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Synthesis And Biological Evaluation of Some Novel 3,4-Dichloroacetophenone Chalcones as Potential Antifungal, Anti Tubercular, And Cytotoxic Agents

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Abstract
Chalcones containing a variety of rings were found to possess significant biological activities including antibacterial, antifungal, anti-inflammatory, anticancer, anti-HIV, antimalarial, anthelmintic, MAO inhibition, and anti-angiogenic activities. The present study was to synthesize a series of novel 3,4-dichloro acetophenone derivatives including chalcones by Claisen-Schmidt condensation, to characterize the synthesized chalcones using suitable IR, ^{1}H NMR, ^{13}C NMR, mass spectra and elemental analyses data, to screen the synthesized chalcones for their antifungal, anti-tubercular, and cytotoxic activities, to identify the active compounds for further exploitation. The composition of the synthesized compounds was confirmed by elemental analysis and the results were also in close agreement with those of the calculated values. Results of the antifungal activity clearly notify that the chalcones exhibited significant antifungal activity with altered MIC values against the tested organisms, but not as much of the standard fluconazole. The results of the anti-tubercular activity of novel chalcones 3a-3o divulged that all the compounds exhibited considerable activity against the M. tuberculosis H_{37}Rv strain. The titled compounds (3a-3o) were evaluated for their in vitro cytotoxic activity against prostate cancer cell line DU-145 by using MTT assay. Most potent chalcone of the series was 3o containing 2”-thienyl rings at portion-B, with an of MIC 5µg/mL which is equal to that of the positive control methotrexate.

Keywords: 3,4-dichloroacetophenone, Antifungal, Antitubercular, Cytotoxic, Chalcone

1. Introduction

1.1 Structure-Based Drug Design: An attempt can be made to directly design a therapeutic molecule when there is occasionally, albeit still quite infrequently, enough molecular knowledge about the physiological target (for example, the crystal structure of an enzyme indicating the precise geometry of the active site).[2],[3]

1.2 Analogue-Based Drug Design: The therapeutic utility of a pioneer drug has not yet been clinically validated; its development is more unpredictable than usual. On the other hand, creating an analogue of a well-known drug has the significant advantage that the analogue's anticipated therapeutic utility has al-

ready been demonstrated. This reduces the total risk of success by a sizable amount. But there are still a lot of obstacles to clear, particularly regarding safety and pharmacokinetic behavior. However, it is crucial to establish at the outset of the study program the anticipated therapeutic advantage of the analogue over the lead medicine. Improvement in the use as a medicine must be the goal.[3],[7]

1.3 Importance of Finding New Antimicrobials: For infectious diseases to be successfully treated, antimicrobial medicines must be used. Even though several different drug classes are frequently used to treat human infections, the identification and development of new antimicrobial medicines is crucial for several reasons. In the last ten years, the development of resistance in organisms that are common human diseases has grown. Among them are intermediate and methicillin/oxacillin-resistant Staphylococcus aureus Vancomycin-resistant Staphylococcus aureus Extended spectrum beta lacta masses are produced by Enterococcus gram-negative bacilli. carbapenem-resistant All commonly prescribed antibiotics are ineffective against strains of Klebsiella pneumonia, Pseudomonas, and Acinetobacter. The number of antimicrobials that can be employed to treat species has been reduced because of this rising resistance. For specific classes of species, new antimicrobials are also required. Antimicrobials that can treat infections brought on by fungus and mycobacteria are extremely few. Due to the fatal interaction between drug-resistant tuberculosis and HIV infection, the growth of extensively drug-resistant (XDR) and multidrug-resistant (MDR) tuberculosis (TB) poses a significant barrier to successful TB control. To combat these deadly infectious diseases, there is a strong need for discovering and synthesizing innovative medications with potent selectivity shorter length of treatments and less toxic anti mycobacterial and antimicrobial agents.[5]

1.4 Tuberculosis And Importance of Finding Novel Anti-Tubercular Agents: A deadly infectious disease that is typically spread through the air is tuberculosis (TB), which is caused by the bacteria Mycobacterium tuberculosis (M. tb). The tubercle bacilli infection affects more than one-third of the world's population, and 5 to 10% of those people eventually get sick. The World Health Organization (WHO) estimated that 1.6 million people died from TB in 2005.The co-infection of the HIV-AIDS virus promotes the growth of TB. HIV/AIDS weakens the human immune system, and the spread of TB becomes out of hand. Additionally, the advent of TB strains that are resistant to both the most potent anti-TB medications, isoniazid, and rifampicin, as well as multiple drug-resistant TB (MDR-TB) and exceptionally drug-resistant TB (XDR-TB), classifies the illness as one that currently contributes to human death. It's going to take new strategies to stop the organisms.[6],[11].

