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SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME NOVEL 3, 4-DICHLOROACETOPHENONE CHALCONE DERIVATIVES

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ABSTRACT

Chalcones are the most important precursors for the synthesis of numerous flavonoids and isoflavonoids. These are condensation products of aromatic aldehyde and acetophenone in the presence of a catalyst. The reactions monitored by TLC using Silica gel-G as the adsorbent. The compounds were purified by column chromatography. All the titled (3a-o) compounds were characterized by elemental analysis, IR and ^{1}HNMR spectral data and microanalysis. All the synthesized compounds were screened for biological activities such as antifungal, antitubercular and anticancer activity by using suitable reference standards. All the compounds subjected for biological screening. The compounds 3h and 3o possessed good antifungal activity, 3f and 3h shown excellent antitubercular activity, and the compound 3o shown good cytotoxic activity. The synthesized compounds evaluated for antifungal, antitubercular and anticancer activity. The compounds 3f, 3o, 3h good activity as antifungal, antitubercular, and anticancer agents. These studies may be useful for further developments in future.

Keywords: Flavonoids, Condensation, Antifungal, antitubercular, anticancer activity and cytotoxic activity

INTRODUCTION

Chalcone **[1]** is an aromatic ketone and an enone that forms the central core for a variety of important biological compounds, which are collectively known as chalcones or chalconoids. Different names for chalcones are benzylideneacetophenone or phenyl styryl-ketone or benzalacetophenone or α-phenyl-β-benzoylethylene. These compounds have an absorption maximum at 280nm to 340nm **[2]**. Chalcones are abundantly present in nature starting from ferns to higher plants **[3]**. Chalcones have a crystal structure. The dihedral angle between the two phenyl rings is 13.0(1)°, and the dihedral angle from the plane of C7/C8/C9 to the phenyl rings (C1 to C6 and C10 to C15) are 13.8(1)° and 2.6(1)° respectively, indicating that the central C7-C8-C9 fragment lies nearly in the phenyl ring plane of C10 to C15, but rather more displaced out of the other benzene ring of C1 to C6. The atom shapes a crisscross chain by C-H···π (arene) hydrogen bonds along the *c* axis. There also exist intermolecular hydrogen bonding interactions involving C11 acting as H-bond donor, via H11, to oxygen in the adjacent molecules at -*x*,1-*y*,1-*z*, resulting in a three-dimensional network **[4]**. They can be readily synthesized in a laboratory by the Claisen-Schmidt condensation reaction which is very easy and simple to conduct as well as inexpensive **[5]**. The structure of chalcone with a plane, centroid, and exclusion sphere has been shown in **Figure 1**.

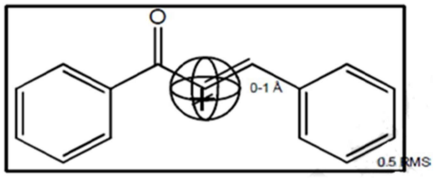


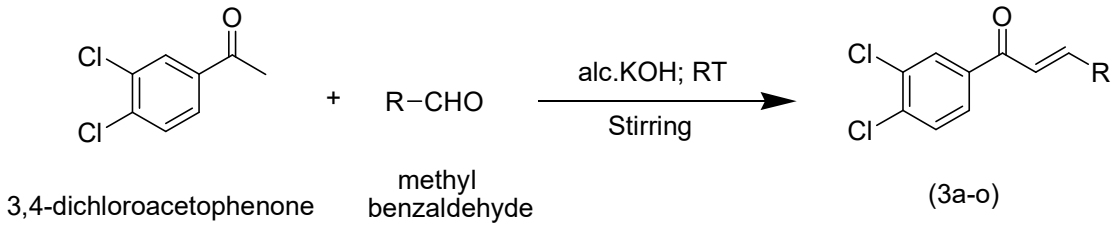
Figure 1: Structure of Chalcone showing plane, centroid and exclusion sphere

