Int. J. Drug Dev. & Res.
| July-September 2013 | Vol. 5 | Issue 3 | ISSN 0975-9344 |
www.ijddr.in

Full Length Original Research Paper

Synthesis of some new chalcone derivatives and evaluation of their Anticancer activity

Afzaye Rasool,

Rakesh Panda and

Dr. Monica Kachroo*

*Department of Pharmaceutical Chemistry, Al-Ameen college of Pharmacy, Bangalore, 560027

Corresponding Authors:
Dr. Monica Kachroo
E-mail id:
monicakachroo@gmail.com

Abstract: Chalcones which are also known as a,β-unsaturated ketones is an important class of organic compounds and reported to possess a wide spectrum of biological activities such as antibacterial, antifungal, anticancer, anti-inflammatory etc. The biological activity of chalcone is mainly because of an enone pharmacophore in their structures, the importance of which is well documented in the literature. Although a number of drugs are available in the market, the thirst for discovering a new drug with better pharmacokinetic profile, lesser toxicity has become imperative for obvious reasons and also due to the fast development of microbial resistance towards existing molecules. Therefore in the present study some novel chalcones have been synthesized for biological activities like anti-cancer , antibacterial and antioxidant activity. Cyclic ketones having a-hydrogens was treated with various aromatic aldehydes in alcohol, in the presence of potassium hydroxide to form corresponding a,β-unsaturated compounds. The structures of these compounds are supported by their UV, IR, NMR and Mass spectral data. The compounds have been evaluated for their anti-cancer, antibacterial and antioxidant activities.

Keywords: Chalcones, anticancer, antibacterial, antioxidant.

INTRODUCTION:

The chalcones are a, β-Unsaturated ketones containing the reactive keto ethylene group -CO-CH=CH-, the presence of a,β-Unsaturated carbonyl system in chalcone makes it biologically active. Some substituted chalcones and their derivatives have been reported to exhibit a wide variety of biological properties such as anthelmintic[1], anti-microbial[2], antimycobacterial[4], antifungal[5], anticancer[6-9], anti-oxidant[10], and anti-inflammatory[11] activity etc.

In the present work attention has been focused on the synthesis of chalcones from various aldehyde moieties and its derivatives. The structure of various synthesized compound was assigned on the basis of UV, IR, ^{1}H-NMR, ^{13}C-NMR, and Mass spectral data. The synthesized compounds were further screened for anticancer, antibacterial and antioxidant activity.

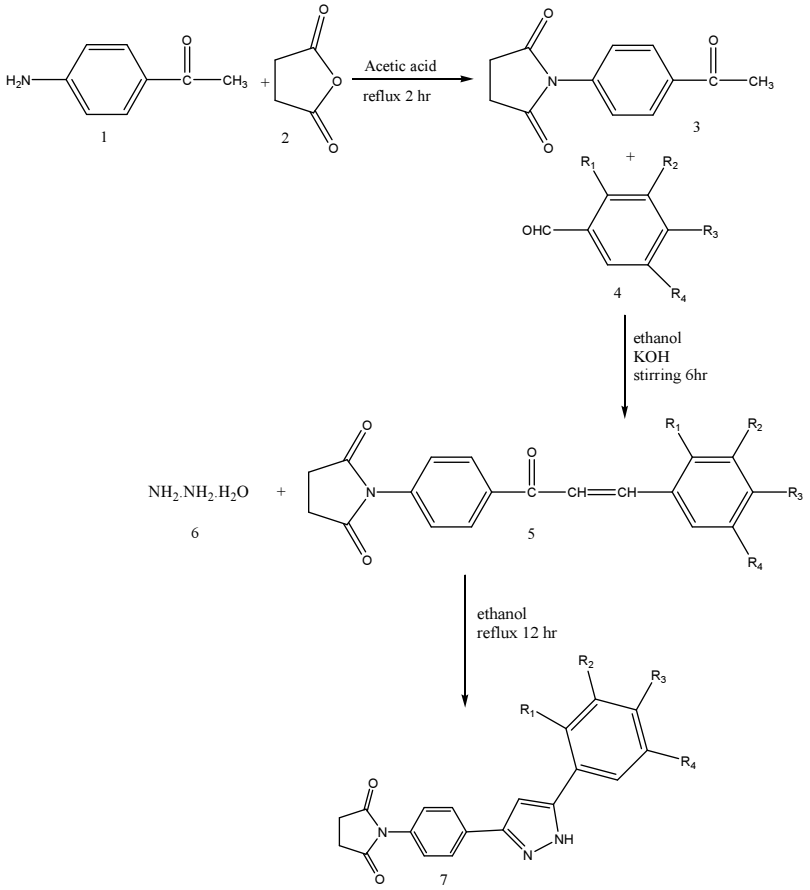
Material and method:

