Original Article

Synthesis and Biological Activity of Novel 2,5-Dichloro-3-Acetylthiophene Chalcone Derivatives

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ABSTRACT

Objective: A series of novel 2, 5-dichloro-3-acetylthiophene chalcone derivatives were synthesized by Claisen-Schmidt condensation and evaluated for them *in vitro* antifungal, antitubercular activities and cytotoxic activity against DU145 (prostate) cancer cell line. **Methods:** Among all series of synthesized compounds, four compounds were displayed proximity of antifungal activity (MIC ≤ 8.0 μg/mL) towards the fungus species *Aspergillus niger* (ATCC 6275, An) and *Candida tropicalis* (ATCC 1369, Ct) and compared against fluconazole standard (MIC ≤ 1.0 μg/mL) by agar-diffusion and tube dilution methods. **Results:** One compound was shown a promising antimycobacterial activity (MIC~3.12 μg/mL) correlated with pyrazinamide (MIC~3.12 μg/mL) and also another five compounds exhibited better activity (MIC~6.25 μg/mL) towards *M. tuberculosis* H_{37}Rv species. All series of compounds were treated with DU145 cells and tested for cytotoxicity by MTT assay. Among all, one compound was shown similar cytotoxicity activity (IC_{50} ~5 ± 1 μg/mL) and the other compound was shown closer activity (IC_{50}~10 ± 2μg/mL) compared with a methotrexate (IC_{50} ~5 ± 1 μg/mL). The spectral studies (FT-IR, Mass, ^{1}HNMR and ^{13}CNMR) studies provided new molecular scaffolds with detailed structure-activity relationship. **Conclusion:** These novel chalcones with thienyl ring structure would be promising new scaffold for establishing specific targets on fungal, mycobacteria species and other cancer cell lines.

Key words: 2, 5-Dichloro-3-acetylthiophene chalcone derivatives, Claisen-Schmidt condensation, Cytotoxic activity, DU145 (prostate) cancer cell line, Structure-activity relationship, Antifungal activity, Antitubercular activity.

INTRODUCTION

Chalcones are a group of compounds with two aromatic rings connected by a keto-vinyl chain, constitute an important class of naturally occurring flavonoids exhibiting a wide spectrum of biological activities.[1] Chalcones are abundantly present in nature starting from ferns to higher plants and most of them are polyhydroxylated in the aryl rings. In plants, chalcones are converted to the corresponding (2S)-flavanones in a stereospecific reaction catalysed by the enzyme chalcone isomerase. This structural and biogenetic resemblance between chalcones and flavanones explained clearly why they often pre-existed as natural products. Chalcones occurs widely in nature particularly in colored flowers. These are colored compounds because of the presence of the chromophore -CO-CH=CH-, which deepens in the presence of other auxochromes. The presence of a reactive α,β-unsaturated keto (also called as 1,3-diaryl-2-propen-1-one) functional group is partly responsible for their activity.[2]

The chalcones are important intermediates in the synthesis of various five, six and seven membered heterocyclics like pyrazoles, isoxazoles, pyrimidines, 1,5-Benzothiazepines and other heterocyclic systems. Chalcones play a pivotal role in synthesizing a range of remedial compounds. There are various methods to synthesize chalcones. A number

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of techniques and schemes have been reported for the synthesis of chalcones. Amongst all the methods Aldol condensation and Claisen-Schmidt condensation still hold high position. Other renowned techniques include Suzuki coupling reaction, Wittig reaction, Fridel-Crafts acylation of cinnamoyl chlorides, Photo-Fries rearrangement of phenyl cinnamates etc.

A number of synthetic routes have been reported for synthesis of chalcones while their general synthesis involves Claisen-Schmidt condensation under homogeneous conditions in the presence of acid or base.[3,4,5] Traditionally, strong alkaline media including natural phosphates, Ba $(OH)_2$, KOH, NaOH, LiHMDS etc., have been employed for their synthesis.[6,7,8,9,10,11] The use of several Lewis acids (p-toluene sulfonic acid, B_2O_3, $RuCl_3$, $AlCl_3$, BF_3 and dry HCl) has also been demonstrated.[12,13,14,15,16,17] The most important catalysts were used and listed as solid sodium hydroxide (NaOH) and aqueous NaOH. Andreson *et al*, in 2001, introduced first time a solid sodium hydroxide method in a hydroxy chalcone derived from cinnamon.[18]

Chalcones were obtained by various schemes of synthesis published in the literature. Several synthetic routes have been reported for synthesis of chalcones while their general synthesis involves Claisen-Schmidt condensation under homogeneous conditions in the presence of acid or base.[3,4,5] 2-hydroxyacetophenone and benzaldehyde react in the presence of 0.1M NaOH to give the chalcone.[19] Liquid phase Claisen–Schmidt condensation between 2'-hydroxyacetophenone and benzaldehyde was carried out over a zinc oxide supported metal oxide catalyst under solvent free conditions to form 2'-hydroxychalcone.[20] 2',4',5'-trimethoxyacetophenone, when condensed with equimolar proportions of aromatic aldehydes in the presence of 30% alcoholic alkali at room temperature yield chalcones.[21] Claisen-Schmidt condensation between benzaldehyde and acetophenone by sonochemical and thermally activated reactions over zeolite as catalyst under solvent free conditions give chalcone.[22] 4-acetyl-3-aryl-syndnones when subjected to grinding with various aryl aldehydes in the presence of a base catalyst under solvent free conditions yield syndnone chalcones.[23] Condensation of 2-naphthylmethyl ketones with substituted aryl aldehydes in the presence of NaOH under methanol as solvent gave the corresponding chalcones.[24] Cinnamic acid condenses with resorcinol in chloroform in the presence of boron trifluoride to yield the chalcone.[25] *para*-methoxyphenylethenylboronic acid undergoes Suzuki coupling reaction with 3,4-dimethoxybenzoyl chloride in the presence of tetrakis(triphenylphosphine) palladium(0) and cesium carbonate as catalyst and anhydrous toluene as solvent to form 3',4'4''-trimethoxychalcone[37].[26] *O*-acylated and *N*-acylated chalcones are produced in high yields by condensation of *O*-acylated and *N*-acylated acetophenones with aromatic aldehydes in the presence of Borontrifluoride-etherate.[27] 5,6-trimethylbenzene-1,3-diol undergoes Friedel-Crafts acylation reaction with 3-phenylpropionyl chloride in the presence of aluminium trichloride as catalyst to form 2',6'-dihydroxy-3',4',5'-dimethylchalcone.[28] 2-acetyl-1-napthol upon grinding with various diversely substituted aromatic aldehydes in the presence of a base results in different array of chalcones. The key notable advantages of grinding technique include mild reaction conditions, non-hazardous and environmentally safer and give excellent yields in short reaction time.[29] β-chlorovinylketone condenses with phenolic ether (anisole) in the presence of stannic chloride to form chalcone.[30,31]

The compounds with chalcone as backbone have been reported to possess varied biological and pharmacological activities, including antimicrobial, anti-inflammatory, analgesic, cytotoxic, antitumor, antimalarial, antitubercular, antiviral, anti-HIV, antiulcerative, antileishmanial, antioxidant, antiprotozoal, antihistaminic, antifedent, immunomodulatory, and anticonvulsant, antihyperglycemic, antihyperlipidemic and antiplatelet activities. Several pure chalcones have been approved for clinical use or tested in humans. Clinical trials have shown that these compounds reached reasonable plasma concentration and they are well tolerated. For this reason, they are an object of continuously growing interest amongst the scientists. However, much of the pharmacological potential of chalcones is still not utilized. These chalcones are continued to attract considerable scientific attention because of their association with a variety of biological activities.