Various manufactured courses have been accounted for the amalgamation of chalcones while their overall combination includes Claisen-Schmidt buildup under homogeneous conditions within the sight of corrosive or base **[6-8]**. It was demonstrated from the writing chalcones are additionally valuable intermediates for the combination of a few synthetic and pharmacological classes of helpful specialists having heterocyclic constructions in them. Also, a number of chalcones with novel substituents were earlier isolated from a number of plants. These chalcones were endowed with significant biological activities. The chalcones containing a variety of rings were found to possess significant biological activities including antibacterial **[9]**, antifungal **[10]**, anti-inflammatory **[11-13]**, anticancer **[14-16]**, anti-HIV **[17]**, antimalarial **[18]**, anthelmintic **[19]**, MAO

inhibition **[20]**, and anti-angiogenic **[21]** activities. Based on these observations, it was considered worthwhile to synthesize some new chalcones containing 3,4-dichlorophenyl ring by Claisen-Schmidt condensation reaction. The main aim of present work is to synthesize and evaluate the antifungal, antitubercular and anticancer activity of synthesized compounds.

MATERIALS AND METHODS

All the chemicals used in the synthesis were obtained from commercial sources. The 3, 4-dichloroacetophenone was purchased from Aldrich Chemical Co. (Milwaukee, Wisconsin, USA).The organic solvents such as methanol, acetone, chloroform, and ethyl acetate were of spectral grade and used as such without further refinement. Some of the solvents were purchased from S.D Fine Chem. Ltd, Mumbai, India and the local manufacturers. The reactions were monitored using TLC. All the melting points were determined in open capillaries, using Boitus melting point apparatus, articulated in ^{o}C and are uncorrected. The ^{1}H and ^{13}C NMR spectra of the compounds were recorded on Bruker AMX 400 MHz spectrophotometer using TMS as an internal standard and the values are expressed in δ ppm. The mass spectra of the compounds were recorded on Agilent 6100 QQQ ESI mass spectrophotometer method. Elemental analyses were carried out with a Carlo Erba 1108 elemental analyzer. The results of elemental analyses (C, H, and N) were within ± 0.4% of the calculated values.



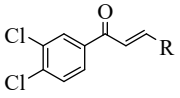
Scheme 1: Procedure for the synthesis of titled compounds (3a-o)

General Procedure for the synthesis of titled compounds (3a-3o):

Synthesis of *(E)*-1-(3, 4-dichlorophenyl)-3-(p-tolyl) prop-2-en-1-one (3a): A mixture of 3, 4-dichlororacetophenone (0.001 mol)and appropriate aldehyde (0.001 mol) was stirred in 7.5 ml of ethanol and then few drops of 50% of 7.5 ml of alcoholic KOH solution was added drop wise to it. The mixture was stirred for 24 hours. After stirring, it was acidified with 1:1 of HCl and H_2O which resulted in the formation of precipitate. Then the precipitate was filtered under vacuum, the

solid was washed with water and purified by recrystallization using ethanol as solvent. The general information of synthesized compounds was mentioned in below **Table 1.** All the prepared compounds were characterized by elemental analysis and the spectral studies were shown in **Table 2 & 3** respectively.

Table 1: General structure and information of the synthesized compounds (3a-o)



(3a-o)

