CDCl_3$): 7.58 (d, J=9.86, 2H), 7.57 (d, J=9.84, 2H), 7.04 (m, J=3.00, 4H), 4.23 (s, J=14.17, 3H), 3.48 (d, J=7.12, 1H), 3.47 (d, J=7.12, 1H), 238.8. 262.6, 272.8, MS (ESI): 272.73 (M*+1).

. 3-(4-(dimethylamino) phenyl)-1- (2-methoxyphenyl) prop-2-ene-1-one (4)

Dark Brownish red solid, mp 94-96°C; 1H NMR (400 MHz, CDCl3): 7.11 (d, J=15.77, 2H), 7.09 (m, J=7.46, 4H), 7.01 (m, J=7.46, 4H), 4.21 (s, J=6.9, 3H), 3.47 (s, J=6.9, 1H), 3.44 (d, J=6.9, 1H), 2.41 (s, d=6.9, 6H), 115.0 , 253.8 281.8, MS (ESI): 281.36 (M*+1).

. 3-(3,4-(dimethoxyphenyl)-1- (4-methoxyphenyl)prop-2-ene-1-one (5)

Pale yellow solid, mp 82-84°C; 1H NMR (400 MHz, CDCl3): 8.04 (s, J=6.9, 2H), 7.72 (d, J=15.5, 1H), 7.41 (d, J=15.5, 1H), 7.24 (d, J=8.4, 1H), 6.99 (d, J=6.9, 2H), 6.90 (d, J=8.4, 1H), 8.04 (s, J=6.9, 2H), 3.95 (s, J=9.2, 6H), 3.89 (s, J=6.9, 3H), 249.0 , 298.7 299.7, MS (ESI): 298.34 (M*+1).

. 1-(4-methoxyphenyl)-3- (3, 4, 5, trimethoxyphenyl)prop-2-ene-1-one (6)

Pale yellow solid, mp 144-146°C;1H NMR (400 MHz, CDCl3): 8.04 (s, J=6.9, 2H), 7.72 (d, J=15.5, 1H), 7.42 (d, J=15.5, 1H), 7.00 (d, J=8.4, 2H), 6.87 (d, J=6.9, 2H), 3.93 (s, J=9.2, 9H), 3.90 (s, J=6.9, 3H), 328.7 , 328.7 , MS (ESI): 328.36 (M*+1).

. 3- (4-chlorophenyl)-1-(4-methoxyphenyl) prop-2-ene-1-one (7)

Pale yellow solid, mp 138-140°C ;1H NMR (400 MHz, CDCl3): 8.04 (s, J=6.9, 2H), 7.75 (d, J=15.7, 1H), 7.58 (d, J=6.7, 2H), 7.52 (d, J=15.7, 1H), 7.39 (d, J=6.69, 2H), 6.99 (d, J=6.9, 2H), 3.9 (s, J=6.9, 3H) 238.8. 272.7, 274.7,275.7 MS (ESI): 272.73 (M*+1).

. 3-(4-(dimethylamino) phenyl)-1- (4-methoxyphenyl) prop-2-ene-1-one (8)

Yellow solid, mp 130-132°C;1H NMR (400 MHz, CDCl3): 8.03 (d, J=6.9, 2H), 7.79 (d, J=15.4, 1H), 7.55 (d, J=8.9, 2H), 7.36 (d, J=15.5, 1H), 6.97 (d, J=6.8, 2H), 6.70 (d, J=8.9, 2H), 3.89 (s, J=6.9, 3H), 3.05 (s, J=6.9, 6H), 134.8, 272.6,281.8 MS (ESI): 281.36 (M*+1).

.1-(4-methoxyphenyl)-3-(o-tolyl)prop-2-ene-1-one(9)

Pale Yellow solid, mp 98-100°C;1H NMR (400 MHz, CDCl3): 8.10 (d, J=15.5, 1H), 8.05 (d, J=6.9, 2H), 7.69 (d, J=7.8, 1H), 7.47 (t, J=15.5, 1H), 7.29 (d, J=6.9, 1H), 7.23 (d, J=8.0, 2H), 3.99 (d, J=6.9, 1H), 3.91 (d, J=6.9, 1H), 3.80 (d, J=6.9, 3H), 2.48 (s, J=6.9, 3H), 144.8, 242.6,266.8 MS (ESI): 267.35 (M*+1).