Based on these observations, it was considered worthwhile to synthesize some new heterocyclic chalcones containing dichlorothiophene ring by Claisen-Schmidt condensation reaction. Additionally, the dielectrophilic ketovinyl chain between of chalcones is highly reactive and acts as an important chemical synthon for constructing different five, six and seven membered heterocyclic scaffolds containing different hetero atoms like nitrogen, oxygen and sulphur atoms by abridgment with a variety of binucleophile reagents.

In the proposed work, it was contemplated to synthesize a series of novel 2, 5-dichloro-3-acetylthiophene chalcones by Claisen-Schmidt condensation. The spectral characterizations were carried out on the synthesized 2, 5-dichloro-3-acetylthiophene derivatives using suitable IR, ^{1}H NMR, ^{13}C NMR, mass spectra and elemental analyses data. All these 20 compounds (**1-20**) have been

screened for their antifungal, antimycobacterial and cytotoxic activities.

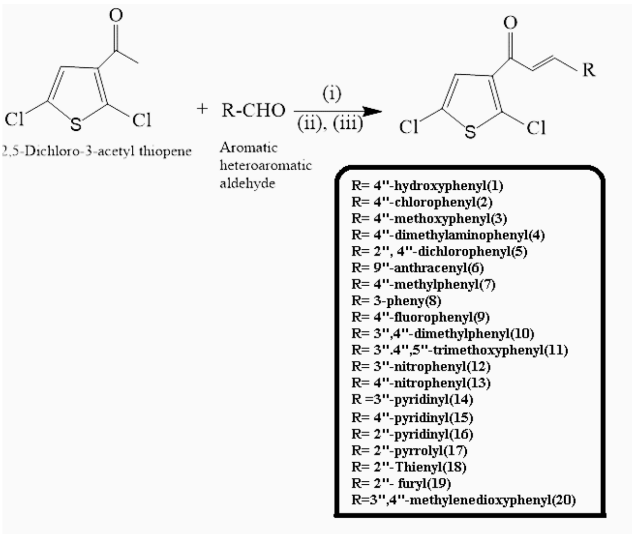
Therefore, owing to our interest in the synthesis of biologically active compounds, it has been envisioned to prepare novel chalcone derivatives with 2, 5-dichloro-3-acetyl thiopene using aryl or heteroaryl aldehydes by Claisen-Schmidt condensation reaction which may lead us towards 20 bioactive compounds with possessing different biological activities.

MATERIALS AND METHODS

Chemistry

The synthetic protocol was designed and followed to achieve target molecule involve with simple step (Scheme-I), which incites from synthesis of new chalcones with 2,5-dichloro-3-acetyl thiopene treated with different substituted aromatic and heteroaromatic aldehydes (**Compounds 1-20**) in an aqueous solution of KOH (40%) with continuous stirring. The mixture was kept for 24 h at room temperature and it was acidified with 1:1 mixture of hydrochloric acid and water, then it was filtered under vacuum and the product was washed with water. Then, it was purified by column chromatography and crystallized from a mixture of ethyl acetate and hexane (1:1) (Scheme-I).

Reagents and conditions: (i) potassium hydroxide (KOH), (ii) Room temperature condition; (iii) Methanol; The structural group of R in scheme-1 is specified below (**1-20**) and the synthesis was carried out with individual type of aromatic or heteroaromatic aldehydes to synthesize various chalcone derivatives with 2,5-dichloro-3-acetylthiopene ring (**1-20**) as in the Table 1. The



Scheme-1: Synthesis of chalcone derivatives

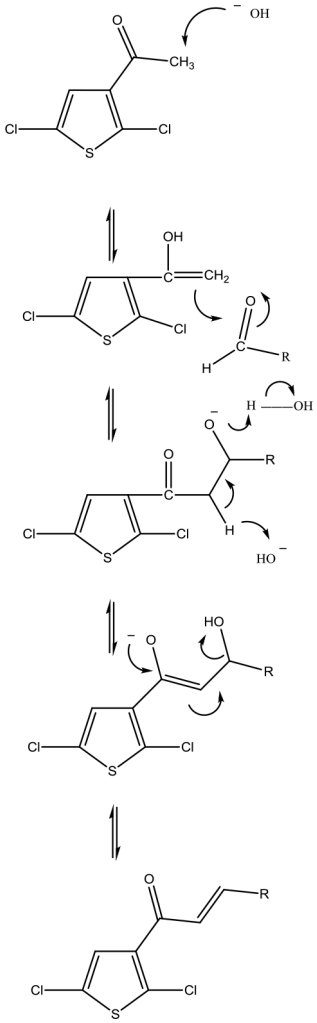


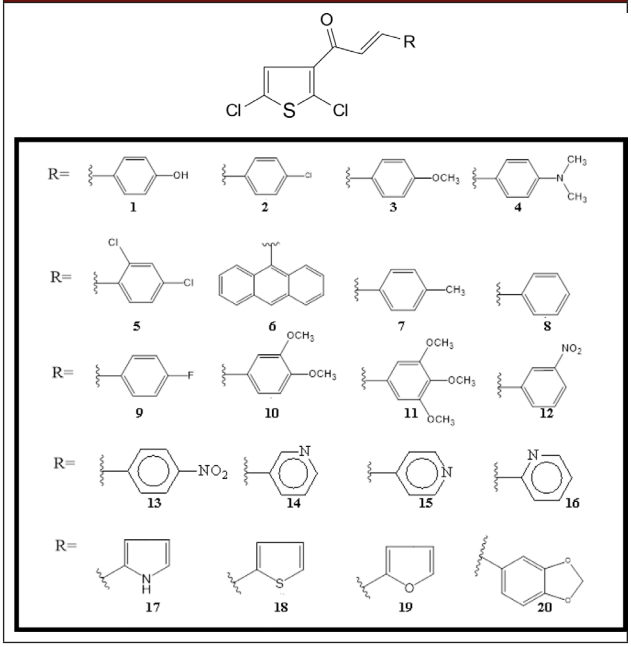
Figure 1: Mechanism of formation of chalcones by Claisen-Schmidt condensation.

mechanism of formation of novel synthetic chalcone was shown below in Figure 1.