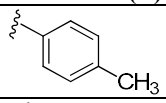
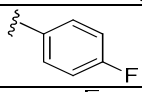
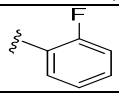
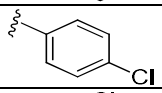
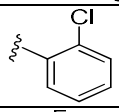
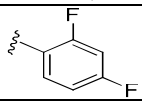
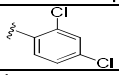
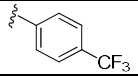
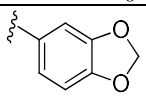
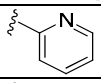
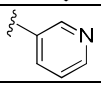
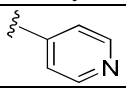
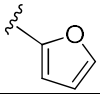
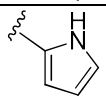
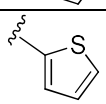
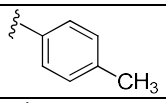
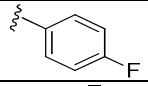
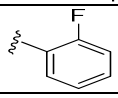
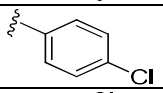
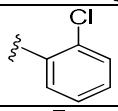
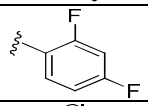
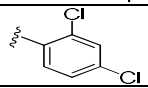
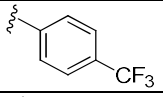
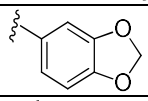
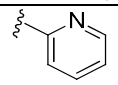
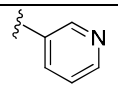
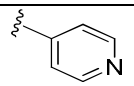
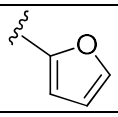
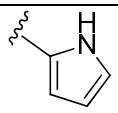
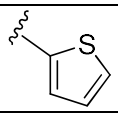
S.No	Compound	Substituent (R)	IUPAC Name
1.	3a		1-(3,4-dichlorophenyl)-3-(p-tolyl)prop-2-en-1-one (3a)
2.	3b		1-(3,4-dichlorophenyl)-3-(4-fluorophenyl)prop-2-en-1-one (3b)
3.	3c		1-(3,4-dichlorophenyl)-3-(2-fluorophenyl)prop-2-en-1-one (3c)
4.	3d		1-(3,4-dichlorophenyl)-3-(4-chlorophenyl)prop-2-en-1-one (3d)
5.	3e		1-(3,4-dichlorophenyl)-3-(2-chlorophenyl)prop-2-en-1-one (3e)
6.	3f		1-(3,4-dichlorophenyl)-3-(2,4-difluorophenyl)prop-2-en-1-one (3f)
7.	3g		1-(3,4-dichlorophenyl)-3-(2,4-dichlorophenyl)prop-2-en-1-one (3g)
8.	3h		1-(3,4-dichlorophenyl)-3-(4-trifluoromethylphenyl)prop-2-en-1-one (3h)
9.	3i		3-(benzo[d][1,3]dioxol-5-yl)-1-(3,4-dichlorophenyl)prop-2-en-1-one (3i)
10.	3j		1-(3,4-dichlorophenyl)-3-(pyridin-2-yl)prop-2-en-1-one (3j)
11.	3k		1-(3,4-dichlorophenyl)-3-(pyridin-3-yl)prop-2-en-1-one (3k)
12.	3l		1-(3,4-dichlorophenyl)-3-(pyridin-4-yl)prop-2-en-1-one (3l)
13.	3m		1-(3,4-dichlorophenyl)-3-(furan-2-yl)prop-2-en-1-one (3m)
14.	3n		1-(3,4-dichlorophenyl)-3-(1*H*-pyrrol-2-yl)prop-2-en-1-one (3n)
15.	3o		1-(3,4-dichlorophenyl)-3-(thiophen-2-yl)prop-2-en-1-one (3o)

Table 2: Elemental and Physical Data of Compounds (3a-o)

S. No.	R	Molecular Formula	RMM	M.P (^{o}C)	P .Y (%)	Elemental analysis					
						%Calculated			%Found		
						C	H	N	C	H	N
3a		$C_{16}H_{12}Cl_2O$	291.17	222	62	66.00	4.15	---	65.56	4.01	---
3b		$C_{15}H_9FCl_2O$	295.14	188	81	61.04	3.07	---	60.50	2.49	---
3c		$C_{15}H_9FCl_2O$	295.14	121	68	61.04	3.07	---	60.50	2.49	---
3d		$C_{15}H_9Cl_3O$	311.59	201	85	57.82	2.91	---	57.01	2.26	---
3e		$C_{15}H_9Cl_3O$	311.59	133	72	57.82	2.91	---	57.01	2.26	---
3f		$C_{15}H_8F_2Cl_2O$	313.13	265	83	57.54	2.58	---	57.12	2.22	---
3g		$C_{15}H_8Cl_4O$	346.04	299	91	52.06	2.33	---	52.01	2.04	---
3h		$C_{16}H_9F_3Cl_2O$	345.14	169	75	55.68	2.63	---	55.25	2.29	---
3i		$C_{16}H_{10}Cl_2O_3$	321.15	106	50	59.84	3.14	---	59.33	2.95	---
3j		$C_{14}H_9Cl_2ON$	277.01	156	43	60.46	3.26	5.04	60.08	3.15	4.75
3k		$C_{14}H_9Cl_2ON$	277.01	191	53	60.46	3.26	5.04	60.08	3.15	4.75
3l		$C_{14}H_9Cl_2ON$	277.01	247	66	60.46	3.26	5.04	60.08	3.15	4.75
3m		$C_{13}H_8Cl_2O_2$	265.99	172	55	58.46	3.02	---	58.21	2.61	---
3n		$C_{13}H_9Cl_2ON$	265.01	238	46	58.67	3.41	5.26	58.17	3.32	5.21
3o		$C_{13}H_8Cl_2OS$	266	194	58	55.14	2.85	---	55.01	2.67	---