. 3-(4-(dimethylamino) phenyl)-1- (3, 4, 5-trimethoxyphenyl) prop-2-ene-1-one (10)

Dark Yellow solid, mp 140-142°C; 1H NMR (400 MHz, CDCl3): 7.19 (d, J=15.4, 2H), 7.16 (d, J=8.8, 2H), 7.14 (s, J=8.8, 2H), 3.46 (s, J=6.9, 9H), 3.18 (d, J=6.9, 1H), 3.15 (d, J=6.9, 1H), 2.28 (s, J=8.8, 6H), 252.8, 341.7,342.7 MS (ESI): 341.41 (M*+1).

. 3- (o-tolyl))-1- (3, 4, 5-trimethoxyphenyl) prop-2-ene-1-one (11)

Pale Yellow solid, mp 136-138°C ;1H NMR (400 MHz, CDCl3): 7.46 (s, J=7.4, 2H), 7.28 (d, J=6.9, 1H), 7.19 (t, J=6.9, 1H), 7.16 (t, J=6.9, 1H), 7.15 (t, J=6.9, 1H), 4.28 (s, J=7.0, 9H), 3.34 (d, J=7.0, 1H), 3.14 (d, J=7.0, 1H), 3.08 (s, J=7.0, 3H): 114.9, 252.7,312.8 MS (ESI): 312.37 (M*+1).

TUMOR CELL GROWTH INHIBITION ASSAY

The anti-proliferation effect of compounds 1–11 against human hepatoma cancer Hep G2 were performed by the Sulforhodamine B (SRB) assay method as we described previously.

NITRIC OXIDE (NO) DETERMINATION

The inhibitory assay for inflammatory mediator NO was performed by NO inhibition assay methods as we described previously.

STATISTICAL ANALYSIS

Each experiment was performed in triplicate and repeated three times (n = 9). The results were expressed as means ± SD. Statistical comparisons were made. In case of *In Vitro* anticancer screening SRB assay method

GI50 values (Concentration of drug causing 50% inhibition of cell growth) against ADR (Adriamycin, Positive control compound). In NO inhibition assay graph was plotted (Absorbance V/S Concentration) for ascorbic acid inhibition and sodium nitrite as standard was performed in triplicate and repeated three times. The results were expressed as means ± SD.

CHEMISTRY

The preparation of the 2-methoxy chalcone , 4-methoxy chalcone & 3',4',5' –trimethoxy chalcone analogues (Table 1) were carried out via Claisen–Schmidt condensation (Scheme 1). Thus, an appropriate commercially available aryl aldehydes were reacted with inexpensive 2-methoxy chalcone , 4-methoxy chalcone and 3,4,5-trimethoxy acettophenone in ethanol/KOH at 0–5 °C. Upon completion, the cooled reaction mixture was poured into ice water and treated with HCl yielded the desired 2-methoxy chalcone analogue 4-methoxy chalcone analogue & 3' ,4' ,5' –trimethoxy chalcone analogues (1–11) with an average yield of 12–72% (Table 1,).[22] The melting point was determine by open capillary method and are uncounted.. When needed, the crude product was purified by silica gel column chromatography.. The structure of compounds 1–11 were established with I.R, NMR and mass spectrometry measurements. Coupling constants (J) from the proton nuclear magnetic resonance (1H NMR) spectra clearly indicated that compounds 1–11 were geometrically pure and were exclusively Trans (E) isomers. To our knowledge these compounds has not been described in the literature up till now. Their spectral data are presented herein.

