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DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL CHALCONES AND 2-AMINO-3-CYANOPYRIDINE AND 2-AMINO-3-CYANOPYRAN DERIVATIVES

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ABSTRACT

Heterocyclic compounds are a significant group of therapeutic agents owning a diverse range of biological activities. One of the important ways of obtaining these heterocyclic compounds is through sound methods of organic synthesis. This proved to be the most efficient approach in the discovery of new drugs over the years and contributed greatly to the new therapeutic agents currently available in the market. A number of synthons can be used to prepare a wide variety of starting compounds which can be cyclised to novel 2-Amino-3-pyridinecarbonitrile and 2-Amino3-pyrancarbonitrile compounds in good yield. One such group is the bio chromophoric molecules joined by a three-carbon open chain linkage called the "Chalcones". In the present study, an attempt is made to synthesize some new substituted pyridines and pyrans from chalcones which in turn are obtained by Clasein-Schmidt condensation of a variety of aryl/ hetero arylketones and aldehydes

INTRODUCTION

Chalcones are well-known synthons for the synthesis of many new heterocyclic compounds with good pharmacological profile.They are one of-a-kind templates with several biological functions. They are secondary metabolites of terrestrial plants that act as precursors to the formation of flavonoids. Chalcones are α, β-unsaturated ketones with a reactive ketoethylenic group. One or more fluorine atoms can be found in a variety of medications used to treat various conditions including antibacterial, antifungal, antiviral, antineoplastic agents, steroids, selective COX-II inhibitors, antiulcer, antihyperlipidemic, and antischizophrenic agents. An introduction of fluorine into the benzenoid part in α, βunsaturated ketones enhances their biological activity. Clinical investigations have revealed that these medications achieve acceptable plasma concentrations and arewell tolerated. As a result, they are a subject of ever hire interest among scientists. However, much of the pharmacological potential of chalcones is still not utilized. Chalcones with a unique, αβ-unsaturated carbonyl bridge connected on either side by two aromatic or heteroaromatic rings occur frequently in nature, attracting the interest of chemists to the synthesis of a variety of such modified chalcones. These natural chalcones have been reported to have a variety of pharmacological functions. Chalcone synthase is a key enzyme found in many lower plants that is involved in the biosynthesis of chalcones, which are the precursors for a variety of structurally distinct flavonoids, stilbenes, and aurones [1,2]. A thioester linked tetra peptide was recently cyclised by a regiospecific Claisen-type reaction to yield the naringenin system [3, 4,5]. 6′-deoxy naringenin chalcone was obtained from naringenin chalcone in the presence of chalcone reductase and chalcone synthase.[6] The chalcone scaffolds are often found in naturally occurring molecules with antineoplastic and antimicrobial activities. [7,8,9,10] More than 92 thousand chalcones have been isolated from plants so far. These chemicals have been found to be beneficial in the treatment of a variety of diseases and disorders, including cancer, diabetes, HIV, tuberculosis, and malaria. [11,12,13,14,15]

Cyanopyridines: Pyridine is a heterocyclic nucleus composed of one nitrogen and five carbon atoms. N appears in the first and second positions. It is a structure with a planar. The Pyridine ring is found in several natural substances including nicotine and nucleic acid. Because it is a polar and ionisable aromatic derivative, it modifies the pharmacokinetic properties of lead molecules and can thus be used as a treatment to enhance solubility and bioavailability parameters in order to improve the solubility of lead molecules by inserting fluorine element in it. Pyridine derivatives occupy a unique position in the realm of medicinal chemistry. The incorporation of the Pyridine ring is a critical synthetic strategy in drug discovery. Because of the remarkable therapeutic significance of Pyridine-related medicines, medicinal chemists have been inspired to prepare a greater number of newer chemotherapy agents. Substituted Pyridine medications have a wide range of significance in clinical drugs for treating numerous conditions. Diverse methods for the preparation of substituted pyridine, as well as their different structural combinations are available.