*Relative molecular mass (RMM), *Melting Point (M.P), and *Percent Yield (%)

Table 3: IR (cm^{-1}) and ^1H NMR (δ, ppm) Spectral data of Compounds (3a-o)

S.No	IR data (cm^{-1})	^1HNMR data (δ, ppm)
3a	1652 (C=O), 1608 (C=C of Ar), 1512 (CH=CH), 832 (C-Cl)	2.32 (3H, s, Ar-CH_3), 7.26 (1H, d, *J* = 17 Hz, -CO-CH=), 7.75 (1H, d, *J* =17 Hz, =CH-Ar), 7.18-7.81 (7H, Ar-H)
3b	1660 (C=O), 1576 (C=C of Ar), 1528 (CH=CH), 832 (C-Cl), 928 (C-F)	7.18 (1H, d, *J* = 17 Hz, -CO-CH=), 7.72 (1H, d, *J* =17 Hz, =CH-Ar), 7.09-7.75 (7H, Ar-H)
3c	1642 (C=O), 1585 (C=C quadrant of Ar), 1518(CH=CH),829 (C-Cl), 929 (C-F)	7.22 (1H, d, *J* = 17 Hz, -CO-CH=), 7.65 (1H, d, *J* =17 Hz, =CH-Ar), 7.10-7.80 (7H, Ar-H)
3d	1648 (C=O), 1578 (C=C of Ar), 1508 (CH=CH), 835 (C-Cl), 841 (C-Cl)	7.41 (1H, d, *J* = 17 Hz, -CO-CH=), 7.85 (1H, d, *J* =17 Hz, =CH-Ar), 7.32-8.12 (7H, Ar-H)
3e	1648 (C=O), 1581 (C=C of Ar), 1505 (CH=CH), 835 (C-Cl), 831 (C-Cl)	7.45 (1H, d, *J* = 17 Hz, -CO-CH=), 7.82 (1H, d, *J* =17 Hz, =CH-Ar), 7.36-8.21 (7H, Ar-H)
3f	1656 (C=O), 1581 (C=C of Ar), 1512 (CH=CH), 829 (C-Cl), 926 (C-F)	7.28 (1H, d, *J* = 17 Hz, -CO-CH=), 7.75 (1H, d, *J* =17 Hz, =CH-Ar), 7.33-8.05 (6H, Ar-H)
3g	1659 (C=O), 1582 (C=C of Ar), 1509 (CH=CH), 826 (C-Cl), 822 (C-Cl)	7.31 (1H, d, *J* = 17 Hz, -CO-CH=), 7.80 (1H, d, *J* =17 Hz, =CH-Ar), 7.45-8.25 (6H, Ar-H)
3h	1652 (C=O), 1586 (C=C quadrant of Ar), 1521 (CH=CH), 922 (C-F)	7.35 (1H, d, *J* = 17 Hz, -CO-CH=), 7.69 (1H, d, *J* =17 Hz, =CH-Ar), 7.22-8.09 (7H, Ar-H)
3i	1647 (C=O), 1580 (C=C of Ar), 1510 (CH=CH), 1245 (O-CH_2-O), 841 (C-Cl)	6.15 (2H,s,-O-CH_2O-), 7.01 (1H, d, *J* = 17 Hz, -CO-CH=), 7.72 (1H, d, *J* =17 Hz, =CH-Ar), 7.12-7.56 (6H, Ar-H)
3j	1656 (C=O), 1600 (C=C of Ar), 1598 (C=N), 1512 (CH=CH), 1377 (C-N), 822 (C-Cl)	7.18 (1H, d, *J* = 17 Hz, -CO-CH=), 7.61 (1H, d, *J* =17 Hz, =CH-Ar), 6.37-8.15 (7H, Ar-H)
3k	1655 (C=O), 1602 (C=C of Ar), 1591 (C=N), 1510 (CH=CH), 1371 (C-N), 828 (C-Cl)	7.19 (1H, d, *J* = 17 Hz, -CO-CH=), 7.71 (1H, d, *J* =17 Hz, =CH-Ar), 7.11-8.25 (7H, Ar-H)
3l	1652 (C=O), 1605 (C=C of Ar), 1586 (C=N), 1509 (CH=CH), 1378 (C-N), 829 (C-Cl)	7.14 (1H, d, *J* = 17 Hz, -CO-CH=), 7.69 (1H, d, *J* =17 Hz, =CH-Ar), 7.22-8.19 (7H, Ar-H)
3m	1649 (C=O), 1581 (C=C of Ar), 1515 (CH=CH), 836 (C-Cl)	7.20 (1H, d, *J* = 17 Hz, -CO-CH=), 7.72 (1H, d, *J* =17 Hz, =CH-Ar), 7.19-7.91 (6H, Ar-H)
3n	1656 (C=O), 1601 (C=C of Ar), 1581 (C=N), 1512 (CH=CH), 1375 (C-N), 3233 (N-H), 821 (C-Cl)	5.12 (1H, s, -NH), 7.08 (1H, d, *J* = 17 Hz, -CO-CH=), 7.68 (1H, d, *J* =17 Hz, =CH-Ar), 6.45-7.95 (6H, Ar-H)
3o	1661 (C=O), 1611 (C=C of Ar), 1519 (CH=CH), 628 (C-S), 823 (C-Cl)	7.14 (1H, d, *J* = 17 Hz, -CO-CH=), 7.72 (1H, d, *J* =17 Hz, =CH-Ar), 6.69-8.14 (6H, Ar-H)