1.5 Importance of The Development of Cytotoxic Agents Against Prostate Cancer: It is clear from the literature review that several chalcones, pyrazolines, and 1, 5-benzothiazepines had cytotoxic action when evaluated in vitro on different cancer cell lines. When tested for cytotoxic activity on prostate cancer cell lines, some of the chalcones, pyrazolines, and 1,5-benzothiazepines previously produced in our lab were found to be effective. Additional research is still ongoing in partnership with other labs. Based on these early findings, it was deemed worthwhile to screen these chemicals for similar effect on prostate cancer cell lines. When a bodily region's cells start to multiply uncontrollably, cancer develops. Unlike cancer cells, which do not divide and expand in an organized manner, normal cells do. They keep expanding and displacing healthy cells. Even though there are many different types of cancer, they are always characterized by unchecked cell proliferation. Different cancers may act in very different ways. Breast cancer and lung cancer, for instance, are two completely distinct disorders. They develop at various rates and react to various therapies. The requirement for treatment in cancer patients is due to this. Cancer cells can occasionally separate from tumors and travel through the bloodstream or

lymphatic system to other regions of the body. They can locate new locations and grow new tumors. Metastasis is the medical term for this. Cancer is still given the name of the bodily part where it first appeared, even though it has spread to a new location. For instance, prostate cancer is always referred to as prostate cancer even if it spreads to the bones. It is still breast cancer if it travels to the lungs. It is known as a recurrence when cancer reappears in a patient who initially appeared to be in remission after treatment.[9]

2. Materials and Method: Methanol, acetone, chloroform, and ethyl acetate are examples of spectral grade organic solvents that were used without further purification. The production of anhydrous methanol involved fractional distillation and storage on type 4A molecular sieves. The acetone that was present in the methanol was eliminated using the next procedure. A mixture of 500 ml methanol, 25 ml furfural, and 60 ml 10% sodium hydroxide solution was refluxed for 12 hours before being distilled. Due to the presence of formaldehyde, the first few millilitres of the distillate were discarded. Commercial ethanol was then distilled to produce ethanol, which was then used, after being refluxed for 6 hours over lit calcium oxide. Some of the solvents came from regional companies and S.D Fine Chem. Ltd. in Mumbai, India. All the chemicals used in the synthesis were purchased from conventional commercial sources. The 3, 4-dichloroacetophenone came from Aldrich Chemical Co. in Milwaukee, Wisconsin, the United States. 2-Aminothiophenol and phenyl hydrazine hydrochloride were purchased from a local supplier. When relevant, the suitable solvent systems are noted. Silica gel-G (Merck grade) was utilized as an adsorbent during TLC reaction monitoring. Silica gel (Merck grade, 100–200 mesh) has been used for column chromatography. n-Hexane, ethyl acetate, and mixtures of ethyl acetate and methanol (1%, 2%, 5%, and 10%), as well as hexane and ethyl acetate (5%, 10%, 15%, 25%, 50%, and 75%) were used in the gradient elution of the column. Fractions of each were collected in 100 mL volumes. TLC, a UV lamp, and the application of 10% sulfuric acid to the plates were used to analyse the chemical separation. All melting points were determined using the melting point apparatus in open capillaries, reported in oC, and are uncorrected. The values of the compounds' 1H NMR spectra were calculated using TMS as an internal standard, and they are displayed on either a Bruker AMX 400 MHz or an Advance 400 MHz NMR spectrophotometer in ppm. Bruker AMX 400 MHz NMR spectrophotometer was used to record the compounds' 13C NMR spectra. The mass spectra of the substances were captured using an Agilent 6100 QQQ ESI mass spectrophotometer. A Carlo Erba 1108 elemental analyser was used for the elemental analysis. The C, H, and N elements' outcomes were within 0.4% of the estimated values.

The serial tube dilution approach was used in the current experiment to calculate the MIC. The test organisms were injected into tubes of broth medium that contained progressive dosages of several substances using this technique. In the tubes where the chemical concentration was below the inhibitory level and the culture became murky after the proper incubation, growth occurred. The tubes were clear, and no development was visible above the inhibitory threshold. Fungi: The screening organisms are Aspergillus Niger (ATCC 6275, an) and Candida tropicalis (ATCC 1369, Ct).

The National Centre for Cell Science (NCCS), located in Pune, India, provided the DU-145 (prostate cancer) cell line. The following items were acquired from Sigma Chemicals in St. Louis, MO: DMEM (Dulbecco's Modified Eagles Medium), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), Trypsin, and EDTA. Arrow Labs provided the foetal bovine serum (FBS), while Tarson provided the 96 well flat bottom tissue culture plates.

2.1 Biological evaluation: Antimicrobial evaluation can be done by two methods, they are:

2.1.1 Agar diffusion method: In this technique petri dishes of agar are prepared by pouring melted agar inoculated with microorganisms. Bores are made in agar plate and antimicrobial substances are placed in the cups. Plates are incubated at a temperature of 37°C for 24 hours. The antimicrobial substance diffuses through agar around its cup and produces a clear zone of inhibition. The diameter of this zone can be measured and an estimation of degree of activity of the antimicrobial substance can be obtained.

2.1.2 Tube dilution method: The antimicrobial drugs are diluted in growth medium to a concentration that covers their clinically relevant range. Each tube, along with a control tube devoid of any antimicrobial agent, receives an equal amount of broth containing 105-106 bacteria per millilitre. After incubating the tubes for a night, they are checked for visual turbidity. This technique is used to assess the susceptibility of liquid media to antimicrobials. This establishes a substance's minimal inhibitory concentration (MIC). Responses of an organism to unidentified chemicals are contrasted with responses to a preparation of the standard reference medication that has a known composition and concentration. Fluconazole is the conventional medication utilized in the current study for antifungal activity.