The IR spectral data revealed that all compound shows aromatic C=C stretching in the region of 1600-1570 cm-1. The aromatic C-H stretching is not significant, but the -OCH3 stretch in methoxy substituted chalcones shows absorbance at 1280 cm-1. The IR studies revealed the C=O and C=C stretching due to α,β-unsaturated carbonyl groups and the olefins are trans-olefins as they shows absorbance at around 970 cm-1 due to olefinic C-H bending. All compounds shows α,β-unsaturated carbonyl stretching in the region 1665-1650 cm-1. For o-substituted benzene, with four adjacent hydrogen the absorbance is at 775-750 cm-1. For m-substituted benzene with three adjacent hydrogen the absorbance is at 780 cm-1. For p-substituted benzene, with two adjacent hydrogen the absorbance at 850-800 cm-1.

NMR spectra of methoxy substituted chalcones shows absorbance at 3.9 ppm which includes presence of 9H of three- OCH3 groups, A complex spectral peak of 11H with the shift between 6.8 to 8.30 ppm i.e. for benzylic, and alkene.

Table. 1: 2-methoxy chalcone , 4-methoxy chalcone & 3',4',5' –trimethoxy chalcone 1–11 prepared via Aldol condensation reaction.

Compounds	R1	R2	R3	R4	R5	R6	R7	R8	R9	Molecular Formula	% Yield	Rf
1	-OCH3	-	-	-	-H	-H	$-NO_2$	-H	-H	C16H17O4	53.0%	0.71
2	-OCH3	-	-	-	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	-H	C19H20O5	48.9%	0.66
3	-OCH3	-	-	-	-H	-H	-Cl	-H	-H	C17H16ClO2	47.0%	0.44
4	-OCH3	-	-	-	-H	-H	$-N(CH_3)_2$	-H	-H	C18H19NO2	11.8%	0.40
5	-	-	-OCH3	-	-H	$-OCH_3$	$-OCH_3$	-H	-H	C18H18O4	50.0%	0.40
6	-	-	-OCH3	-	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	-H	C19H20O5	60.0%	0.40
7	-	-	-OCH3	-	-H	-H	-Cl	-H	-H	C16H13ClO2	77.0%	0.40
8	-	-	-OCH3	-	-H	-H	$-N(CH_3)_2$	-H	-H	C18H19NO2	53.7%	0.40
9	-	-	-OCH3	-	$-CH_3$	-H	-H	-H	-H	C18H19O2	53.0%	0.66
10	-	-OCH3	-OCH3	-OCH3	-H	-H	$-N(CH_3)_2$	-H	-H	C20H23NO4	64.0%	0.53
11	-	-OCH3	-OCH3	-OCH3	$-CH_3$	-H	-H	-H	-H	C19H20O4	25.0%	0.50

RESULTS AND DISCUSSION

BIOLOGICAL EVALUATION

***IN VITRO* ANTICANCER ACTIVITY OF CHALCONES IN HUMAN HEPATOCELLULAR CARCINOMA HEP-G2 CELLS**

Experimental Procedure for SRB Assay {23, 24, 25, 26]

Four synthesized compounds with best docking scores were submitted for *In Vitro* anticancer activity against Human Hepatocellular Carcinoma Hep-G2 Cells.The cell lines were grown in appropriate medium containing 10% fetal bovine serum and 2 mM L-glutamine. For present screening experiment, 5000 cells/well were inoculated into 96 well microliter plates in 100 µL. After cell inoculation, the microliter plates were incubated at 37° C, 5 % CO2, 95 % air and 100 % relative humidity for 24 h prior to addition

of synthesized compounds. Synthesized compounds were solubilized in appropriate solvent at 100mg/ml and diluted to 1mg/ml using water and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate (1mg/ml) was thawed and diluted to 100 µg/ml, 200 µg/ml, 400 µg/ml and 800 µg/ml with complete medium containing test article. Aliquots of 10 µl of these different drug dilutions were added to the appropriate microliter wells already containing 90 µl of medium, resulting in the required final drug concentrations i.e.10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml. After compound addition, plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 µl of cold 30 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4 % (w/v) in 1 % acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1 % acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm reference wavelength. Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent Growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells * 100. Using the six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as:

$$[Ti/C] \times 100\ \%$$

Table-2 Growth inhibition of tumor cell proliferation against Hepg2 Cell Line by 2-methoxy chalcone 4-methoxy chalcone & 3',4',5' –trimethoxy chalcone analogues 1–11 as calculated from dose–response curves.