- Melting points of the synthesized compound was determined using Thiele's melting point apparatus and was found uncorrected.
- Purity of the compounds was checked by thin layer chromatography using silicagelG in solvent system n-hexane-ethyl acetate (3:1) and the sport were located under iodine vapour and UV light.
- The UV spectra of the synthesized compounds were recorded on UV–Visible

spectrophotometer (model Shimadzu 8700) using alcohol and the values of wave length (λ max) were reported in nm.

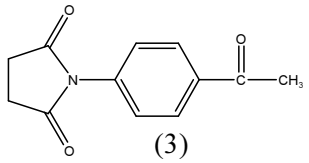
- IR spectra of all compounds were recorded on FTIR spectrometer (model Shimadzu 8700) in the range of 400 -4000 using KBr.
- ^{1}H NMR spectra were recorded on Amx - 400 MHz NMR spectrometer using $CDCl_3$ and chemical shifts (δ) are reported in parts per million downfield using Tetramethylsilane (TMS).
- ^{13}C NMR (400 MHz) spectra were recorded in deuterated $CDCl_3$ in Amx-400 liquid state NMR spectrometer .Chemical shifts (δ) are reported in parts per million.
- Mass spectra were recorded on Mass spectrophotometer (model Shimadzu) by MS

Synthetic pathway



Procedure for the preparation of 1-(4-Acetyl-phenyl)-pyrrolidine-2, 5-dione

4-Aminoacetophenone (0.01M), succinic anhydride (0.01M) and acetic acid (40 ml) was added in round bottom flask, refluxed for 2 hr and kept overnight, filtered and recrystalized from ethanol. The elution was done with n-hexane: ethyl acetate (4: 1) crystallized from ethanol as white colour, yield 52 %, m.p 129-131^{0}C, Rf- 0.6 (structure-3)



(3)

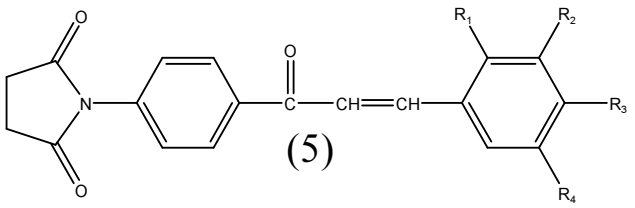
Procedure for the preparation of 1-(4(3-Substituted phenyl) acryloyl)phenyl} pyrrolidine-2, 5-dione

A mixture of 1-(4-acetyl-phenyl)-pyrrolidine-2, 5-dione (0.01M) and aryl aldehyde (0.01M) was stirred in ethanol (40 ml) and an aqueous solution of KOH (40%, 15 ml) was added to it. The stirring

was continued for 6 hr and the mixture was kept overnight at room temperature and it was then poured into crushed ice and acidified with HCl. The solid separated was filtered and recrystallized from ethanol. (Table-1)

Procedure for the preparation of 1-{4[5-substitutedphenyl)-1*H*-pyrazol-3yl] phenyl} Pyrrolidine-2, 5 Dione.