2.1.3 Preparation of the sample solution: Each test substance was given 2.048 mg in separate vials. Methanol (2 mL) was then added. The result was a solution with a concentration of 1.024 mg/mL.

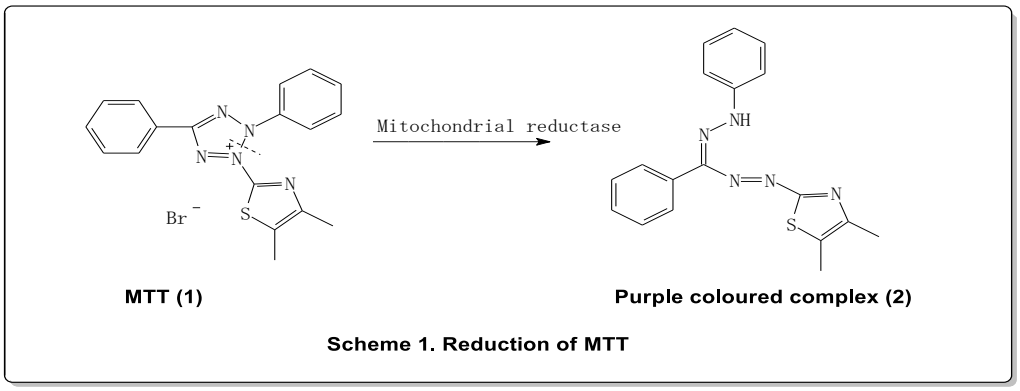
2.1.4 Preparation of the inoculums: The test bacteria was diluted in sterile nutrient broth medium so that the suspension contained around 107 cells/ml after being cultured at 370C in nutrient agar medium. The inoculum was this suspension.

2.1.5 Procedure: Eleven test tubes were used, nine of which were marked 1, 2, 3, 5, 6, 7, 8, and the remaining two were designated as medium and medium inoculum, respectively. Each of the 11 test tubes received 1 mL of nutrient broth medium. These test tubes were cotton-plugged before being autoclaved at a pressure of 15 lbs/sq.in to sterilize them. After cooling, 1 mL of the sample solution was added to the first test tube, thoroughly mixed, and then transferred to the second test tube with 1 mL of the remaining contents. After thoroughly combining the contents of the second test tube, 1 mL of this combination was once more transferred to the third test tube. The ninth test tube was added after continuing this serial dilution process. Each of the nine test tubes received 10 l of suitably diluted inoculum, which was then well mixed in. To track the growth of the organism in the medium, 10 l of the inoculum were put to the test tube TMI. To verify the sterility of the media, a controlled test tube TM containing only the medium was employed. For 18 hours, all of the test tubes were incubated at 370C.

To make sure that the methanol has no inhibitory impact in the utilized dilutions, a similar experiment using medium, methanol, and inoculum without compound was also carried out. The test tube number in which the organism's initial signs of growth were noticed was documented. The concentration utilized in the test tube number shortly before the test tube number where the first sign of growth was noticed was considered the MIC. The MIC values for each chemical were calculated using this method.

2.1.6 Experimental work: The MTT assay was used to assess the test compounds' in vitro cytotoxicity, which included chalcones, pyrazolines, and 1, 5-benzothiazepines. This colorimetric assay evaluates the degree to which mitochondrial succinate dehydrogenase reduces the yellow 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT). The MTT enters the cells and travels to the mitochondria, where it is converted to a formazan product that is insoluble and colored (dark purple). The released, solubilized formazan reagent is then detected spectrophotometrically at 570 nm after the cells have been solubilized with DMSO. Since only metabolically active cells can

reduce MTT, the level of activity is a gauge of the viability of the cells. It is possible to determine an agent's efficiency in killing cells by creating a dose-response curve by comparing the amount of dark purple formazan produced by treated cells to the amount produced by untreated control cells.



Scheme 1. Reduction of MTT

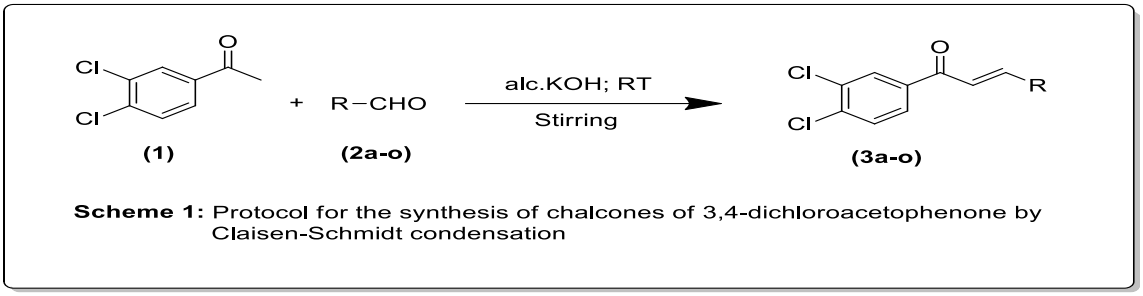
2.1.6.1 Maintenance of cell lines: DU-145cell band was developed as adherent in DMEM media. The ability was maintained in a humidified atmosphere with 5% CO2.

2.1.6.2 Preparation of samples for cytotoxicity: Test compound stock solutions (T1 to T20, 10 mg/mL) were generated in DMSO, and from there, various dilutions with sterile water were made to obtain the final medication concentrations of 10, 50, 100, and 200 mg/ml.