1.00	Human Hepatocellular Carcinoma Hep-G2															
	% Control Growth															
	Drug Concentrations (µg/ml)															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
Comp.	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
01	22.3	-17.9	-50.4	-51.2	35.0	7.2	-23.3	-28.5	34.4	34.6	-29.9	-29.9	30.6	8.0	-34.5	-36.5
03	93.8	91.9	73.8	63.0	97.3	90.1	76.9	60.7	116.0	105.3	81.0	62.0	102.4	95.8	77.3	61.9
09	121.6	107.0	120.3	100.1	119.6	118.0	120.5	98.9	135.2	134.7	135.2	108.0	125.5	119.9	125.3	102.3
11	117.8	109.8	117.2	106.5	124.4	108.9	109.4	113.6	135.0	128.4	123.0	122.1	125.7	115.7	116.5	114.1
ADR	-38.6	-50.4	-57.2	-44.2	-31.4	-45.6	-52.2	-25.8	-41.7	-42.3	-51.7	-29.0	-37.2	-46.1	-53.7	-33.0

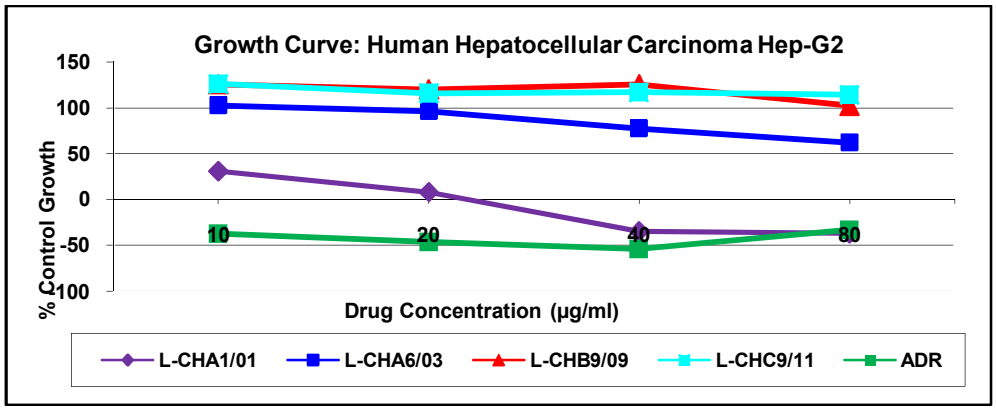


Fig. 3: Growth Curve He-G2

Table 3: GI50 Values of By 2-Methoxy Chalcone , 4-Methoxy Chalcone & 3',4',5' –Trimethoxy Chalcone 1–11analogues Against Adr (Adriamycin) Positive Control Compound.

Particulars	Drug concentration(µgm/ml) calculated from graph		
Hep-G2/ Comp.	LC50	TGI	GI50*
01	>80	28.6	<10
03	NE	NE	>80
09	NE	NE	>80
11	NE	NE	>80
ADR	NE	<10	<10

DIFFERENT DEFINITIONS

LC50 = Concentration of drug causing 50% cell kill

GI50 = Concentration of drug causing 50% inhibition of cell growth

TGI = Concentration of drug causing total inhibition of cell growth ADR = Adriamycin, Positive control compound

NE: Non- evaluable data. Experiment needs to be repeated using different set of drug concentrations

Note: Erratic data can result due to less solubility of the compound

GI50 value of ≤ 10^-6 molar (i.e. 1 µmolar) or ≤ 10µg/ml is considered to demonstrate activity in case of pure compounds. For extracts, GI50 value ≤ 20µg/ml is considered to demonstrate activity.