General experimental method

All the melting points were determined in open capillaries, using Boitus melting point apparatus, expressed in °C and are uncorrected. The 1H NMR spectra of the compounds were recorded either on Bruker AMX 400 MHz or Advance 300 MHz NMR spectrophotometer using TMS as an internal standard and the values are expressed in δ ppm. The 13C NMR spectra of the compounds were recorded on Bruker AMX 400 MHz NMR spectrophotometer using TMS as an internal standard and the values are expressed in δ ppm. The mass spectra of the compounds were recorded either on Agilent 6100 QQQ ESI mass spectrophotometer method. Elemental analyses were carried out with a Carlo Erba

Table 1: R is replaced with aryl and heteroaryl moieties.



1108 elemental analyzer. The results of elemental analyses (C, H, N) were within ± 0.4% of the calculated values. All IR Spectra were recorded using Perkin-Elmer ATR-FTIR Spectrophotometer.

The organic solvents such as methanol, acetone, chloroform and ethyl acetate were of spectral grade and were used as such without further purification. Anhydrous methanol was obtained by fractional distillation and storing over type 4A molecular sieves. The acetone present in methanol was removed by using the following procedure. A mixture of 500 mL of methanol, 25 mL of furfural and 60 mL of 10% sodium hydroxide solution was refluxed for 12 h, then the mixture was distilled and the first few milliliters of the distillate was rejected as it contains trace amount of formaldehyde. Ethanol obtained by distillation of commercial ethyl alcohol was refluxed over ignited calcium oxide for 6 h and distilled at atmospheric pressure and then used. Some of the solvents were purchased from the local manufacturers and S.D Fine Chem. Ltd, Mumbai, India.

All the chemicals used in the synthesis were obtained from standard commercial sources. 2,5-dichloro-3-acetylthiophene was purchased from Aldrich Chemical Co. (Melwaukee, Wisconsin, USA). Reactions were monitored by TLC using silica gel-G (Merck grade) as the adsorbent and the solvent systems are indicated at appropriate places. Silica gel (100-200 mesh, Merck grade) has been used for column chromatography. The column was subjected to gradient elution using n-hexane, mixtures of hexane and ethyl acetate (5%, 10%, 15%, 25%, 50% and 75% hexane in ethyl acetate), ethyl acetate and mixtures of ethyl acetate and methanol (1%, 2%, 5% and 10% ethyl acetate in methanol). Fractions each of 100 mL were collected. The separation of the compounds was checked on TLC under UV lamp and by spraying the plates with 10% sulphuric acid.

General procedure for the preparation and structural features of compounds (1-20)

All compounds (**1-20)** were synthesized by a mixture of 2,5-dichloro-3-acetylthiophene (0.001 mol) and the appropriate aryl or heteroaryl aldehyde (0.001 mol) were stirred in methanol (10 mL). To this mixture an aqueous solution of KOH (40%, 5 mL) was added with continuous stirring. The mixture was kept for 24 h at room temperature and it was acidified with 1:1 mixture of hydrochloric acid and water, then it was filtered under vacuum and the product was washed with water and purified by column chromatography and crystallized from a mixture of ethyl acetate and hexane (1:1).

Compound 1 ($C_{13}H_8Cl_2O_2S$) analyzed for m.p.177°C, yield 88% and exhibited the molecular ion [M +H]+ at m/z 300 in its mass spectrum. The mass spectrum was interpreted with an isotope satellite signal (37Cl) of one-third intensity at m/z 302. The IR spectrum showed the characteristic intense absorption bands at ν_{max} 3422 cm^{-1}, (-OH), 1657 cm^{-1}, (-C=O), 1585 cm^{-1} (-C=C- quadrant of Ar), 1511 cm^{-1} (-CH=CH-), 632 cm^{-1} (C-S) and 875 cm^{-1} (C-Cl). The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) showed two doublets (*J*=17 Hz) at δ 7.35 and 7.84 characteristic of the -CO-CH= and =CH-Ar respectively. This was also confirmed the *trans* geometry at the double bond for the molecule. The spectrum also accounted for all the five aromatic protons appearing in between δ 6.84 and 7.84. The phenolic hydroxyl resonated as a singlet at δ 5.44. The ^{13}C NMR (δ ppm) spectrum exhibited the characteristic signals at 184.10 (C1), 119.65 (C2), 145.77 (C3), 129.83 (C2'), 147.20 (C3'), 133.83 (C4') and 135.57 (C5'). The spectrum also showed the carbon signals characteristic of the phenyl ring at 127.66, 132.11 and 117.23. The results of elemental analysis were also in close agreement with those of the calculated values. Based on the above spectral data and elemental analysis, the structure of the compound 1 was confirmed as 1-(2', 5'-dichloro-3'-thienyl)-3-(4''-hydroxyphenyl)-2-propen-1-one.

1-(2'',5''-dichloro-2''-thiopenyl)-3-(4''-chlorophenyl)-2-propen-1-one(**2**): colorless needles, m.p.133°C, yield **90%**, ATR-FTIR (cm^{-1}):ν_{max} 1649(C=O), 1589 (C=C, quadrant of Ar),1518 (-CH=CH-), 853(C-Cl), 663(C-Cl in Ar), 641(C-S). ^{1}HNMR (400 MHZ, TMS): δ (ppm)7.73 (1H, d, J=17 Hz, -CO-CH=), 7.88 (1H, d,

J=17 Hz, =CH-Ar), 7.25 (1H, s, C-4'-H), 7.55 (2H, d, J=7Hz, C-2"-H and -C-6"-H), 7.39 (2H, dd, J=7 Hz, C-3"-H and C-5"-H). HRMS (EI): m/e 317.0 $[M+H]^+$, calculated for $C_{13}H_7Cl_3OS$: 317.0.

1-(2",5"-dichloro-2"-thiopenyl)-3-(4"-methoxyphenyl)-2-propen-1-one (3): colorless needles, m.p.77ºC, yield 87%, ATR-FTIR (cm^{-1}): ν_{max} 1643(C=O), 1578 (C=C, quadrant of Ar),1521 (-CH=CH-), 1168(O-CH_3), 843(C-Cl), 629(C-S). ^{1}HNMR (400 MHZ, TMS): δ (ppm) 6.99 (1H, d, J=17 Hz, -CO-CH=), 7.56 (1H, d , J=17 Hz, =CH-Ar), 7.21 (1H, s, C-4'-H), 7.69 (2H, d, J=7 Hz, -C-2"-H and -C-6"-H), 7.02 (2H, d, J=7 Hz , C-3"-H and -C-5"-H). HRMS (EI): m/e 313.0 $[M+H]^+$, calculated for $C_{14}H_{10}Cl_2O_2S$: 313.0.

1-(2',5'-dichloro-2'-thiopenyl)-3-(4"-dimethylaminophenyl)-2-propen-1-one (4): colorless needles, m.p.145ºC, yield 82%, ATR-FTIR (cm^{-1}): ν_{max} 1652 (C=O), 1584 (C=C quadrant of Ar), 1524 (CH=CH), 1187 (N(CH_3)$_2$), 633 (C-S), 852 (C-Cl).^{1}HNMR (400 MHZ, TMS): δ (ppm) 6.73 (1H, d, J=17 Hz, -CO-CH=), 7.44 (1H, d, J=17 Hz, =CH-Ar), 3.12 (1H, s, N(CH_3)$_2$), 7.06 (1H, s, C-4'-H), 7.78 (2H, d, J=7 Hz, C-2"-H and C-6"-H), 7.65 (2H, d, J=7 Hz, C-3"-H and C-5"-H). HRMS (EI): m/e 326.0 $[M+H]^+$, calculated for $C_{15}H_{13}Cl_2NOS$: 326.0.