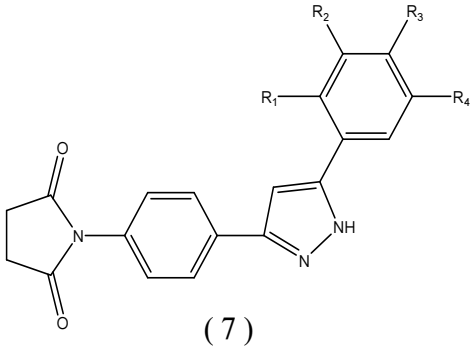
A mixture of chalcone (0.01M), hydrazine hydrates (0.01M) and ethanol 25ml was refluxed for 12 hr .The mixture was concentrated by distilling out of the solvent under reduced pressure and poured into ice water. The precipitate obtained was filtered, washed and recrystalized with ethanol.



(5)

Compound Code	R_1	R_2	R_3	R_4	Molecular formula	Molecular weight	M.P ^{0}C	% of yield
1a	OCH_3		OCH_3		$C_{21}H_{19}NO_5$	365	147-148	62
1b		OCH_3	OCH_3	OCH_3	$C_{22}H_{21}NO_6$	395	161-163	91.4
1c		NO_2			$C_{19}H_{14}N_2O_5$	350	158-160	78.2
1d	Cl				$C_{19}H_{14}O_3NCl$	339	137-138	95
1e	OH			OCH_3	$C_{20}H_{17}NO_5$	351	142-144	92
1f			NO_2		$C_{19}H_{14}N_2O_5$	350	132-134	92
1g			OH		$C_{19}H_{15}NO_4$	321	162-164	91
1h			OC_2H_5		$C_{21}H_{19}NO_4$	349	132-134	85
1i	NO_2				$C_{19}H_{14}N_2O_5$	350	96-98	75
1j	Cl		Cl		$C_{19}H_{13}O_3NCl_2$	350	118-120	85
1k	$N(C_2H_5)_2$				$C_{19}H_{16}N_2O_3$	320	95-98	79

Table 1: Physiochemical parameters of 1-{4(3-Substituted phenyl) acryloyl)phenyl} pyrrolidine-2, 5 dione



(7)

Compound Code	R_1	R_2	R_3	R_4	Molecular formula	Molecular weight	M.P ^{0}C	% of yield
2a	OCH_3		OCH_3		$C_{21}H_{19}NO_4$	377	110-112	77.7
2b		OCH_3	OCH_3	OCH_3	$C_{22}H_{21}N_3O_5$	407	110-112	21.1
2c		NO_2			$C_{19}H_{14}N_4O_4$	362	110-112	81.2
2d	Cl				$C_{19}H_{14}N_3O_2Cl$	361	115-120	32.3
2e	OH			OCH_3	$C_{20}H_{17}N_3O_4$	363	118-120	33.3
2f			OC_2H_5		$C_{21}H_{19}N_3O_3$	361	45-48	27

Table 2: Physiochemical parameters of Procedure of 1-{4[5-substitutedphenyl)-1*H*-pyrazol-3yl] phenyl} Pyrrolidine-2, 5 Dione.