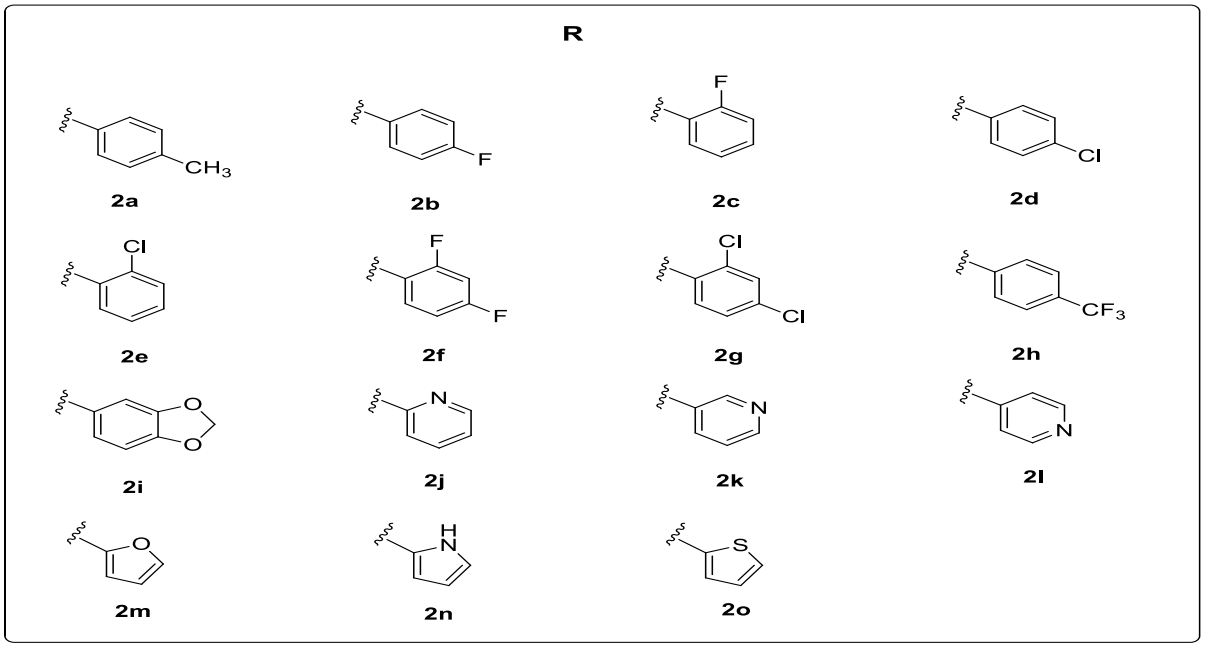
2.1.7 Cytotoxicity evaluation: To allow the cells to recuperate, they were seeded in 96-well plates at a density of 1x104 (as measured using the Typhon blue exclusion dye method) and then left to sit for 24 hours. Fresh media containing varying dilutions of the test substances were added to the medium after incubation. The plates were then kept in DMEM/MEM with 10% FBS medium for an additional 48 hours at 370°C. 90 l of brand-new, FBS-free DMEM was added after the medium had been removed for incubation. The aforesaid media was replaced by adding 200 l of DMSO to each well (to dissolve the blue formazan crystals) and incubating at 370C for 10 min. The above wells were first treated with 10 l of MTT reagent (5 mg/mL of stock solution in DMEM without FBS) and then incubated at 370C for 3–4 h. On a spectrophotometer, the absorbance at 570 nm was measured. For purposes of comparison, methotrexate was utilized as the standard medication. For three independent determinations, the assay was carried out in triplicate. The cytotoxicity was indicated as IC50 (g/mL), which is the concentration of the substance that reduced the growth rate of the tum or cells by 50% as compared to the control, untreated cells. The plot of percent inhibition against concentration was used to calculate IC50 values.

Synthesis and characterization of chalcones: The reaction follows base catalysed condensation of aromatic aldehyde and aromatic ketone by Claisen-Schmidt reaction.

2.1.8 General procedure for the synthesis of chalcones: 3, 4-dichloroacetophenone (1) and the appropriate aryl or heteroaryl aldehyde (2a-o) were combined and agitated in ethanol (7.5 mL), to which dropwise additions of an alcoholic solution of KOH (50%, 7.5 mL) were made. The mixture was stirred continuously for 24 hours before being acidified with a 1:1 solution of hydrochloric acid and water. After the precipitate had formed, it was vacuum filtered, extensively washed in water to eliminate any water-soluble contaminants, and then dried. To create crystals of the chalcone derivatives (3a-o), the dried precipitate was further recrystallized from ethanol.