1-(2",5"-dichloro-2"-thiopenyl)-3-(2",4"-dichlorophenyl)-2-propen-1-one (**5**): colorless needles, m.p.165ºC, yield **93%**, ATR-FTIR (cm^{-1}):ν_{max} 1647(C=O), 1576 (C=C, quadrant of Ar),1522 (-CH=CH-), 855(C-Cl), 661(C-S), 680(C-Cl). ^{1}HNMR (400 MHZ, TMS): δ (ppm) 7.32 (1H, d, J=17 Hz, -CO-CH=), 7.91 (1H, d, J=17 Hz, =CH-Ar), 7.16 (1H, s, -C-4'-H), 7.51 (1H, s, -C-3"-H), 7.65 (1H, d, J=7 Hz, -C-5"-H), 7.72 (1H, d, J=7 Hz, -C-6"-H). HRMS (EI): m/e 352.0 $[M+H]^+$, calculated for $C_{13}H_6Cl_4OS$: 352.0.

1-(2",5"-dichloro-2"-thiopenyl)-9"-anthryl-2-propen-1-one (6): colorless needles, m.p.83ºC, yield 91%, ATR-FTIR (cm^{-1}):ν_{max} 1648(C=O), 1585 (C=C, quadrant of Ar), 1522 (-CH=CH-), 863(C-Cl), 651(C-S). ^{1}HNMR (400 MHZ, TMS): δ (ppm) 7.39 (1H, d, J=17 Hz, -CO-CH=), 7.75 (1H, d, J=17 Hz, =CH-Ar), 8.11 (1H, s, C-4'-H), 7.19-7.82 (10H, Ar-H. HRMS (EI): m/e 383.0 $[M+H]^+$, calculated for $C_{21}H_{12}Cl_2OS$: 383.0.

1-(2",5"-dichloro-2"-thiopenyl)-3-(4"-methlyphenyl)-2-propen-1-one (7): colorless needles, m.p.96ºC, yield 86%, ATR-FTIR (cm^{-1}):ν_{max} 1648(C=O), 1582(C=C, quadrant of Ar), 1519 (-CH=CH-), 863(C-Cl), 644(C-S). ^{1}HNMR (400 MHZ, TMS): δ (ppm) 7.41 (1H, d, J=17 Hz,-CO-CH=), 7.59 (1H, d, J=17 Hz, =CH-Ar), 7.20 (1H, s, -C-4'-H), 7.71 (2H, d, J=8 Hz, -C-2"-H and -C-6"-H), 7.18 (2H, d, J=6.5 Hz, -C-3"-H and -C-5"-H). HRMS (EI): m/e 297.0 $[M+H]^+$, calculated for $C_{14}H_{10}Cl_2OS$: 297.0.

1-(2",5"-dichloro-2"-thiopenyl)-3-phenyl-2-propen-1-one (8): colorless needles, m.p.122ºC, yield 81%, ATR-FTIR (cm^{-1}): ν_{max} 1652(C=O), 1585(C=C, quadrant of Ar), 1523 (-CH=CH-), 868(C-Cl), 657(C-S). ^{1}HNMR (400 MHZ, TMS): δ (ppm) 7.63 (1H, d, J=17 Hz, -CO-CH=), 7.79 (1H, d, J=17 Hz, =CH-Ar), 7.20 (1H, s, C-4'-H), 7.66 (2H, m, C-2"-H and C-6"-H), 7.41 (3H, m, C-3"-H, C-4"-H and C-5"-H). HRMS (EI): m/e 283.0 $[M+H]^+$, calculated for $C_{13}H_8Cl_2OS$: 283.0.

1-(2",5"-dichloro-2"-thiopenyl)-3-(4"-fluorophenyl)-2-propen-1-one (9): colorless needles, m.p.221ºC, yield 92%, ATR-FTIR (cm^{-1}): ν_{max} 1654(C=O), 1575(C=C, quadrant of Ar), 1528 (-CH=CH-), 1124(C-F), 877(C-Cl), 659(C-S). ^{1}HNMR (400 MHZ, TMS): δ (ppm) 7.28 (1H, d, J=17 Hz, -CO-CH=), 7.86 (1H, d, J=17 Hz, =CH-Ar), 7.15 (1H, s, C-4'-H), 7.67 (2H, d, J=8 Hz, C-2" and 6"-H), 7.06 (2H, d, J=8 Hz, C-3" and 5"-H). HRMS (EI): m/e 301.0 $[M+H]^+$, calculated for $C_{13}H_7Cl_2FOS$: 301.0.

1-(2",5"-dichloro-2"-thiopenyl)-3-(3",4"-dimethoxyphenyl)-2-propen-1-one (10): colorless needles, m.p.123ºC, yield 90%, ATR-FTIR (cm^{-1}): ν_{max} 1646(C=O), 1580(C=C, quadrant of Ar), 1519 (-CH=CH-), 1139(O-CH_3), 869(C-Cl), 649(C-S). ^{1}HNMR (400 MHZ, TMS): δ (ppm) 6.92 (1H, d, J=17 Hz, -CO-CH=), 7.80 (1H, d, J=17 Hz, =CH-Ar), 7.22 (1H, s, C-4'-H), 7.27 (1H, s, C-2"-H), 3.86-3.90 (6H, 2x-OCH3), 7.06 (1H, d, J=7 Hz, C-5"-H), 7.34 (1H, d, J=7 Hz, C-6"-H). HRMS (EI): m/e 343.0 $[M+H]^+$, calculated for $C_{15}H_{12}Cl_2O_3S$: 343.0.

1-(2",5"-dichloro-2"-thiopenyl)-3-(3",4",5"-trimethoxyphenyl)-2-propen-1-one (11): colorless needles, m.p.129ºC, yield 85%, ATR-FTIR (film) (cm^{-1}): ν_{max} 1648(C=O), 1584(C=C, quadrant of Ar), 1526 (-CH=CH-), 1168O-CH_3), 872(C-Cl), 640(C-S). ^{1}HNMR (400 MHZ, TMS): δ (ppm) 7.71 (1H, d, J=17 Hz, -CO-CH=), 7.90 (1H, d, J=17 Hz, =CH-Ar), 3.90-3.95 (9H, s, 3X-OCH_3), 7.22 (1H, s, C-4'-H), 7.27 (2H, s, C-2"-H and C-6"-H). HRMS (EI): m/e 373.0 $[M+H]^+$, calculated for $C_{16}H_{14}Cl_2O_4S$: 373.0.