Test values under GI50 column indicate activity.

We next evaluated the four chalcones for their *In Vitro* anti- proliferation screening using a 48-h continuous exposure Sulforhodamine B (SRB) assay. Concentrations of compounds that inhibited tumour cell growth by 50% relative to an untreated control (ADR), or GI50 = Concentration of drug causing 50% inhibition of cell growth for both human cancer cell lines HepG2 (human hep-atoma cancer cell line), are shown in Table 2.

The GI50 values were determined by interpolation from growth curves. A total of 80 µgm/ml was chosen as a maximal concentration because much higher doses do not normally reach the blood plasma,19 and we are looking for compounds with high activity (low GI50) which could eventually be used in the future as a drug for chemotherapy with very low to no side effects.

Anti-proliferation results for Hep G2 cells treated with each compound indicate that compound 1, (2-methoxy chalcone analogue) showed the highest activity with GI50 value of less than 10 µg/ml. The compounds displaying the potent anti-proliferation effect in Hep G2 cells from 2-methoxy chalcone analogue was 1 (4 --NO2), & 3 (4 --Cl) (Table 2) whereas compound 5 from (4-methoxy chalcone analogue) & compound 2 from (3, 4, 5, trimethoxy chalcone analogue) shows moderate anti-proliferation activities. This clearly indicated that replacing a electron withdrawing group specially nitro or chloro in 2-methoxy chalcone analogue increases tremendously anti-proliferation activity against Hep G2 cell lines. Future investigation of chalcone 1 and the other structural modifications noted herein as Hep G2 cell growth inhibitors may provide further SAR insights.

NITRIC OXIDE INHIBITION ASSAY:

PRINCIPLE: Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions.

CHEMICALS/ REAGENTS:

Sodium nitroprusside , Sulphanilamide, Orthophosphoric acid, Naphthaleneamide, Sodium nitrite , Ascorbic Acid , Sodium dihydrogen Orthophosphate , Disodium Hydrogen phosphate , Sodium hydroxide for adjusting pH.

PROCEDURE:

Each sample was dissolved in the concentration 1mg/ml in ethanol as stock and from the above solution each sample was diluted to 100ug/ml with ethanol. Each sample was then incubated at 25º C with 1 ml Sodium nitroprusside (5mM) (148.9 mg of Sodium nitroprusside was dissolved in 100 ml of standard phosphate buffer) for 5 hours. After 5 hours, 0.5 ml of each sample was allowed to react with 0.5 ml of Griess reagent(0.1% naphthylethylene diamine dihydrochloride, 1% sulphanilamide, 2% phosphoric acid). The absorbance of chromophore formed was read at 546nm. Similarly, for standard, Ascorbic acid (1mg/ml) was made into different concentration of 5,10,15,20 and 25 ug/ml in distilled water. To assess the concentration of Sodium nitrite, present after inhibition was also calculated utilizing the standard curve of sodium nitrite. [27]

Formula used was:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of test}}{\text{Absorbance of Control}} \times 100$$

Table 4: Ascorbic acid Inhibition

Ascorbic Acid Inhibition		
Concentration	Absorbance	% Inhibition
5	0.2620	13.25
10	0.2197	27.25
15	0.1887	37.52
20	0.1558	48.41
25	0.1248	58.68
Blank	0.3020	-------

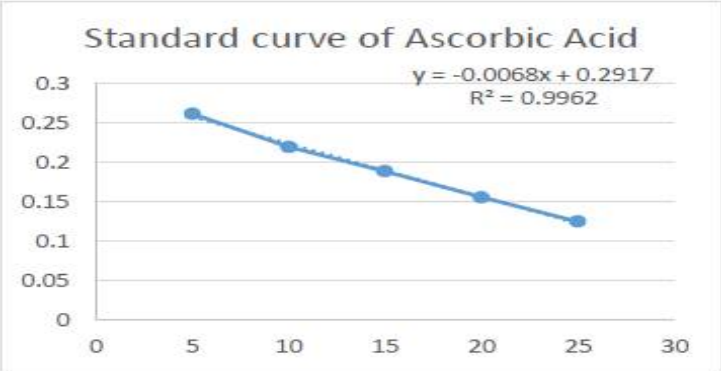


Fig. 4: Standard Curve of Ascorbic Acid

Table 5: Sodium Nitrite Std

Sodium Nitrite Std	
Concentration	**Absorbance**
1.0	0.200
2.5	0.432
5.0	0.867
10	1.305
15	1.736
20	2.089