4H-pyrans: Because of their significance, the preparation of novel heterocyclic compounds has long been a contentious topic. Heterocyclic compounds originate in nature and are necessary for humans. The significance of organic reactions has been recognized, and significant impact has been made to synthesize and create a unique 4H-Pyrans library. The pharmacological actions of 4H-pyrans and their analogues such as spasmolytic, diuretic, anticoagulant, antineoplastic, and antihypersensitive activity are of great interest. Furthermore, newer chalcones can be used as intermediates in the production of newer 4H-Pyrans derivatives. In contrast, 4H-pyrans serve as the foundation for a wide range of natural products. A number of 2-amino-4H-pyrans are employed as photoactive materials, pigments, and possible eco-agrochemicals and accordingly, several schemes have been revealed for the preparation of these compounds.

The aims and objectives of the present study are:

1. To synthesize and characterize some novel fluorinated chalcones from4trifluoromethyacetophenone by reaction with various aryl and heteroaryl carboxaldehydes (Scheme-1).

2. To synthesize and purify the 2-amino-3-cyano pyridines and their derivatives obtained from chalcones (Scheme -2).

3. To synthesize and purify the 2-amino-3-cyano pyrans and their derivatives obtained from chalcones (Scheme -3).

4. To screen all the synthesized novel chalcones, 2-amino-3-cyano pyridines and2amino-3-cyano pyran derivatives for their antimicrobial and anticancer (lung A549, breast MCF-7 and cytotoxicity HEK 293 cell line) activities.

5. To identify the active compounds for further exploitation.

EXPERIMENTAL PART

Chemical protocol

Chemicals: 4’-Acetylbenzotrifluoride,Pyrrole-

3-carbox aldehyde, Pyridine-3 carboxaldehyde, 2-hydroxybenzcarboxaldehyde,3-hydroxy benzcarboxaldehyde,4hydroxy benzcarbox aldehyde,3-methoxy benzcaroxaldehyde,2-methoxy benzcarboxaldehyde,2,4-dimethyl benzcarboxaldehyde, 3-Fluoro benzcarbox aldehyde, 3-Bromo benzcarboxaldehyde, Ammonium acetate, Pyridine, Malononitrile, NaOH, H_2SO_4, HCl, Column Silica gel and Iodine vapors

Reagents: Sulphuric acid spray reagent (10% H_2SO_4/Methanol)

Solvents: Ethyl Acetate, Hexane, Methanol, Ethanol, DMF, trichloromethane, DMSO

Apparatus / Instruments: Round bottomed flask, Reflux condenser, hot water bath, conical flasks, beakers, test tubes, thermometer, glass rods, hot air oven, refrigerator, TLC plates, capillary tubes and melting point apparatus.

Analytical protocol: The M.P is uncorrected using open capillaries Method (EZmelt, Stanford Research Systems) for synthesised compounds. The Silica gel-G of TLC was used to predict the compound purity with appropriate solvent systems. BRUKER FTIR with KBr disc was used and the numericals are given in cm^{-1}. BRUKER with NMR spectrophotometer of 400 MHz was used, numericals of chemical shifts are in form of delta ppm, using TMS as an internal standard for both ^1H NMR and ^{13}C NMR SHIMADZU ESI-MS with Positive electrospray ionisation method was used and the values are expressed in m/z. Thermo Finnigan flash (EA 1112 CHNS Analyzer) elemental analyser was used for elemental analyses.

LIST OF SYNTHESISED COMPOUNDS:

The synthesised compound of 1-(4-(trifluoromethyl) phenyl)-3-(1H-pyrrol-3-yl) prop2-en-1-one (C1)

The synthesised compound of 1-(4-(trifluoromethyl) phenyl)-3-(pyridin-3-yl) prop-2 en-1-one (C2)

The synthesised compound of 1-(4-(trifluoromethyl) phenyl)-3-(2 hydroxyphenyl) prop-2-en-1-one (C3)

The synthesised compound of 1-(4-(trifluoromethyl) phenyl)-3-(3 hydroxyphenyl) prop-2-en-1-one (C4) The synthesised compound of 1-(4-(trifluoromethyl) phenyl)-3-(4 hydroxyphenyl) prop-2-en-1-one (C5)

