ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Synthesis and *In-vitro* anti-cancer activity of some substituted Chalcone derivatives

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ABSTRACT

A series of chalcones have been synthesized and evaluated for their cytotoxic activity. The title compounds (G_{1-10}) were prepared by the esterification of paracetamol in the presence of acetic anhydride and conc. Sulphuric acid to o-acetyl-p-acetamido phenol (1a).Which on Fries rearrangement to give corresponding acetophenone (2a). Further condensation of acetophenone with various aldehydes gave chalcone derivatives (G_{1-10}). The structure of chalcone derivatives have been established on the basis of spectral (IR, ^{1}HNMR, Mass) data. The synthesized chalcone derivatives were evaluated for *in vitro* cytotoxic activity by MTT method using two breast cancer cell lines.

Keywords: Chalcone, synthesis, anticancer activity

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ISSN: 0975-8585

INTRODUCTION

Cancer is one of the most dangerous, fast propagating with quite high mortality rate disease of present century even in the developed country. The situation is even worse in the under developed country due to lack of knowledge, poverty and non-availability of quality drugs [1]. As a result of the dramatic increase in cancer, in the recent years serious attention has been towards the discovery and development of new anticancer drugs. There is considerable interest in the chemotherapeutic activity of chalcone derivatives. This includes antibacterial, anti-inflammatory, antimalarial, antifungal, antitumor, antimicrobial, antiviral, diuretics and analgesic [2-6]. Chalcones and their derivatives are a group of compounds reported to exhibit promising anticancer activity. These compounds are precursor of flavonoids which are abundant in edible plants. The chemical structure of chalcone (1,3-diphenyl-2-propen-1-ones) consists of two aromatic rings joined by a three carbon α, β- unsaturated carbonyl system. The above prepared compounds are α, β- unsaturated ketones (chalcones) containing the ketoethylenic group (– CO – CH=CH-).

In the present communication, we report the reaction of corresponding acetophenone (2a) with various aromatic aldehydes to form substituted chalcone derivatives (G_{1-10}).

MATERIAL AND METHODS

Experimental

All melting points were recorded in Digital melting point apparatus and are uncorrected. The IR spectra were recorded on Perkin Elmer FTIR spectrometer using KBr. ^{1}HNMR spectra were recorded on Bruker Avance II 400MH_Z NMR. The chemical shift's were reported as parts per million downfield from tetra methyl silane as internal standard. Mass spectra were performed on LC-MSD-Tranp-SL2010A SHIMADZU using DMSO as solvent. The purity of the compound was checked by TLC using precoated silica gel G plate method R_f (Table-1) using ethyl acetate: glacial acetic acid: water.

Synthesis of o-acetyl -p-acetamido phenol (1a)

Take a mixture of paracetamol (5gm, 0.098mol), acetic anhydride (10ml, 0.033mol) and concentrated sulphuric acid (1ml, 0.001mol) in 250ml round bottom flask equipped with a reflux condenser. Shake the mixture thoroughly and heat the content on a water bath at 50-60°C with occasional stirring for about 15min. Cool the flask and add water (100ml) into it. Filter the solid separated under suction and wash with water. Recrystallized the crude product with 50% ethanol to get o-acetyl- p-acetamido phenol. [Yield: 78%; m.p.147^{0}C; IR (KBr, cm^{-1}) 3320(NH), 3103(=C-H), 2913(ArC-H), 1615(ArC=C), 1702(C=O), 1105(C-O)].

Synthesis of 5-acetamido-2-hydroxy acetophenone (2a)

o- acetyl- p-acetamido phenol (34gm, 199mmol) was added drop wise on anhydrous $AlCl_3$ (40gm, 293mmol) and the mixture was heated at 130°C for 3hrs. To the cooled reaction mixture crushed ice was added slowly and the resulting solution was extracted with diethyl ether. The organic fraction was dried over anhydrous $MgSO_4$ and the solvent was evaporated to obtain oil. On distillation under reduced pressure 5-acetamido-2-hydroxy acetophenone was obtained. [Yield: 72%; m.p.137^{0}C; IR (KBr, cm^{-1}) 3398(OH), 3143(=C-H), 2943(ArC-H), 1587(ArC=C), 1743(C=O), 1119 (C-O)].