1-(2",5"-dichloro-2"-thiopenyl)-3-(3"-nitrophenyl)-2-propen-1-one (12): colorless needles, m.p.172ºC, yield 91%, ATR-FTIR (film) (cm^{-1}): ν_{max} 1655(C=O), 1597(C=C, quadrant of Ar), 1533 (-CH=CH-), 1522(N=O,asymmetric),1343(N=O,symmetric),879(C-Cl), 657(C-S). ^{1}HNMR (400 MHZ, TMS): δ (ppm) 7.43 (1H, d, J=17 Hz, -CO-CH=), 7.81 (1H, d, J=17 Hz, =CH-Ar), 7.22 (1H, s, C-4'H), 8.44 (1H, d, J=2 Hz, C-2"-H), 8.28 (1H, m, C-4"-H), 7.93 (2H, m, C- 5"-H

and C-6''-H). HRMS (EI): m/e 328.0 $[M+H]^+$, calculated for $C_{13}H_7Cl_2NO_3S$: 328.0.

1-(2'',5''-dichloro-2''-thiopenyl)-3-(4''-nitrophenyl)-2-propen-1-one (13): colorless needles, m.p.167ºC, yield 95%, ATR-FTIR (film) (cm^{-1}): ν_{max} 1646(C=O), 1579(C=C, quadrant of Ar), 1529(-CH=CH-), 1512(N=O, asymmetric), 1313(N=O, symmetric), 879(C-Cl), 657(C-S). ^{1}HNMR (400 MHZ, TMS): δ (ppm) 7.53 (1H, d, J=17 Hz, -CO-CH=), 7.81 (1H, d, J=15.6 Hz, =CH-Ar), 7.13 (1H, s, C-4'-H), 7.82 (2H, d, J=7 Hz, C-2'' and 6''-H), 8.21 (2H,d, J=7 Hz, C-3'' and 5''-H. HRMS(EI): m/e 328.0 $[M+H]^+$, calculated for $C_{13}H_7Cl_2NO_3S$: 328.0.

1-(2'',5''-dichloro-2''-thiopenyl)-3-(3''-pyridinyl)-2-propen-1-one (14): colorless needles, m.p.133ºC, yield 80%, ATR-FTIR (film) (cm^{-1}): ν_{max} 1662(C=O), 1524(C=C, quadrant of Ar), 1588(C=N), 882(C-Cl), 644(C-S). ^{1}HNMR (400 MHZ, TMS): δ (ppm) 6.86 (1H, d, J=17 Hz,-CO-CH=), 7.18 (1H, d, J=17 Hz, =CH-Ar), 6.65 (1H, s, C-4'-H), 8.56 (2H, d, J=7 Hz, C-2''-H and C-6''-H), 7.09 (2H, d, C-3''-H and C- 4''-H). HRMS (EI): m/e 284.0 $[M+H]^+$, calculated for $C_{12}H_7Cl_2NOS$: 284.0.

1-(2'',5''-dichloro-2''-thiopenyl)-3-(4''-pyridinyl)-2-propen-1-one (15): colorless needles, m.p.141ºC, yield 76%, ATR-FTIR (film) (cm^{-1}): ν_{max} 1658(C=O), 1527(C=C, quadrant of Ar), 1590(C=N), 880(C-Cl), 654(C-S). ^{1}HNMR (400 MHZ, TMS): δ (ppm) 6.76 (1H, d, J=17 Hz,-CO-CH=), 7.11 (1H, d, J=17 Hz, =CH-Ar), 6.98 (1H, s, C-4'-H), 8.14 (2H, d, J=7 Hz, C-2''-H and C-6''-H), 8.35 (2H, d, J=7 Hz, C-3''-H and C-5''-H). HRMS (EI): m/e 284.0 $[M+H]^+$, calculated for $C_{12}H_7Cl_2NOS$: 284.0

1-(2'',5''-dichloro-2''-thiopenyl)-3-(2''-pyridinyl)-2-propen-1-one (16): colorless needles, m.p.127ºC, yield 82%, ATR-FTIR (film) (cm^{-1}): ν_{max} 1666(C=O), 1530(C=C, quadrant of Ar), 1586(C=N), 878(C-Cl), 635(C-S). ^{1}HNMR (400 MHZ, TMS): δ (ppm) 6.58 (1H, d, J=17 Hz, -CO-CH=), 7.12 (1H, d, J=17.2 Hz, =CH-Ar), 7.18 (1H, s, J=7.0 Hz, C-4'-H), 7.78-8.10 (4H, m, C-3'',4'', C-5'' and C-6''-H). .HRMS (EI): m/e 284.0 $[M+H]^+$, calculated for $C_{12}H_7Cl_2NOS$: 284.0.

1-(2'',5''-dichloro-2''-thiopenyl)-3-(2''-pyrrolyl)-2-propen-1-one (17): colorless needles, m.p.105ºC, yield 69%, ATR-FTIR (film) (cm^{-1}): ν_{max} 1658(C=O), 1543(C=C, quadrant of Ar), 1242 (-C=N-), 3342(-NH), 852(C-Cl), 645(C-S). ^{1}HNMR (400 MHZ, TMS): δ (ppm) 21 (1H, d, J= 17 Hz, -CO-CH=), 7.86 (1H, d, J=17 Hz, =CH-Ar), 4.52 (1H, s, -NH), 7.52 (1H, s, J=7.0 Hz, C-4'-H), 7.12-8.15 (3H, Ar-H). HRMS (EI): m/e 272.0 $[M+H]^+$,calculated for $C_{11}H_9Cl_2NOS$: 272.0

1-(2'',5''-dichloro-2''-thiopenyl)-3-(2''-thienyl)-2-propen-1-one (18): colorless needles, m.p.172ºC, yield 91%, ATR-FTIR (film) (cm^{-1}): ν_{max} 1655(C=O), 1582(C=C, quadrant of Ar), 1524(-CH=CH-), 852(C-Cl), 651(C-S). ^{1}HNMR (400 MHZ, TMS): δ (ppm) 7.33 (1H, d, *J* = 17 Hz, -CO-CH=), 7.81 (1H, d, *J* =17 Hz, =CH-Ar), 7.21 (1H, s, J=7.0 Hz, C-4'-H), 6.99-8.29 (3H, Ar-H). HRMS (EI): m/e 289.0 $[M+H]^+$, calculated for $C_{11}H_6Cl_2O_2S_2$: 289.0.

1-(2'',5''-dichloro-2''-thiopenyl)-3-(2''-furyl)-2-propen-1-one (19): colorless needles, m.p.141ºC, yield 66%, ATR-FTIR (film) (cm^{-1}): ν_{max} 1648(C=O), 1578(C=C, quadrant of Ar), 1518(-CH=CH-), 1095(C-O),848 (C-Cl), 640(C-S). ^{1}HNMR (400 MHZ, TMS): δ (ppm) 7.21 (1H, d, *J* = 17 Hz, -CO-CH=), 7.75 (1H, d, *J* =17 Hz, =CH-Ar), 7.33 (1H, s, J=7.0 Hz, C-4'-H), 7.10-7.85 (3H, Ar-H). HRMS (EI): m/e 273.0 $[M+H]^+$, calculated for $C_{11}H_6Cl_2O_2S$: 273.0.