The synthesised compound of 1-(4-(trifluoromethyl) phenyl)-3-(3 methoxyphenyl) prop-2-en-1-one (C6) The synthesised compound of 1-(4-(trifluoromethyl) phenyl)-3-(2 methoxyphenyl) prop-2-en-1-one (C7)

The synthesised compound of 1-(4-(trifluoromethyl) phenyl)-3-(2,4 dimethyl phenyl) prop-2-en-1-one (C8) The synthesised compound of 1-(4-(trifluoromethyl) phenyl)-3-(3-fluorophenyl) prop2-en-1-one (C9)

The synthesised compound of 1-(4-(trifluoromethyl) phenyl)-3-(3-bromophenyl) prop2-en-1-one (C10) The synthesised compound of 2-amino-6-(4-(trifluoromethyl) phenyl)-4-(1H-pyrrol3yl) pyridine-3-carbonitrile (N1) The synthesised compound of 2-amino-6-(4-(trifluoromethyl) phenyl)-4-(pyridine 3 yl) pyridine-3-carbonitrile (N2)

The synthesised compound of 2-amino-6-(4-(trifluoromethyl) phenyl)-4- (2 hydroxyphenyl) pyridine-3-carbonitrile (N3) The synthesised compound of 2-amino-6-(4-(trifluoromethyl) phenyl)-4- (3 hydroxyphenyl) pyridine-3-carbonitrile (N4) The synthesised compound of 2-amino-6-(4-(trifluoromethyl) phenyl)-4- (4 hydroxyphenyl) pyridine-3-carbonitrile (N5)

The synthesised compound of 2-amino-6-(4-(trifluoromethyl) phenyl)-4- (3 methoxyphenyl) pyridine-3-carbonitrile (N6)

The synthesised compound of 2-amino-6-(4-(trifluoromethyl) phenyl)-4- (2 methoxyphenyl) pyridine-3-carbonitrile (N7)

The synthesised compound of 2-amino-6-(4-(trifluoromethyl) phenyl)-4- (2,4dimethyl phenyl) pyridine-3-carbonitrile (N8) The synthesised compound of 2-amino-6-(4-(trifluoromethyl) phenyl)-4- (3 fluorophenyl) pyridine-3-carbonitrile (N9) The synthesised compound of 2-amino-4-(3-bromophenyl)-6-(4- (trifluoromethyl)phenyl) pyridine-3-carbonitrile (N10) The synthesised compound of 2-amino-6-(4-(trifluoromethyl) phenyl)-4-

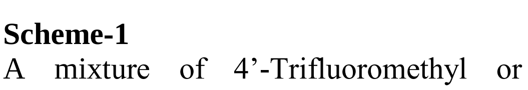
(1H-pyrrol 3yl) pyran-3-carbonitrile (O1)
The synthesised compound of 2-amino-6-(4-(trifluoromethyl) phenyl)-4-(pyridin-3-yl) pyran -3-carbonitrile (O2) The synthesised compound of 2-amino-6-(4-(trifluoromethyl) phenyl)-4- (2 hydroxyphenyl) pyran-3-carbonitrile (O3) The synthesised compound of 2-amino-6-(4-(trifluoromethyl) phenyl)-4- (3 hydroxyphenyl) pyran-3-carbonitrile (O4)
The synthesised compound of 2-amino-6-(4-(trifluoromethyl) phenyl)-4- (4 hydroxyphenyl) pyran-3-carbonitrile (O5) The synthesised compound of 2-amino-6-(4-(trifluoromethyl) phenyl)-4- (3 methoxyphenyl) pyran-3-carbonitrile (O6) The synthesised compound of 2-amino-6-(4-(trifluoromethyl) phenyl)-4- (2 methoxyphenyl) pyran-3-carbonitrile (O7)
The synthesised compound of 2-amino-6-(4-(trifluoromethyl) phenyl)-4- (2,4 dimethyl phenyl) pyran-3-carbonitrile (O8) The synthesised compound of 2-amino-6-(4-(trifluoromethyl) phenyl)-4- (3 fluorophenyl) pyran-3-carbonitrile (O9) The synthesised compound of 2-amino-4-(3-bromophenyl)-6-(4(trifluoromethyl)phenyl) pyran-3-carbonitrile (O10)

1 SYNTHESIS AND CHARACTERIZATION OF CHALCONES (C1-C10)

Scheme-1

Synthesis of 1-(4-(trifluoromethyl) phenyl)-3-(3-methoxyphenyl) prop-2-en-1-one (C6)

Claisen-Schmidt condensation involves the reaction between ketone (K) and an aryl carboxaldehyde (1-10) in appropriate solvent to form the chalcone [16].