Compound code	λ_{max} (nm)	Mass m/e	IR (KBr) V_{max} cm^{-1}	^1H NMR δ ppm	^{13}C NMR
1a	365		3002(Ar C-H) 2927(Ali C-H) 1602(C=C) 1307(OCH_3)	δ 7.5-8.13 4H, (m, 2H of ArH + 2H of CH=CH) ; δ 6.40-6.7 3H (m, ArH of dimethoxy benzene) ; δ 3.84-3.88 6H (s,2xOCH_3) ; δ 1.8 4H (s, 2xCH_2 of succinimide)	
1b	329		3100 (Ar C-H) 2935(Ali C-H) 1589(C=C) 1280 (OCH_3) 1178 (C-N)	δ 7.94 1H (s, CH=CH) ; δ 7.95 1H (s, CO CH=CH) ;δ 7.2-7.82 4H (m ,ArH) ;δ 6.60-6.85 2H (m, ArH of trimethoxy benzene) ;δ 3.8-3.9 9H (s, 3xOCH_3); δ 1.8 4H (s, 2xCH_2 of succinimide)	
1d	317	395(M+2)	3059 (Ar C-H) 2923 (AliC-H) 1649 (C=O) 1608 (C=C) 1178 (C-N)	δ 8.17 1H (s, CH=CH); δ 8.09 1H (s, CO CH=CH); δ 6.62-7.95 8H (m, ArH); δ 1.21-1.90 4H (s, 2xCH_2 of succinimide).	188.36 (C=Oenone), 151.56 (C=O,succinimide), 139.36 (Ci), 135.66, 131.61, 134.15,125.36 [(Ca), (Cc) and (Ce), (Cd), (Cb) and (Cf) respectively of C_6H_4-succinimide].128. 13(Ch), 131.6, 131.07, 130.6, 128.74, 127.35 [(Ca'), (Cb'), (Cc'), (Cd' and Ce') Cf' respectively of o-Chloro C_6H_3]

Table 3: Spectral characteristics of 1-(4(3-Substituted phenyl) acryloyl)phenyl} pyrrolidine-2,5-dione.

Compound code	λ_{max} (nm)	IR (KBr) V_{max} cm^{-1}
2a	314	3358(N-H) 1596(C=N)
2b	327	3361(N-H) 1600(C=N)
2c	327	3357(N-H) 1591(C=N)
2d	382	3342(N-H) 1612(C=N)
2e	522	3213(N-H) 1599(C=N)

Table 4: Spectral characteristics of 1-{4[5-substitutedphenyl)-1*H*- pyrazol3yl] phenyl} Pyrrolidine-2, 5-Dione.

Biological activity:

1. Anticancer studies[6-9]:

- Method: Trypan blue exclusion method(Dalton lymphoma ascities)
- Animal used: Tumor bearing mice
- Chemical used: Phosphate buffered saline

Method:

- Cells were aspirated from the peritoneal cavity of tumor bearing mice.
- The cells were washed three times using PBS.
- The viability of the cells were checked using trypan blue (cell viability should be above 98%)
- The number of cells were counted using haemocytometer and after approximate dilution cell number adjusted to 1×10^{-7} cell/ml
- The experiment was setup by incubating different concentration of the drug with 1×10^{6} cells
- Final volume of the assay mixture was made upto 1ml using PBS and incubated at 37 ^{0} C for about 3 hours.
- 0.1ml of trypan blue was added after incubation and number of dead cell was counted using haemocytometer.

The number of stained and unstained cells was counted separately and percentage cell death was calculated using the formula:

$$\% \text{ cytotoxicity} = \left(\frac{\text{No. of dead cell}}{\text{No. of live cell+No. of dead cell}} \right) \times 100$$

Drug conc	Percent cell Death (DLA)						
µg/ml	1a	1b	1d	1h	2a	2b	2e
200 µg	57%	9%	15%	2%	27%	21%	84%
100 µg	39%	6%	10%	42%	14%	14%	75%
50 µg	25%	2%	7%	34%	8%	5%	60%
20 µg	15%	0	5%	20%	5%	2%	48%
10 µg	5%	0	0	8%	2%	0	33%