Scheme 1: Protocol for the synthesis of chalcones of 3,4-dichloroacetophenone by Claisen-Schmidt condensation

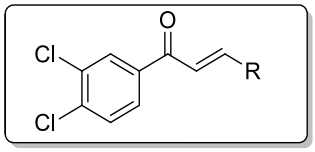


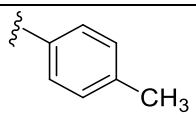
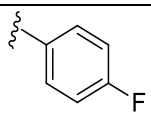
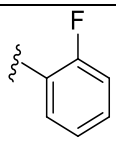
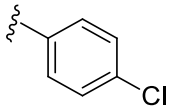
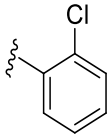
Synthesis of *(E)*-1-(3, 4-dichlorophenyl)-3-(p-tolyl) prop-2-en-1-one (3a): 7.5 ml of ethanol was mixed with 0.001 mol of 3, 4-dichlororacetophenone and 0.001 mol of 4-methyl Benzaldehyde, and a few drops of an alcoholic KOH solution (50 percent, 7.5 ml) were then added drop by drop. Precipitation formed when the mixture was agitated for 24 hours and was made acidic by adding 1:1 HCl to water. It was then refined by recrystallization using ethanol as the solvent after being vacuum-filtered and washed with water. The molecular ion [M] + was seen in the mass spectrum of compound 3a when C16H12Cl2O, with a melting point of 222°C, was examined. The spectrum also showed an isotope satellite signal (^{37}Cl) of 1/3rdintensity atm/z 293. The IR (cm^{-1}), spectrum showed the characteristic intense absorption and sat1652 (C=O), 1608 (C=C of AR), 1512 (CH=CH) and 832 (C-Cl). The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) showed two doublets (J=17 Hz) at δ 7.26 and 7.75 characteristic of the CO-CH= and = CH-AR respectively. This also proved the Trans geometry at the double bond for the molecule. The spectrum also accounted for all the seven aromatic protons appearing in between δ 7.18 and 7.81. The protons of the methyl group resonated as a singlet at δ2.32. The characteristic signal sats for the 13C NMR (ppm) spectra were 185.15 (C-1), 121.67 (C-2), 148.76 (C-3), 129.22 (C-2'), 147.89 (C-3'), 132.52 (C-4'), and 135.44 (C-5'). At 127.66, 117.11, and 133.23 in the spectrum, the carbon signals typical of the phenyl ring were also visible. The outcomes of the elemental analysis and the calculated values had similar results. The structure of compound 3a was determined to be (E)-1-(3, 4-dichlorophenyl)-3-(p-tolyl) prop-2-en-1-one based on the spectrum data and elemental analyses mentioned above. Compounds (3b-3o) were also created by using the above synthetic technique. List of new chalcones synthesized are:

[1] (E)-1-(3,4-dichlorophenyl)-3-(p-tolyl) prop-2-en-1-one (3a)
[2] (E)-1-(3,4-dichlorophenyl)-3-(4-fluorophenyl) prop-2-en-1-one (3b)
[3] (E)-1-(3,4-dichlorophenyl)-3-(2-fluorophenyl) prop-2-en-1-one (3c)
[4] (E)-1-(3,4-dichlorophenyl)-3-(4-chlorophenyl) prop-2-en-1-one (3d)
[5] (E)-1-(3,4-dichlorophenyl)-3-(2-chlorophenyl) prop-2-en-1-one (3e)
[6] (E)-1-(3,4-dichlorophenyl)-3-(2,4-difluorophenyl) prop-2-en-1-one (3f)
[7] (E)-1-(3,4-dichlorophenyl)-3-(2,4-dichlorophenyl) prop-2-en-1-one (3g)
[8] (E)-1-(3,4-dichlorophenyl)-3-(4-trifluoromethylphenyl) prop-2-en-1-one (3h)
[9] (E)-3-(benzo[d] [1,3] dioxol-5-yl)-1-(3,4-dichlorophenyl) prop-2-en-1-one (3i)
[10] (E)-1-(3,4-dichlorophenyl)-3-(pyridin-2-yl) prop-2-en-1-one (3j)
[11] (E)-1-(3,4-dichlorophenyl)-3-(pyridin-3-yl) prop-2-en-1-one (3k)
[12] (E)-1-(3,4-dichlorophenyl)-3-(pyridin-4-yl) prop-2-en-1-one (3l)
[13] (E)-1-(3,4-dichlorophenyl)-3-(furan-2-yl) prop-2-en-1-one (3m)
[14] (E)-1-(3,4-dichlorophenyl)-3-(1H-pyrrol-2-yl) prop-2-en-1-one (3n)
[15] (E)-1-(3,4-dichlorophenyl)-3-(thiophen-2-yl) prop-2-en-1-one (3o)

3. Results

Table 1: Physical characterization of chalcones (3a-3h)



Compound	R	Molecular Formula	Relative Molecular Mass	Melting Poin (°C)	Percentage Yield
3a		$C_{16}H_{12}Cl_2O$	291.17	222	62
3b		$C_{15}H_9FCl_2O$	295.14	188	81
3c		$C_{15}H_9FCl_2O$	295.14	121	68
3d		$C_{15}H_9Cl_3O$	311.59	201	85
3e		$C_{15}H_9Cl_3O$	311.59	133	72

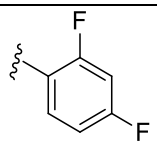
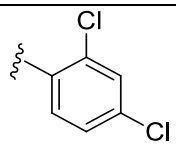
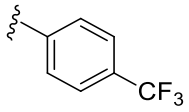
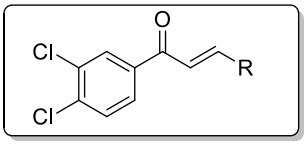
3f		$C_{15}H_8F_2Cl_2O$	313.13	265	83
3g		$C_{15}H_8Cl_4O$	346.04	299	91
3h		$C_{16}H_9F_3Cl_2O$	345.14	169	75