1-(2'',5''-dichloro-2''-thiopenyl)-3-(benzo[*d*][1,3] dioxol-5''-yl)-2-propen-1-one (20): colorless needles, m.p 77ºC, yield 46%, ATR-FTIR (film) (cm^{-1}): ν_{max} 1645(C=O), 1586(C=C, quadrant of Ar), 1520(-CH=CH-), 1084(C-O) ,859(C-Cl), 640(C-S). ^{1}HNMR (400 MHZ, TMS): δ (ppm) 5.86 (2H, s, -O-CH_2-O-), 7.28 (1H, d, *J* = 17 Hz, -CO-CH=), 7.81 (1H, d, *J* =17 Hz, =CH-Ar), 7.18 (1H, d, J=7.0 Hz, C-4'-H), 6.82-8.05 (3H, Ar-H). HRMS (EI): m/e 327.0 $[M+H]^+$, calculated for $C_{14}H_8Cl_2O_3S$: 327.0.

General considerations

All the above chalcone analogues were exhibited characteristic absorption bands in the IR spectra (cm^{-1}) in between 1640-1660 (C=O), 1570-1605 (C=C quadrant of Ar), 1500-1550 (HC=CH) and at other regions of the spectrum depending upon the specific substituents present in each compound. The ^{1}H NMR spectra of the chalcones revealed the characteristic ethylenic protons of the chalcone system in between δ 6.85 and 8.10. The spectra also showed the peaks accounting for the aromatic protons and for the different substituent protons in between the corresponding regions of the spectrum. The ^{13}C NMR spectra of the chalcones exhibited the characteristic peaks of the carbonyl carbon in between δ 185-192, apart from the peaks corresponding to the other carbons. The mass spectra obtained by positive mode ionization method revealed the $[M+H]^+$ ions, whereas the spectra obtained by EI method revealed the molecular ion. The elemental analyses carried out for all the compounds supported the given molecular formulae.

Biological materials

The pure cultures of Fungal Species *Aspergillus niger* (ATCC 6275, An) and *Candida tropicalis* (ATCC 1369, CT) were procured from ATCC. The preliminary antitubercular screening for test compounds was obtained for *M. tuberculosis* H37Rv. The strain is supplied and obtained from Center for Cellular and Molecular Biology (CCMB), Hyderabad, India. All laboratory findings were performed under good laboratory conditions and ICH guidelines. DU-145 (prostate cancer) cell line was obtained from National Centre for Cell Science (NCCS), Pune, India. DMEM (Dulbeccos Modified Eagels Medium), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], Trypsin, EDTA were purchased from Sigma chemicals (St. Louis, MO). Fetal bovine serum (FBS) was purchased from Arrow Labs, 96 well flat bottom tissue culture plates were purchased from Tarson.

Antifungal Activity

Agar diffusion method

In this technique, petri dishes of agar are prepared by pouring melted agar inoculated with microorganisms. Bores are made in the agar plate and antimicrobial substances are placed in the cups. The plates are incubated at a temperature of 37ºC for 24 h. The antimicrobial substance diffuses through the agar around its cup and produces a clear zone of inhibition. The diameter of this zone can be measured and an estimation of the degree of activity of the antimicrobial substance can be obtained.[32,33,34]

Tube dilution method

The dilutions of the antimicrobial agents are prepared in growth medium such that the concentration of drug covers its clinical significant range. An equal volume of broth containing 105-106/ bacteria / mL is added to each tube and to a control tube that contain no antimicrobial agent. The tubes are examined for visible turbidity after overnight incubation. This method is used for determining antimicrobial susceptibility in liquid media. This determines the minimum inhibitory concentration (MIC) of a substance. Responses of an organism to unknown compounds are compared with response to the preparation of known composition and concentration of the standard reference drug. The standard drugs used in the present work for antifungal activity is *fluconazole*.[35,36,37,38,39]

Antitubercular activity

The preliminary antitubercular screening for test compounds was obtained for *M. tuberculosis* H37Rv. The MIC of each drug was determined by broth dilution assay and is defined as the lowest concentration of drug, which inhibits ≤ 99% of bacterial population present at the beginning of the assay. A frozen culture in Middle brook 7H9 broth supplemented with 10% albumin-dextrose-catalase and 0.2% glycerol was thawed and diluted in broth to 105 cfu/mL (colony forming unit/mL) dilutions. Each test compound was dissolved in DMSO and then diluted in broth twice at the desired concentration. The final concentration of DMSO in the assay medium was 1.3%. Each U-tube was then inoculated with 0.05 mL of standardized culture and then incubated at 37 °C for 21 days. 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*.[40,41,42]

MTT Cytotoxicity assay

The cells were seeded in 96 well plates at a density of 1x104 (counted by Tryphan blue exclusion dye method) per well and were incubated for 24 h to recover. After incubation, the medium was replaced with fresh media containing different dilutions of the test compounds. Then the plated were incubated for additional 48 h at 37°C in DMEM/MEM with 10% FBS medium. Following incubation, the medium was removed and replaced with 90 µl of fresh DMEM without FBS. To the above wells, 10 µl of MTT reagent (5 mg/mL of stock solution in DMEM without FBS) was added and incubated at 37ºC for 3-4 h, there after the above media was replaced by adding 200 µl of DMSO to each well (to dissolve the blue formazan crystals) and incubated at 37C for 10 min. The absorbance at 570 nm was measured on a spectrophotometer. *Methotrexate* was used as reference drug for comparison. The assay was performed in triplicate as three independent determinations. The cytotoxicity was expressed as IC_{50} (µg/mL) which is the concentration of the compound that inhibited proliferation rate of the DU-145 prostate cancer cells.[43,44] by 50% as compared to the control untreated cells. IC_{50} values were determined from the percent inhibition versus concentration plot.[45,46]

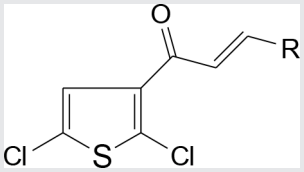
RESULTS

Biological activity

Antifungal activity

In present study, MIC was determined using serial tube dilution technique. In this technique, the tubes of broth medium containing graded doses of compounds were inoculated with the test organisms. After suitable incubation, growth occurred in those tubes where the

Table 2: Antifungal activity of chalcones (1-20).



Compound	R	An	Ct
1	4”-hydroxyphenyl	16	16
2	4”-chlorophenyl	16	16
3	4”-methoxyphenyl	8	16
4	4”-dimethylaminophenyl	8	16
5	2”,4”-dichlorophenyl	8	16
6	9”-anthracenyl	8	16
7	4”-methylphenyl	8	16
8	phenyl	62.5	62.5
9	4”-fluorophenyl	8	62.5
10	3”,4”-dimethoxyphenyl	8	8
11	3”,4”,5”-trimethoxyphenyl	8	4
12	3”-nitrophenyl	16	31.25
13	4”-nitrophenyl	62.5	31.25
14	3”-pyridinyl	16	16
15	4”-pyridinyl	4	4
16	2”-pyridinyl	8	16
17	2”-pyrrolyl	4	8
18	2”-thienyl	8	8
19	2”-furyl	8	62.5
20	3”,4”-methylenedioxyphenyl	4	8
21	Fluconazole	≤1	≤1

An = Aspergillus niger; Ct = Candida tropicalis

concentration of the compound was below the inhibitory level and the culture become turbid. No growth was noticed above the inhibitory level and the tubes remained clear.