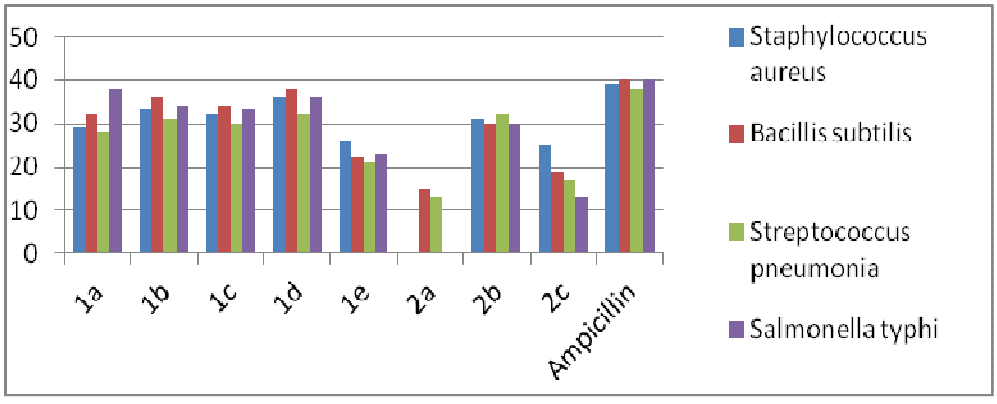
Table 5: Anticancer activity of chalcone by using DLA method

2. Antibacterial activity[2]:

The synthesised compound were screened for their antibacterial activity against two gram positive bacterial strains *B.subtilis*(NCIM 2697), *S.aureus*(NCIM 2079) and two gram negative bacterial strains *S.pneumonia*(NCIM 5082), *S.typhi*(NCIM 2263) by using cup plate method.The zone of inhibition was measured in mm, under similar condition the controlled experiment was carried out using antibiotics(Ampicillin) as a standard drug for comparison.

Compound code	Zone of inhibition (in mm)			
	Staphylococcus aureus	*Bacillus subtilis*	*Streptococcus pneumonia*	*Salmonella typhi*
Ampicillin	39	40	38	40
1a	29	32	28	38
1b	33	36	31	34
1c	32	34	30	33
1d	36	38	32	36
1h	-	18	13	-
2a	-	15	13	-
2b	31	30	32	30
2c	25	19	17	13

Table 6: *in-vitro* antibacterial activity of chalcones determined by agar diffusion method



Bar diagram of antibacterial activity

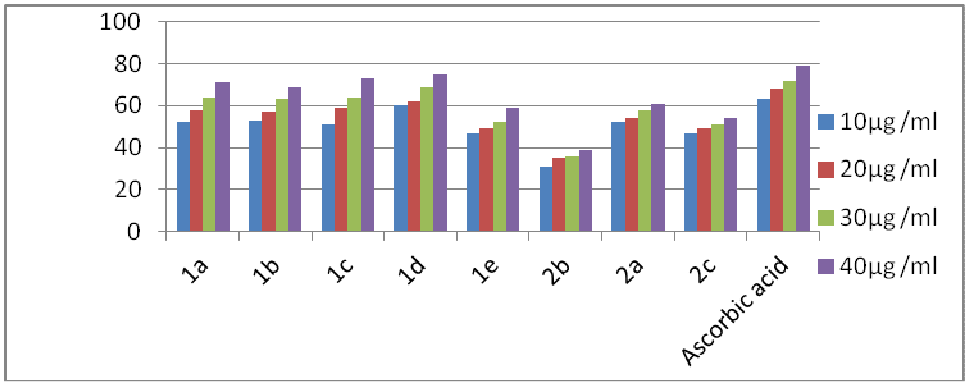
3. Antioxidant activity[10]:

All the synthesized compounds were evaluated for their *in-vitro* free radical scavenging activity by DPPH (2, 2-diphenyl-1-picryl hydrazyl) reduction method using ascorbic acid as the standard.