Table 2: Physical characterization of chalcones (3i-3o)



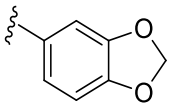
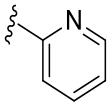
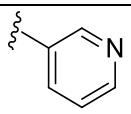
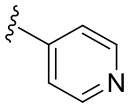
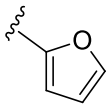
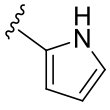
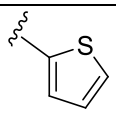
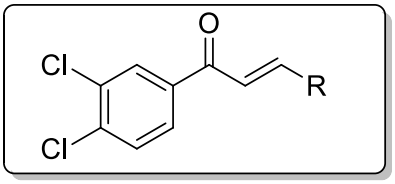
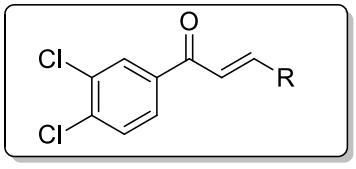
Compound	**R**	**Molecular Formula**	**Relative Molecular Mass**	**Melting Poin (ºC)**	**Percentage Yield**
3i		$C_{16}H_{10}Cl_2O_3$	321.15	106	50
3j		$C_{14}H_9Cl_2ON$	277.01	156	43
3k		$C_{14}H_9Cl_2ON$	277.01	191	53
3l		$C_{14}H_9Cl_2ON$	277.01	247	66
3m		$C_{13}H_8Cl_2O_2$	265.99	172	55
3n		$C_{13}H_9Cl_2ON$	265.01	238	46
3o		$C_{13}H_8Cl_2OS$	266	194	58

Table 3. Elemental Analysis of chalcones (3a-3o)



Compound	Calculated (%)			Found (%)		
	C	H	N	C	H	N
3a	66.00	4.15	---	65.56	4.01	----
3b	61.04	3.07	---	60.50	2.49	----
3c	61.04	3.07	---	60.50	2.49	---
3d	57.82	2.91	---	57.01	2.26	---
3e	57.82	2.91	---	57.01	2.26	---
3f	57.54	2.58	---	57.12	2.22	---
3g	52.06	2.33	---	52.01	2.04	---
3h	55.68	2.63	---	55.25	2.29	---
3i	59.84	3.14	---	59.33	2.95	---
3j	60.46	3.26	5.04	60.08	3.15	4.75
3k	60.46	3.26	5.04	60.08	3.15	4.75
3l	60.46	3.26	5.04	60.08	3.15	4.75
3m	58.46	3.02	---	58.21	2.61	---
3n	58.67	3.41	5.26	58.17	3.32	5.21
3o	55.14	2.85	---	55.01	2.67	---

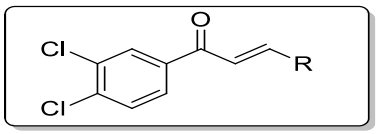
Table 4: IR (KBr disc) spectral data of chalcones (3a-3o)



Compound	Position of absorption band (cm^{-1})
3a	1652 (C=O), 1608 (C=C of AR), 1512 (CH=CH), 832 (C-Cl)
3b	1660 (C=O), 1576 (C=C of AR), 1528 (CH=CH), 832 (C-Cl), 928 (C-F)
3c	1642 (C=O), 1585 (C=C quadrant of AR), 1518(CH=CH),829 (C-Cl), 929 (C-F)
3d	1648 (C=O), 1578 (C=C of AR), 1508 (CH=CH), 835 (C-Cl), 841 (C-Cl)
3e	1648 (C=O), 1581 (C=C of AR), 1505 (CH=CH), 835 (C-Cl), 831 (C-Cl),
3f	1656 (C=O), 1581 (C=C of AR), 1512 (CH=CH), 829 (C-Cl), 926 (C-F)
3g	1659 (C=O), 1582 (C=C of AR), 1509 (CH=CH), 826 (C-Cl), 822 (C-Cl)

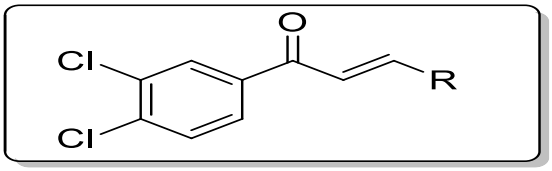
3h	1652 (C=O), 1586 (C=C quadrant of AR), 1521 (CH=CH), 922 (C-F)
3i	1647 (C=O), 1580 (C=C of AR), 1510 (CH=CH), 1245 (O-CH_2-O), 841 (C-Cl)
3j	1656 (C=O), 1600 (C=C of AR), 1598 (C=N), 1512 (CH=CH), 1377 (C-N), 822 (C-Cl)
3k	1655 (C=O), 1602 (C=C of AR), 1591 (C=N), 1510 (CH=CH), 1371 (C-N), 828 (C-Cl)
3l	1652 (C=O), 1605 (C=C of AR), 1586 (C=N), 1509 (CH=CH), 1378 (C-N), 829 (C-Cl)
3m	1649 (C=O), 1581 (C=C of AR), 1515 (CH=CH), 836 (C-Cl)
3n	1656 (C=O), 1601 (C=C of AR), 1581 (C=N), 1512 (CH=CH), 1375 (C-N), 3233 (N-H), 82
3o	1661 (C=O), 1611 (C=C of AR), 1519 (CH=CH), 628 (C-S), 823 (C-Cl)