A similar experiment was repeated with medium, methanol and inoculum without compound, were also performed to ensure that the methanol has no inhibitory effect in the dilutions used. The test tube number in which the first sign of growth of the organism observed was noted. The MIC was taken as that concentration used in the test tube number just prior to the test tube number labelled where the first sign of growth was observed. This procedure was followed to determine the MIC values for all the compounds of series of new chalcone derivatives with 2,5-dichloro-3-acetylthiopene ring (**1-20**). It is shown in the Table 2. The comparison among all series of compounds has been shown in the bar graph as shown below in Figure 2.

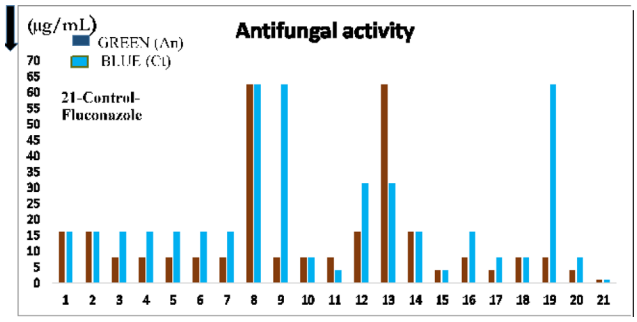


Figure 2: Comparison chart of antifungal activity of new chalcone derivaties with 2,5-dichloro-e-acetylthiopene ring

Antitubercular activity

The preliminary antitubercular screening for all compounds was obtained against species *M. tuberculosis H37Rv*. The MIC of each drug was determined by broth dilution assay and is considered as the lowest concentration of drug, which has inhibited less than

99% of bacterial population present at the beginning of the assay. A frozen culture in Middle brook 7H9 broth supplemented with 10% albumin-dextrose-catalase and 0.2% glycerol was thawed and diluted in broth to 10^5 cfu mL^{-1} (colony forming unit/mL) dilutions. 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**. All new chalcone compounds (**1-20**) were evaluated for antitubercular activity and the results were analyzed as shown in the Figure 3 and detailed values are tabulated in the Table 3.

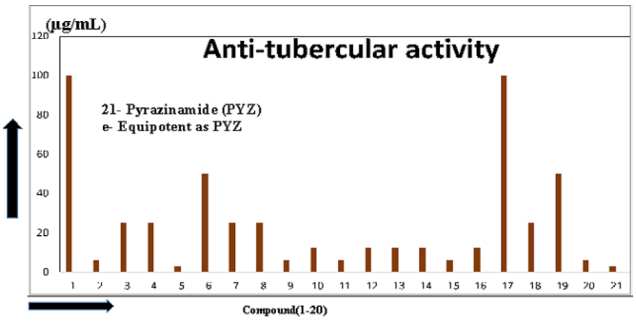
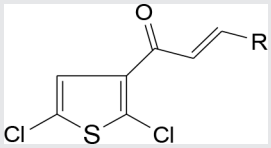


Figure 3: Comparison chart of antitubercular activity of new chalcone derivatives.

Cytotoxicity

After completion of the synthesis, all synthesized compounds (a-t) were evaluated for them *in vitro* cytotoxic activity against DU-145 human prostate cancer cell line. Cell growth inhibition was evaluated by using the standard MTT colorimetric assay after exposure of cells to the test compounds for 72 h and compared against the commercial anticancer agent Methotrexate (MTX) was used as positive control. The results are shown in the bar graph comparison below in Figure 4. All IC_{50} values of new chalcone derivatives (a-t) were listed in the Table 4.

Table 3: Anti-tubercular activity of new chalcones (1-20).



Compound	R	MIC values (µg/mL) of *M. tuberculosis* $H_{37}Rv$
1	4”-hydroxyphenyl	100
2	4”-chlorophenyl	6.25
3	4”-methoxyphenyl	25
4	4”-dimethylaminophenyl	25
5	2”,4”-dichlorophenyl	3.12
6	9”-anthracenyl	50
7	4”-methylphenyl	25
8	phenyl	25
9	4”-fluorophenyl	6.25
10	3”,4”-dimethoxyphenyl	12.5
11	3”,4”,5”-trimethoxyphenyl	6.25
12	3”-nitrophenyl	12.5
13	4”-nitrophenyl	12.5
14	3”-pyridinyl	12.5
15	4”-pyridinyl	6.25
16	2”-pyridinyl	12.5
17	2”-pyrrolyl	100
18	2”-thienyl	25
19	2”-furyl	50
20	3”,4”-methylenedioxyphenyl	6.25
21	Pyrazinamide	3.12

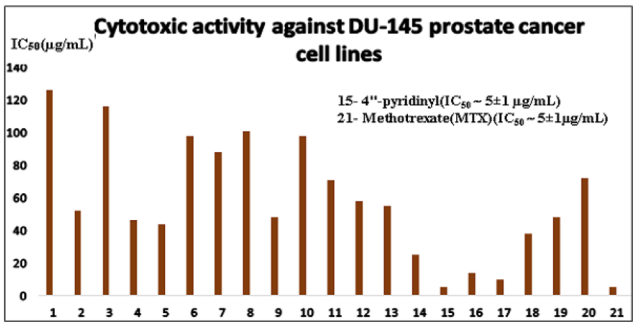
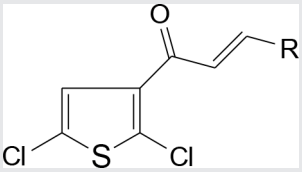


Figure 4: Comparison chart of cytotoxic activity of new chalcone derivaties (1-20).

Table 4: Cytotoxic activity of new chalcones (1-20).



Compound	R	DU-145
1	4”-hydroxyphenyl	126 ± 2
2	4”-chlorophenyl	52 ± 2
3	4”-methoxyphenyl	116 ± 2
4	4”-dimethylaminophenyl	46 ± 2
5	2”,4”-dichlorophenyl	44 ± 2
6	9”-anthracenyl	98 ± 2
7	4”-methylphenyl	88 ± 2
8	phenyl	101 ± 1
9	4”-fluorophenyl	48 ± 2
10	3”,4”-dimethoxyphenyl	98 ± 2
11	3”,4”,5”-trimethoxyphenyl	71 ± 2
12	3”-nitrophenyl	58 ± 2
13	4”-nitrophenyl	55 ± 2
14	3”-pyridinyl	25 ± 2
15	4”-pyridinyl	5 ± 1
16	2”-pyridinyl	14 ± 1
17	2”-pyrrolyl	10 ± 1
18	2”-thienyl	38 ± 2
19	2”-furyl	48 ± 2
20	3”,4”-methylenedioxyphenyl	72 ± 2
21	Methotrexate (MTX)	5 ± 1

Data presented as mean ± SD (n=3). All the compounds and the standard dissolved in DMSO, diluted with culture medium containing 0.1% DMSO. The control cells were treated with culture medium containing 0.1% DMSO.