Covered in Scopus & Embase, Elsevier
Int. J. Drug Dev. & Res., July-September 2013, 5 (3): 309-315

Compound code	% Inhibition			
	10 µg/ ml	20 µg/ ml	30 µg/ ml	40 µg/ ml
Ascorbic acid	63	68	72	79
1a	52	58	64	71
1b	53	57	63	69
1c	51	59	64	73
1d	60	62	69	75
1e	47	49	52	59
2b	31	35	36	39
2a	52	54	58	61
2c	47	49	51	54
2d	32	35	36	39

Table 7: percentage inhibition of free radicals by using DPPH method



Bar diagram of anti-oxidant activity

Result and discussion:

- Structures of synthesized compounds was confirmed and characterized with the help of analytical data such as FTIR, mass spectroscopy, ^{1}H NMR and C^{13} NMR.
- The synthesized compound 1a, 1h and 2d have shown good anti-cancer activity at concentration 100 µg/ml and 200 µg/ml. The compound 2a has shown moderate activity at concentration 200 µg/ml. The compound 1b, 1d and 2b did not exhibit prominent activity.
- Compound 1a, 1b, 1c, 1d and 2d exhibited good antibacterial activity at 100 µg/ ml. 1e, and 2c shows moderate activity while 1f, 1g, and 1i, exhibhited less antibacterial activity.
- 1a, 1b, 1c, 1d, 1e, 2a and 2d exhibited significant antioxidant activity with maximum inhibition at 40 µg/ml. 1g, 1j, 2b and 2c exhibited moderate antioxidant activity whereas 1h exhibited very low activity at 40 µg/ ml.

Acknowledgement:

Authors are thankful to Prof. B.G. Shivananda, Principal, Al-Ameen College of Pharmacy, for providing all the facilities and timely help during the conduct of project in the college.

References:

1) Das BC, Mariappan G, Saha S, Bhomik D, Chiranjib. Anthelmintic and anti-microbial activity of some novel chalcone derivatives. J Chem Pharm.Res.2010; 2(1):113-120

2) Kamboj RC, Arora R, Sharma G, Kumar D, Sharma C, Joshi R, et al. Eco-friendly synthesis and antimicrobial activity of chalcones. Der Pharma Chemica.2010; 2(3):157-170

3) Prasad YR, Kumar PR, Deepti CA, Venkatramana M. Synthesis and antimicrobial activity of some novel chalcones of 2-hydroxy-1-acetonapthone and 3-acetyl coumarin. E J Chem. 2006; 3(13):236-241.

4) Trivedi AR,Dodiya DK,Ravat NR,Shah VH. Synthesis and biological evaluation of some new pyrimidines via a novel chalcones series. ARKIVOC. 2008; (xi):131-141

5) Tiwari B, Pratapwar AS, Tapas AR, Butle SR, Vatkar BS. Synthesis and antimicrobial activity of some chalcone derivatives. Int J ChemTech Res.2010; 2(1):499-503.

6) Shylesh BS, Nair SA, Subramoniam A. Induction of cell-specific apoptosis and protection from Dalton's lymphoma challenge in mice by an active fraction from *Emilia sonchifolia*.Indian J Pharmacol.2005; 37(4):232-237

7) Preethi KL, Kuttan G, Kuttan R. Antitumour activity of *Ruta graveolens* extract.Asian Pacific J Cancer Prev. 2006; 7:439-443

8) Sashidhara KV, Kumar A, Sarkar J, Sinha S. synthesis and invitro evaluation of novel coumarin-chalcone hybrids as potential anticancer agents. Bioorg and Med Che Lett .2010; 20(24):7205-7211

9) Radha R, Kaviman S, Ravichandra V. Antitumour activity of methanolic extract of *Plumeria alba* leaves against Dalton lymphoma ascites in mice. Int J Health Res.2008; 1(2): 79-85

10) Ahmed MR, Sastry VG,Bano N, Ravichandra S, Raghavendra M. Synthesis and cytotoxic, antioxidant activities of new chalcone derivatives. J Chem 2011; 4(2): 289-294

11) Tiwari B, Pratapwar AS, Tapas AR, Butle SR, Vatkar BS. Synthesis and antimicrobial activity of some chalcone derivatives. Int J ChemTech Res.2010; 2(1):499-503.

Article History:----------

Date of Submission: 01-06-2013

Date of Acceptance: 29-06-2013

Conflict of Interest: NIL

Source of Support: NONE