Table 5: ^{1}H NMR spectral data of chalcones (3a-3o)



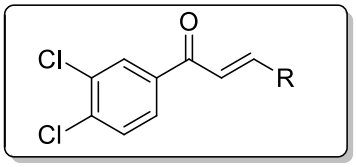
Compound	Chemical shift (δ) in ppm
3a	2.32 (3H, s, Ar-CH_3), 7.26 (1H, d, *J* = 17 Hz, -CO-CH=), 7.75 (1H, d, *J* =17 Hz, =CH-AR), 7.18-7.81 (7H, AR-H)
3b	7.18 (1H, d, *J* = 17 Hz, -CO-CH=), 7.72 (1H, d, *J* =17 Hz, =CH-AR), 7.09-7.75 (7H, AR-H)
3c	7.22 (1H, d, *J* = 17 Hz, -CO-CH=), 7.65 (1H, d, *J* =17 Hz, =CH-AR), 7.10-7.80 (7H, AR-H)
3d	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	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	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	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	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	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	7.18 (1H, d, *J* = 17 Hz, -CO-CH=), 7.61 (1H, d, *J* =17 Hz, =CH-AR), 6.37-8.15 (7H, AR
3k	7.19 (1H, d, *J* = 17 Hz, -CO-CH=), 7.71 (1H, d, *J* =17 Hz, =CH-AR), 7.11-8.25 (7H,
3l	7.14 (1H, d, *J* = 17 Hz, -CO-CH=), 7.69 (1H, d, *J* =17 Hz, =CH-AR), 7.22-8.19 (7H, AR
3m	7.20 (1H, d, *J* = 17 Hz, -CO-CH=), 7.72 (1H, d, *J* =17 Hz, =CH-AR), 7.19-7.91 (6H, AR
3n	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	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)

Table 6: Results of the antifungal activity of chalcones (3a-3o)



S. No	Compound	R	*An*	*Ct*
1	**3a**	4"-methylphenyl	32	32
2	**3b**	4"-fluorophenyl	16	16
3	**3c**	2"-fluorophenyl	8	16
4	**3d**	4"-chlorophenyl	8	16
5	**3e**	2"-chlorophenyl	8	16
6	**3f**	2",4"-fluorophenyl	8	16
7	**3g**	2",4"-dichlorophenyl	8	16
8	**3h**	4"-trifluorophenyl	**4**	**4**
9	**3i**	3",4"-methylenedioxyphenyl	8	62.5
10	**3j**	2"-pyridinyl	8	8
11	**3k**	3"-pyridinyl	**8**	**4**
12	**3l**	4"-pyridinyl	16	31.25
13	**3m**	2"-furfuryl	62.5	31.25
14	**3n**	2"-pyrrolyl	16	16
15	**3o**	2"-thienyl	**4**	**4**
16	**Fluconazole**	----	≤1	≤1

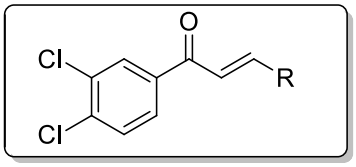
Table 7: Results of the anti-tubercular activity of chalcones (3a-3o)



S. No	Compound	R	MIC values (µg/mL) of *M. tuberculosis* $H_{37}Rv$
1	**3a**	4"-methylphenyl	100
2	**3b**	4"-fluorophenyl	25
3	**3c**	2"-fluorophenyl	25
4	**3d**	4"-chlorophenyl	25
5	**3e**	2"-chlorophenyl	6.25
6	**3f**	2",4"-fluorophenyl	**3.12**
7	**3g**	2",4"-dichlorophenyl	6.25
8	**3h**	4"-trifluorophenyl	**3.12**
9	**3i**	3",4"-methylenedioxyphenyl	25

10	**3j**	2"-pyridinyl	12.5
11	**3k**	3"-pyridinyl	6.25
12	**3l**	4"-pyridinyl	12.5
13	**3m**	2"-furfuryl	12.5
14	**3n**	2"-pyrrolyl	12.5
15	**3o**	2"-thienyl	6.25
16	**Pyrazinamide**		3.12

Table 8: Results of the cytotoxic activity of chalcones (3a-3o)



S. No	Compound	R	DU-145
1	**3a**	4"-methylphenyl	126 ± 2
2	**3b**	4"-fluorophenyl	52 ± 2
3	**3c**	2"-fluorophenyl	116 ± 2
4	**3d**	4"-chlorophenyl	46 ± 2
5	**3e**	2"-chlorophenyl	44 ± 2
6	**3f**	2",4"-fluorophenyl	98 ± 2
7	**3g**	2",4"-dichlorophenyl	88 ± 2
8	**3h**	4"-trifluorophenyl	101 ± 1
9	**3i**	3",4"-methylenedioxyphenyl	48 ± 2
10	**3j**	2"-pyridinyl	98 ± 2
11	**3k**	3"-pyridinyl	71 ± 2
12	**3l**	4"-pyridinyl	58 ± 2
13	**3m**	2"-furfuryl	55 ± 2
14	**3n**	2"-pyrrolyl	25 ± 2
15	**3o**	2"-thienyl	**5 ± 1**
16	**Methotrexate**		5 ± 1