Antifungal activity:

The antifungal activity of the synthesized compounds (3a-o) was assessed by determining the MIC, which is defined as the lowest concentration of the compound that completely inhibited the growth of each strain after overnight incubation. MIC values can be determined by a number of standard test procedures. The most commonly employed methods are tube dilution and agar dilution methods **[22]**. In the contemporary study, MIC was determined using serial tube dilution technique. The responses of an organism to unknown compounds were compared with the response of Fluconazole**,** used as standard reference drug. The antifungal activity of compounds screened against the fungi such as *Aspergillus Niger* (ATCC 6275, *An*) *and Candida Tropicalis* (ATCC 1369, *Ct*).

Anti-tubercular activity:

The preliminary anti tubercular screening **[23-26]** for synthesized compounds was carried against *M. tuberculosis* $H_{37}Rv$. The MIC of each drug was determined by broth dilution assay using pyrazinamide as standard drug. The test solutions were prepared using DMSO as a solvent. The growth in the U-tubes was compared with visibility against positive control (without drug), negative control (without drug and inoculum) and with standard pyrazinamide.

Anticancer Evaluation:

The anticancer activity of the synthesized compounds was evaluated by the MTT assay using the DU-145 cell line which was obtained as a gift from National Centre for Cell Science, Pune, India. The results obtained were compared with Methotrexate as standard reference drug.