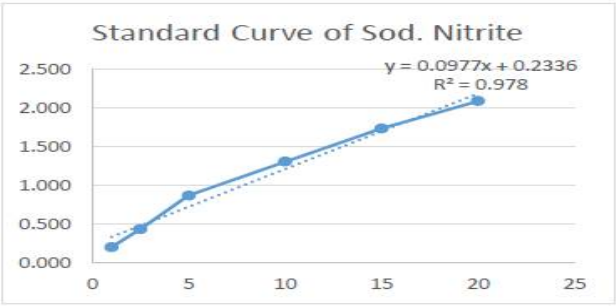


Fig. 5: Standard Curve of Sodium Nitrite

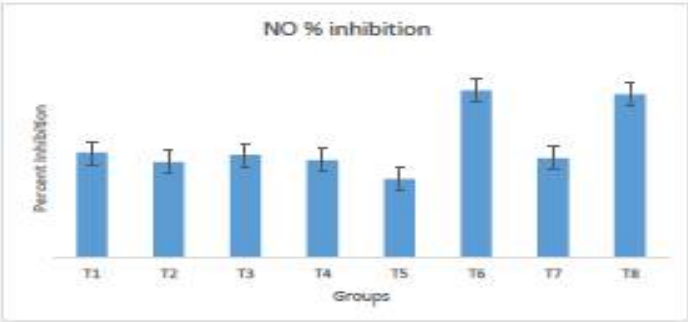
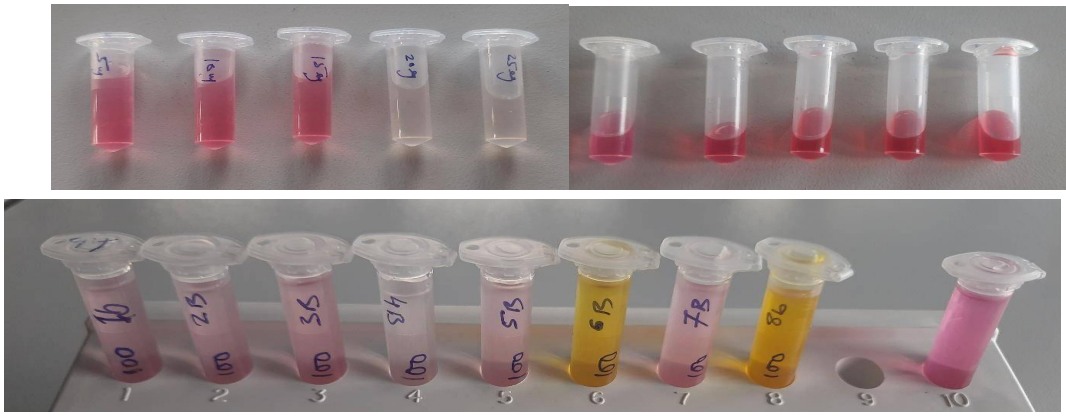


Fig. 6: NO % Inhibition



Standard Ascorbic acid (5-25ug/ml) Standard Nitrite (2.5ug-20ug/ml)

Fig. 8: Sample plus Blank with 100% Nitrite ions

Eight Samples + Blank with 100% nitrite ions

N.B. The lighter the pink colour more is the NO Inhibition as compared to Blank.