4. Conclusion:

According to the unique substituents present in each compound, all of the produced chalcones displayed distinctive absorption bands in the IR spectra (cm^{-1}) between 1640 and 1660 (C=O), 1570 and 1605 (C=C quadrant of AR), 1500 and 1550 (HC=CH), and in other areas of the spectrum. The chalcones' 1H NMR spectra demonstrated the distinctive ethylenic protons of the chalcone system in the range of 6.85 and 8.10. The spectra also revealed peaks between the respective sections of the spectrum that accounted for the aromatic protons as well as the various substituent protons. In addition to the peaks corresponding to the other carbons, the 13C NMR spectra of the chalcone 3a showed the distinctive peaks of the carbonyl carbon in the range of 185–192. While the spectra acquired by the EI approach revealed the molecular ion, the mass spectra obtained by the positive mode ionization method revealed the [M+H] + ions. Elemental analysis was used to confirm the composition of the synthesized compounds, and the results showed strong agreement with the calculated values.

4.1 Biological evaluation

4.1.1 Antifungal activity: Results of the antifungal activity clearly notify that the chalcones exhibited significant antifungal activity with altered MIC values against the tested organisms, but not as much of the standard fluconazole. Most of the compounds were more active against Aspergillus Niger than against Candida tropicalis whereas some possess equal potencies. However, compound 3h and 3o containing 4"-trifluorophenyl and 2"-thienyl scaffolds at ring-B portion of the chalcones were more active against both Aspergillus Niger and with MIC 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 at MIC 8 µg/mL. A Structure-Activity-Relationship study based on the above results indicated the essentiality of 4"-trifluorophenyl and 2"-thienyl moieties for the antifungal activity. Hence molecules composed of thienyl ring with halogen atoms as well as other hetero aromatic rings substituted with electron withdrawing can be attempted as part of SAR and can be synthesized in order to further enhance the activity.

4.1.2 Anti tubercular activity: The results of the anti-tubercular activity of novel chalcones 3a-3o divulged that all the compounds exhibited considerable activity against *M. tuberculosis* H_{37}Rv strain. The compound 3f and 3h containing 2”,4”-difluorophenyl and 4”-trifluorophenyl moieties showed excellent activity at MIC 3.12 µg/mL and is equivalent to that of the standard pyrazinamide. The chalcones, 3e, 3g, 3k, and 3o exhibited activity at MIC 6.25 µg/mL whereas compounds, 3j, 3l, 3m, and3n showed activity at MIC 12.5µg/mL. All the other compounds were somewhat potent with MIC values ranging between 25-100µg/mL. A Structure-Activity-Relationship study based on the above results indicated that with increase in the number of electrons withdrawing hydrophobic substituents on the six membered ring-B portion of chalcone linkage, increased the anti-tubercular activity. Hence it can be concluded that chalcones containing six membered aryl or heteroaryl ring-B with hydrophobic substituents will enhance the anti-tubercular potency. So, molecules composed of this type of rings with different hydrophobic electron withdrawing and electron releasing substituents can be attempted as part of SAR and can be synthesized to further enhance the activity.

4.1.3 Cytotoxic activity: The titled compounds (3a-3o) were evaluated for their in vitro cytotoxic activity against prostate cancer cell line DU-145byusing MTT assay. Most potent chalcone of the series was 3o containing 2”-thienylring at portion-B, with an of MIC 5µg/mL which is equal to that of the positive control methotrexate. The compound 3n, containing2”-pyrrolyl was next in potency with an

MIC of 25µg/mL. Most of the other compounds also exhibited considerable cytotoxic activity at MIC less than 100 µg/mL.

The structure activity relationships based on the above results indicated that five membered heterocyclic rings are most essential for the cytotoxic activity. Further modification of the 2"-thienyland2"-pyrrolyl rings by incorporating different electron withdrawing or releasing groups may enhance the cytotoxic activity of chalcones.

Chalcones having a variety of pharma cophores could be successfully synthesized in good yield, purified and characterized by spectral studies. These compounds were evaluated for anti-fungal, anti-tubercular, and cytotoxic activities. The compound 3h and 3o containing 4"-trifluorophenyl and 2"-thienyl scaffolds at ring-B portion of the chalcones were more active against both Aspergillus Nige*r* and Candida tropicalis with MIC 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 compound 3f and 3h containing 2",4"-difluorophenyl and 4"-trifluorophenyl moieties showed excellent anti-tubercular activity at MIC 3.12 µg/mL and is equivalent to that of the standard pyrazinamide. The chalcones, 3e, 3g, 3k, and 3o exhibited activity at MIC 6.25 µg/mL whereas compounds, 3j, 3l, 3m, and 3n showed activity at MIC 12.5 µg/mL. All the other compounds were somewhat potent with MIC values ranging between 25-100 µg/mL. From the cytotoxicity point of view most potent chalcone of the series was 3o containing 2"-thienyl ring at portion-B, with an of MIC 5 µg/mL which is equal to that of the positive control methotrexate. The compound 3n, containing 2"-pyrrolyl was next in potency with an MIC of 25 µg/mL. Most of the other compounds also exhibited considerable cytotoxic activity at MIC less than 100 µg/mL.

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