Synthesis of Substituted Chalcone derivatives (G_{1-10})

A solution of NaOH (0.05mol) in water (25ml) and ethanol (15ml) was stirred and cooled. To this solution heterocyclic aldehydes (0.05mol) was added by the appropriate ketone (0.05mol). The temperature of the mixture was kept at 25- 30°C and stirring was continued for 3.5hr. After keeping the reaction mixture in the refrigerator overnight, the chalcones that separated out were collected and identified. Similarly other members of G_{1-10} were

ISSN: 0975-8585

prepared and their physical and analytical data were recorded. All the synthesized compounds were screened for anticancer activity. The characteristic data have been given in Table-3.

In- vitro cytotoxic Activity

All substituted chalcone derivatives were screened for *in vitro* cytotoxic activity by Micro culture Tetrazolium Test (MTT) assay method using two Breast cancer cell lines MCF-7 and T47D [7]. The MTT assay is a standard colorimetric assay, which measures changes in color to determination of viable cells. The assay is dependent on the activity of mitochondrial dehydrogenase enzymes that reduce yellow 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to a blue formazan product and the activity of enzyme is directly proportional to cell viability.

The percent cell viability with respect to control is calculated using formula-

$$\% \text{ Cell viability} = \frac{\text{OD of treated cells}}{\text{OD of control cells}} \times 100$$

The results of all synthesized compounds are summarized in Table- 2.

Table 1: Spectral data of substituted chalcone derivatives (G_{1-10})

Compounds	Spectral Data
G_1	IR (KBr, cm^{-1}) 3458 (NH, OH), 3192(=C-H), 1660(C=O), 1591(ArC=C), 1104(C-O),771(C-Cl); ^{1}HNMR (DMSO-d_6) δ 2.0 (s, 3H, OCH_3), 5.0 (s, 1H, OH), 6.7 (m, 9H, ArH), 8.0 (s, 1H, NH); Mass: m/z $316M^+$, $262B^+$
G_2	IR (KBr, cm^{-1}) 3432 (NH, OH), 3158(=C-H), 2933(ArC-H), 1670(C=O), 1601(ArC=C), 1134 (C-O), 774 (C-Cl); ^{1}HNMR (DMSO-d_6) δ 2.0 (s, 3H, OCH_3), 5.0 (s, 1H, OH), 6.9-7.9 (m, 9H, ArH), 8.0 (s, 1H, NH). Mass: m/z $316M^+$, $259B^+$
G_3	IR (KBr, cm^{-1}) 3410 (NH, OH), 3038(=C-H), 2903(ArC-H), 1659(C=O), 1521(ArC=C), 1112 (C-O), 1445(ArC-NO_2); ^{1}HNMR (DMSO-d_6) δ 2.0 (s, 3H, OCH_3), 5.0 (s, 1H, OH), 6.8-7.9 (m, 8H, ArH), 8.0 (s, 1H, NH), 8.4 (s, 1H, CH). Mass: m/z $327M^+$, $262B^+$
G_4	IR (KBr, cm^{-1}) 3370 (NH, OH), 3068(=C-H), 2917(ArC-H), 1657(C=O), 1531(ArC=C), 1102 (C-O), 1470(ArC-NO_2); ^{1}HNMR (DMSO-d_6) δ 2.0 (s, 3H, OCH_3), 5.0 (s, 1H, OH), 6.8-8.1 (m, 10H, ArH), 8.2(s, 1H, NH). Mass: m/z $327M^+$, $271B^+$
G_5	IR (KBr, cm^{-1}) 3332 (NH, OH), 3092(=C-H), 2849(ArC-H), 1667(C=O), 1533(ArC=C), 1103 (C-O), 1345(ArC-NO_2); ^{1}HNMR (DMSO-d_6) δ 2.0 (s, 3H, OCH_3), 5.0 (s, 1H, OH), 6.9-8.1(m, 9H, ArH), 8.2(s, 1H, NH). Mass: m/z $327M^+$, $267B^+$
G_6	IR (KBr, cm^{-1}) 3313(NH, OH), 3062(=C-H), 2916(ArC-H), 1676(C=O), 1590(ArC=C), 1144 (C-O), ^{1}HNMR (DMSO-d_6) δ 2.0 (s, 3H, OCH_3), 5.0 (s, 2H, OH), 6.8-8.0 (m, 9H,ArH), 8.1(s, 1H, NH). Mass: m/z $333M^+$, $289B^+$
G_7	IR (KBr, cm^{-1}) 3295(NH, OH), 3142(=C-H), 2934(ArC-H), 1666(C=O), 1613(ArC=C), 1044 (C-O), ^{1}HNMR (DMSO-d_6) δ 2.0 (s, 3H, OCH_3), 5.0 (s, 2H, OH), 6.6-7.9 (m, 9H, ArH), 8.0 (s, 1H, NH). Mass: m/z $333M^+$, $309B^+$
G_8	IR (KBr, cm^{-1}) 3415(NH, OH), 3158(=C-H), 2981(ArC-H), 1658(C=O), 1590(ArC=C), 1093 (C-O), ^{1}HNMR (DMSO-d_6) δ 2.0 (s, 3H, OCH_3), 3.7 (s, 9H, CH), 5.0 (s, 1H, OH), 6.2-6.9 (m, 5H, ArH), 8.0(s, 1H, NH). Mass: m/z $372M^+$, $212B^+$
G_9	IR (KBr, cm^{-1}) 3432(NH, OH), 3162(=C-H), 2907(ArC-H), 1661(C=O), 1607(ArC=C), 1177(C-O), ^{1}HNMR (DMSO-d_6) δ 2.0 (s, 3H, OCH_3), 2.8 (s, 6H, CH), 5.0 (s, 1H, OH), 6.5-6.9 (m, 9H, ArH), 8.0 (s, 1H, NH). Mass: m/z $325M^+$, $280B^+$
G_{10}	IR (KBr, cm^{-1}) 3366(NH, OH), 3147(=C-H), 2936(ArC-H), 1647(C=O), 1550(ArC=C), 1170 (C-O), ^{1}HNMR (DMSO-d_6) δ 2.0 (s, 3H, OCH_3), 3.7 (s, 3H, CH), 5.0 (s, 2H, OH), 6.5-6.9 (m, 8H, ArH), 8.1 (s, 1H, NH). Mass: m/z $327M^+$, $227B^+$