DISCUSSION

Antifungal activity and SAR

Compounds **11, 15, 17** and **20** have shown proximity in similar inhibitory activity of both fungi species of *Aspergillus niger* (An) and *Candida tropicalis* (Ct) compared to the standard fluconazole. Their structural features have been exploited and analyzed that the aryl or heteroaryl ring substitutions at 2”, 3”, 4” and 5” plays an important role for its activity. Especially, 4”-pyridinyl (**15**), 2”-pyrrolyl (**17**), and 3”,4”-methylenedioxyphenyl (**20**) rings were shown much similar activity for *Aspergillus niger* (An) whereas 3”,4”,5”-trimethoxyphenyl and 4”-pyridinyl were exhibited closer activity compared to fluconazole against *Candida tropicalis* (Ct). Among the above four compounds, compound **15** was being more potent. Nitrogen containing heterocycles have shown promising antifungal activity over the decades and the structural features of compound **(**MIC≤ 4 μg/mL in both fungi) have clearly indicated and exhibited its antifungal activity closely to fluconazole (MIC≤1 μg/mL in both fungi), It is also concluded that both 5-membered and 6-membered heterocycles containing nitrogen have shown promised results above especially **15** and **17** compounds.

Antitubercular activity and SAR

Compounds **2, 5, 9, 11, 15** and **20** have shown good antitubercular activity on species *M. tuberculosis H37Rv* more or less similar to the standard pyrazinamide. Their structural features have been evaluated on the aryl or heteroaryl ring substitutions at 2”, 3”, 4” and 5” plays an important role for its activity. Especially, 2”,4”-dichlorphenyl group (**5**) (MIC ~ 3.12μg /mL) gave promising and similar activity like pyrazinamide (MIC ~ 3.12μg /mL). Whereas 4”-chlorophenyl group (**2**), 4”-fluorophenyl (**9**), 3”, 4”, 5”-trimethoxy phenyl groups (**11**), 4”-pyridinyl ring(**15**) and 3”, 4”-methylenedioxy phenyl ring(**20)** were shown 50% of its activity) (MIC ~ 6.25μg /mL) against standard pyrazinamide (MIC ~ 3.12μg /mL). Among the above six compounds, compound **e** was being more potent. Chloro (-Cl), fluoro (-F), methylene dioxy (O-CH_2-O-), methoxy(-OCH_3) groups with high electronegativity show better antitubercular activity. Among all groups with nitrogen containing heterocycles (**12, 14, 15, 16** and **17**), only compound **15** (4”-pyridinyl group) have shown promising antitubercular activity. It is interpreted that highly electronegative elements (F, Cl, O, N) substituted on phenyl ring offered promising results. Further investigation is needed to ascertain their safety and efficacy.

Cytotoxicity and SAR

As per IC_{50} values of the cytotoxic assay on specific prostate cancer cell line, compounds **15**,**16**, and **17** were sensitive among all synthesized analogues. The highest potency in the culture of DU-145 cells was recorded after treatment with 4”-pyridinyl chalcone analogue (**15**, IC_{50}~5±1 µg/mL), which demonstrated similar potency

upon comparison with the commercial anticancer agent MTX (**21**, IC_{50} ~5±1 µg/mL). A good antiproliferative activity was recorded after treatment of DU-145 cells with compounds 2"-pyridinyl chalcone (**16**, IC_{50} ~14±1 µg/mL) and 2"-pyrrolyl chalocne analogues (**17**, IC_{50} ~10±1 µg/mL). However, the potencies of other analogues (**1-14, 18-20**) were significantly lower than that recorded for **MXT** in the same cell line (IC_{50} >25 µg/mL). All IC_{50} values of new chalcone derivatives (**1-20**) were listed in the Table 4. It is also indicative that among these compounds may show antiproliferative activity in other cell lines and future studies are going on other cell lines with these compounds.

Among the structures of synthesized chalcones mimic with their cytotoxic activities, it has been considered the effect of nitrogen containing heterocyclic moiety (4"-pyridinyl ring) and two halogens present on thiopene ring in a chalcone derivative **15**. The other nitrogen containing heterocyclic moieties (**16**, 2"-pyridinyl moieties; **17**, 2"-pyrrolyl moiety) showed comparable cytotoxic activity, however, it is lower than that of standard MTX and analogue **15**.

CONCLUSIONS

It is concluded that 2,5-dichloro-3-acetylthiophene chalcone derivatives have shown significant antifungal, antitubercular and cytotoxic activities more or less similar to the reference compounds or better than reference compounds. Among these 20 synthesized compounds, compound **15** was predominantly appeared to be promising compound with antifungal, antitubercular and cytotoxic activities better than respective reference compounds. Other compounds like **11**, **16**, **17**, **20** have also shown better and similar activity. These compounds needed to be further studied *in vivo* specifically for its wide biological actions. The role of heterocyclic nucleus present in side chain attached to the chalcone have shown better structure-activity relationship among benzene and its derivatives. Future considerations are considered very specific to the particular novel chalcone derivative for a selective biological action.

ACKNOWLEDGEMENT

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Supplementary data

All data related to the above work and its results of: ATR-FTIR- Spectra, Elemental analysis data, Chemical structure determination, SAR analysis, MTT assay results and copies of ^{1}HNMR and ^{13}C NMR spectra of all compounds are supplied separately in supplementary data.

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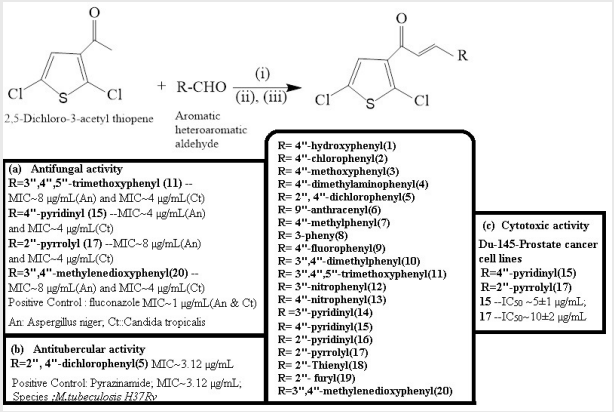
PICTORIAL ABSTRACT



SUMMARY

- The novel 2,5-dichloro-3-acetyl-thiopene chalcone derivatives have been synthesized by Claisen-Schmidt condensation. With the help of physicochemical, spectroscopic techniques like IR, 1HNMR, 13CNMR, MS various compounds have been proposed. Among all, some compounds showed better antifungal, antitubecular and cytotoxicity activities than respective controls. After all these observations and data analysis, it is concluded that some heterocyclic and phenylic chalcone derivatives have shown significant antifungal, antitubercular and cytotoxicity activities. Those active compounds are progressed to further study preclinical and biological studies.

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