RESULTS AND DISCUSSION

The synthesized compounds were screened for different biological activities including antifungal, antitubercular and anticancer activities by employing standard protocols. The results of antifungal activity exhibited that the compounds (3a-o) exhibited moderate to considerable activity. The compound 3h and 3o containing 4"-trifluorophenyl and 2"-thienyl scaffolds at a ring-B portion of the chalcones were more active against both *Aspergillus niger* and *Candida tropicalis* with MIC of 4 µg/mL. Compound 3k was active with MIC 4 µg/mL against *Aspergillus niger*. Most of the other compounds containing halogen atoms like chlorine and fluorine were also active against Aspergillus Niger at MIC 8 µg/mL. The antitubercular activity of compounds indicated that the compounds have some degree of inhibitory activity on the bacteria at both 3.12 and 6.25 µg/mL. Among all the compounds tested, the compound 3f and 3h containing 2",4"-difluorophenyl and 4"-trifluorophenyl moieties showed excellent antitubercular activity at MIC of 3.12µg/mL and is equivalent to that of the standard pyrazinamide. The chalcones, 3e, 3g, 3k, and 3o exhibited activity at MIC of 6.25 µg/mL whereas compounds, 3j, 3l, 3m, and 3n showed activity at MIC of 12.5 µg/mL. The other compounds were somewhat potent with MIC values ranging between 25-100 µg/mL. The compounds also evaluated for anticancer activity, from the cytotoxicity point of view most potent chalcone of the series was 3o containing 2"-thienylring at portion-B, with MIC 5 µg/mL which is equal to that of the standard methotrexate. The compound 3n, containing 2"-pyrrolyl was next in potency with MIC of 25 µg/mL. Most of the other compounds also exhibited considerable

cytotoxic activity at MIC less than 100µg/mL. All the results attained were given in **Table 4**.

Table 4: MIC and IC_{50} values of titled compounds (3a-o)

S.No	R	Antifungal activity		Anti-tubercular	Anticancer IC_{50}value
		A. niger	*C. tropicalis*	MIC values (µg/mL) of *M. tuberculosis* $H_{37}Rv$	DU-145 cancer cell line
3a	4"-methylphenyl	32	32	100	126 ± 2
3b	4"-fluorophenyl	16	16	25	52 ± 2
3c	2"-fluorophenyl	8	16	25	116 ± 2
3d	4"-chlorophenyl	8	16	25	46 ± 2
3e	2"-chlorophenyl	8	16	6.25	44 ± 2
3f	2",4"-fluorophenyl	8	16	3.12	98 ± 2
3g	2",4"-dichlorophenyl	8	16	6.25	88 ± 2
3h	4"-trifluorophenyl	4	4	3.12	101 ± 1
3i	3",4"-methylenedioxyphenyl	8	62.5	25	48 ± 2
3j	2"-pyridinyl	8	8	12.5	98 ± 2
3k	3"-pyridinyl	8	4	6.25	71 ± 2
3l	4"-pyridinyl	16	31.25	12.5	58 ± 2
3m	2"-furfuryl	62.5	31.25	12.5	55 ± 2
3n	2"-pyrrolyl	16	16	12.5	25 ± 2
3o	2"-thienyl	4	4	6.25	5 ± 1
Standard	Fluconazole	≤1	≤1	---	---
	Pyrazinamide	---	---	3.12	---
	Methotrexate	---	---	---	5 ± 1

CONCLUSION

Chalcones having a variety of pharmacophore could be successfully synthesized in good yield, purified and characterized by spectral studies. These compounds were evaluated for anti-fungal, antitubercular, and cytotoxic activities**.** By examining all the activities**,** the compounds 3h and 3o containing 4"-trifluorophenyl and 2"-thienyl scaffolds showed good antifungal activity, the compound 3f and 3h containing 2", 4"-difluorophenyl and 4"-trifluorophenyl moieties showed excellent antitubercular activity and the compound 3o containing 2"-thienylring at portion-B, with MIC 5 µg/mL showed antitumor activity which is equal to that of the positive control methotrexate.

Abbreviations:

1. Thin layer chromatography– TLC
2. Relative molecular mass - RMM
3. Melting Point - M.P
4. Percent Yield – P Y
5. Infrared spectroscopy – IR
6. Nuclear Magnetic Resonance – NMR

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