ISSN: 0975-8585

Table 2: *In vitro* cytotoxic activity for compounds G_{1-10}

S.No	Compounds	(IC_{50})µM	
		MCF-7	T47D
1.	G1	71	72
2.	G2	69	71
3.	G3	56	58
4.	G4	59	62
5.	G5	55	52
6.	G6	71	74
7.	G7	69	72
8.	G8	78	75
9.	G9	82	94
10.	G10	82	89
Standard	Doxorubicin	0.60	0.69

RESULT AND DISCUSSION

The synthesis is based on the esterification of paracetamol in the presence of acetic anhydride and conc. sulphuric acid to gave o-acetyl -p-acetamido phenol (1a) which on Fries rearrangement in the presence of anhydrous $AlCl_3$ to gave 5-acetamido-2-hydroxy acetophenone (2a) which on furture treatment with various aromatic aldehydes in the presence of alkaline medium to gave substituted chalcone derivatives G_{1-10}. All the above reactions are summarized in Scheme-1.

The structures of various synthesized compounds were assigned on the basis of IR, ^{1}HNMR, Mass Spectral data, which are tabulated in Table -1.

The compounds are evaluated for their cytotoxic activity. The IC_{50} value was calculated at the 0.1-100 µM concentration range. The result showed that significant cytotoxicity against both of the cell line and value lies between 52-89 µM. All the compounds showed good cytotoxic activity and the compound G_3, G_4, G_5 showed better activity than other compounds, this may be due to presence of nitro group in the compound.

ISSN: 0975-8585

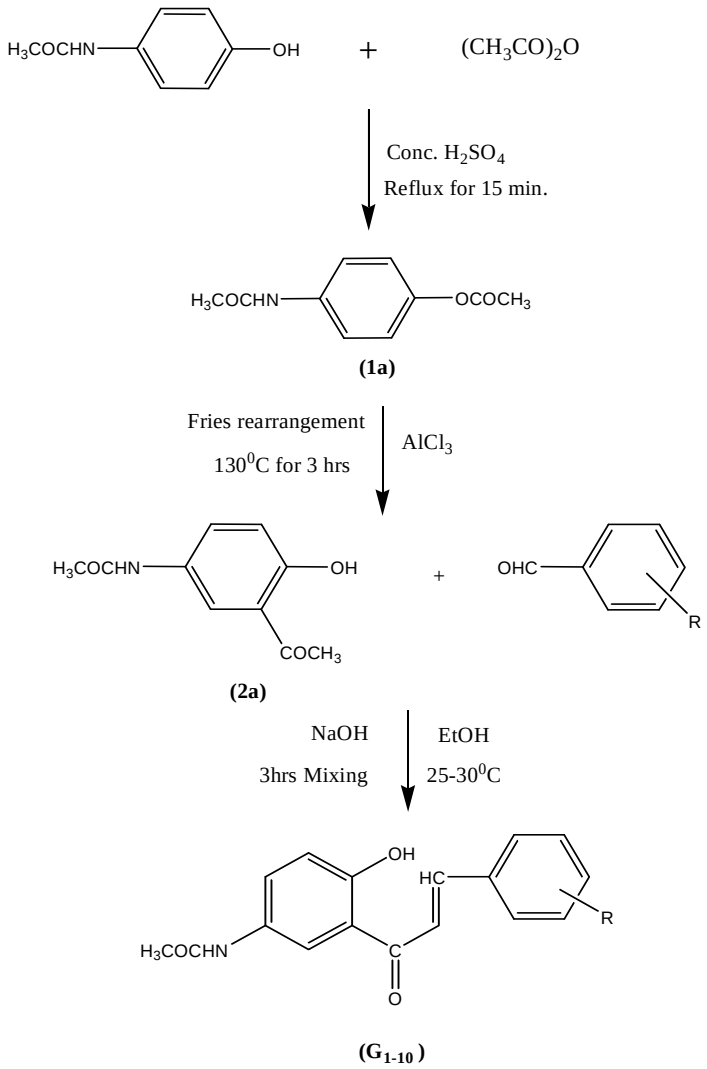
Table 3: Characterization data of substituted chalcone derivatives (G_{1-10})