Table 6: % Nitric Oxide Inhibition

100µg	Absorbance	% inhibition	Concentration Of Nitrite	Conc. Equivalent to Ascorbic Acid
Test 1	0.1558	48.41	-0.80	19.99
	0.1663	44.93	-0.69	18.44
	0.1552	48.61	-0.80	20.07
Mean	0.1591	47.32	-0.76	19.50
SD	0.0062	2.07	0.06	0.92
Test 2	0.1715	43.21	-0.64	17.68
	0.1712	43.31	-0.64	17.72
	0.1719	43.08	-0.63	17.62
Mean	0.1715	43.20	-0.64	17.67
SD	0.0004	0.12	0.00	0.05
Test 3	0.1619	46.39	-0.73	19.09
	0.1615	46.52	-0.74	19.15
	0.1623	46.26	-0.73	19.03
Mean	0.1619	46.39	-0.73	19.09
SD	0.0004	0.13	0.00	0.06
Test 4	0.1695	43.87	-0.66	17.97
	0.1692	43.97	-0.66	18.01
	0.1685	44.21	-0.67	18.12
Mean	0.1691	44.02	-0.66	18.03
SD	0.0005	0.17	0.01	0.08
Test 5	0.1980	34.44	-0.36	13.78
	0.1920	36.42	-0.43	14.66
	0.1950	35.43	-0.40	14.22
Mean	0.1950	35.43	-0.40	14.22
SD	0.0030	0.99	0.03	0.44
Test 6	0.0739	75.53	-1.63	32.03
	0.0732	75.76	-1.64	32.13
	0.0735	75.66	-1.64	32.09
Mean	0.0735	75.65	-1.64	32.08
SD	0.0004	0.12	0.00	0.05
Test 7	0.1668	44.77	-0.68	18.37
	0.1662	44.97	-0.69	18.46
	0.1665	44.87	-0.69	18.41
Mean	0.1665	44.87	-0.69	18.41
SD	0.0003	0.10	0.00	0.04
Test 8	0.0782	74.11	-1.59	31.40
	0.0785	74.01	-1.59	31.35
	0.0781	74.14	-1.59	31.41
Mean	0.0783	74.08	-1.59	31.39
SD	0.0002	0.07	0.00	0.03

N.B. As all the samples at 100ug concentration has showed considerable inhibition of Nitric oxide so the concentration of Sodium nitrite was in negative values; also Test 6 and Test 8 has showed the highest

efficacy in Nitric oxide inhibition assay at the given concentration and 100 µg of the test solutions were equivalent to 31-32 ug of Ascorbic acid approximately.

CONCLUSION

The synthesis and biological evaluation of 2′-Methoxy chalcone , 4'methoxy chalcone & 3', 4', 5'-trimethoxy chalcone analogues 1–11 has been described. The synthetic approach involves Aldol condensation of 2′-Methoxy acetophenone, 4'methoxy acetophenone & 3', 4', 5'-trimethoxy acetophenone and aldehydes in the presence of KOH to generate 2′-Methoxy chalcone , 4'methoxy chalcone & 3', 4', 5'-trimethoxy chalcone analogues in high yields. The prepared compounds 1–11 were evaluated for NO inhibition, and human tumor cell proliferation *In Vitro*. Compound 6 & 8 exhibited potent inhibition NO production whereas compound 1 shows tumor cell proliferation, and may provide a template to design new novels with a dual activity of anti-inflammation and anti-cancer. Compound 6 & 8 selectively inhibited NO production and shows moderate Hep G2 proliferation; while 01 showed selective anti-proliferation affect Hep G2 proliferation.

Taken together, these results indicates that 2′-Methoxy chalcone, 4'methoxy chalcone & 3', 4', 5'-trimethoxy chalcone analogues may consider as potential anti- inflammatory and anti-cancer agents. Further studies will be required to better understand signaling mechanisms of the subject compounds, and needs to be generalized to a larger series bearing other substitution patterns on the ring-B. On the other hand, the most active compounds should be analyzed for effects on normal human cells, in order to determine potential therapeutic windows supporting further studies on experimental tumor-bearing animals' finalized to verify possible *in vivo* anti-cancer activity.

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