Compounds	R	Mol.formula	M.P (^{0}C)	Yield (%)	R_f Value	(%) Elemental analysis Calculated (found)		
						C	H	N
G1	-Cl (*o*)	$C_{17}H_{14}ClNO_3$	124	53.17	0.56	64.67 (64.62)	4.57 (4.43)	4.44 (4.43)
G2	-Cl (*p*)	$C_{17}H_{14}ClNO_3$	120	62.31	0.71	64.67 (64.50)	4.47 (4.37)	4.44 (4.45)
G3	-NO_2 *(o)*	$C_{17}H_{14}N_2O_5$	62	67.25	0.76	62.57 (62.48)	4.32 (4.54)	8.59 (8.62)
G4	-NO_2 (*m*)	$C_{17}H_{14}N_2O_5$	64	65.32	0.72	62.57 (63.34)	4.32 (4.45)	8.59 (8.61)
G5	-NO_2 (*p*)	$C_{17}H_{14}N_2O_5$	85	68.85	0.78	62.57 (62.29)	4.32 (4.29)	8.59(8.58)
G6	-OH *(o)*	$C_{17}H_{15}NO_4$	92	55.21	0.76	68.68 (68.70)	5.09 (5.11)	4.71 (4.60)
G7	-OH (*p*)	$C_{17}H_{15}NO_4$	124	64.98	0.62	68.68 (68.51)	5.09 (5.16)	4.71 (4.65)
G8	-N $(CH_3)_2$ (*p*)	$C_{17}H_{15}NO_6$	65	75.28	0.69	70.35 (70.21)	6.21 (6.19)	8.64 (8.71)
G9	- 3,4,5- Tri - OCH_3	$C_{19}H_{20}N_2O_3$	78	72.68	0.74	65.82 (65.73)	6.78 (6.61)	3.49 (3.52)
G10	3-OH, 4- OCH_3	$C_{18}H_{17}NO_5$	98	63.45	0.75	67.21 (67.25)	6.49 (6.43)	3.92 (3.96)

ACKNOWLEDGEMENT

The authors are thankful to Dr. K.S. Lakshmi, Dean, S.R.M. College of Pharmacy, S.R.M. University for providing laboratory facilities and Tamil Nadu Pharmaceutical Science Welfare Trust, Chennai for awarding scholarship for the project.

ISSN: 0975-8585



(G_{1-10})

SCHEME - 1

REFERENCES

[1] Wolff ME, Burger Medicinal Chemistry and Drug Discovery, 5th edition, 1994,pp-611.
[2] Yajuvendra Jhala S, Pradhuman S, Ranawat S, Shiv Dulawat BL, Verma. Ind J Heterocyclic Chem 2001; 14: 357.
[3] Zdzislawa Nowakoska. Eur J Med Chem 2007;42:125.
[4] Aurangzeb Hasan, Lubna Rasheed, Abdul Malik. Asian J Chem 2007; 19: 937.
[5] Mrek Konieczny T, Wojciech K, Michal S, Andrzej S, Ronald W, Ewa Augustynowicz K, Zofia Z. Eur J Med Chem 2007; 42: 729.
[6] Prabhavat M, Ghiya BJ. Ind J Heterocyclic Chem 1998; 7: 311.
[7] Gupta SK, Drug Screening Methods, 1st edition, 2004, pp- 418.