Scheme-1

A mixture of 4’-Trifluoromethyl or 4’-Trifluoromethoxy ketones (0.001 mole) and the appropriate aryl or heteroaryl aldehyde (0.001 mole) was dissolved in ethanol (7.5 mL) under continuous stirring.Meanwhile, freshly prepared aqueous solution of sodium hydroxide from volumetric flask (40 %, 7.5 mL) was added drop wise into reaction flask. The reaction flask was kept aside overnight.Then, neutralized with dilute hydrochloric acid and immediately precipitate was formed which is filtered under vacuum. Crystals of chalcones were obtained. In case it is impure; it was purified by column chromatography or recrystallized with an appropriate solvent system (hexane and ethyl acetate (1:15)). Compound **C6,** analyzed for $C_{17}H_{13}F_3O_2$, m.p. 145.26^{o}C, exhibited a $[M+H]^+$ at 307.200 m/z. The FTIR (cm^{-1}) spectrum of **C6** revealed the absorption bands at 1650 (C=O), 1628 (C=C Ar), 1512 (CH=CH), 1298 (-CF_3), 1037 (-O-CH3) and 3013 (Ar C-H) cm^{-1}. The ^{1}H NMR spectrum (400 MHz, CDCl3) of compound **C6** revealed the signals at δ 7.47 ppm of CO-CH=, δ: 7.74 ppm of =CH-Ar as doublets (*J* =17 H_Z) ensuring that molecules geometry as trans in ethylenic double bond and δ 3.83 as methoxy group at third position on B ring. Rest of eight protons lie in between 8.14 -6.99 ppm ensuring that aromatic protons of methoxy benzene ring and phenyl rings **C6**. The ^{13}C NMR (δ ppm) spectrum of **C6** compound disclosed the signals at 189.75, 121.88 and 137.34 corresponding to the three carbons of the propenone moiety. The other signals at 189.75 (C-1), 121.88 (C-2), 137.34 (C-3), 138.61 (C-1'), 130.74 (C2'), 130.13(C-6'), 120 (C-3' and 5'), 137.30 (C-4'), 125.00 (C-4,'-), 137.88 (C-1"), 114 (C-2" and C-4"), 58.22 (C-4"-), 158 (C-3"), 125 (C-5") and 117.18 (C-6") are consistent with the proposed structure.

The elemental analysis results were consistent with the calculated values. By adopting the above synthetic procedure, other chalcones were obtained from 4 trifluoromethyl acetophenone by reaction with different aldehydes **(C1-C10)**.

Section-2

SYNTHESIS AND CHARACTERIZATION OF 2-AMINO-3-CYANO PYRIDINES (N1-N10)

Scheme-2

Synthesis of 2-amino-4-(1H-indol-3-yl)-6-[4-

(trifluoromethyl) phenyl] 4H-Pyridine-3-carbonitrile (N6):

Cyclocondensation and elimination reaction involving the reaction between 1-(4(trifluoromethyl) phenyl)-3-(3-methoxyphenyl) prop-2-en-1-one (N6) and malononitrile in appropriate solvent resulted in the formation of 2-amino-3-cyanopyridine.

General Procedure:

0.01 mole of 1-(4-(trifluoromethyl) phenyl)-3-(3-methoxyphenyl) prop-2-en-1-one and 0.01mole of malononitrile were dissolved in ethanol and to this a 0.08 mole of ammonium acetate was added. The mixture was refluxed for a period of 8-15 hrs. The reaction mixture was neutralised. The contents were slowly poured into crushed ice. The product was filtered, rinsed with water and then dried; followed by recrystallisation from DMF.

The compound N6 was analysed for a molecular formula C20H14F3N3O, m.p. 234.73 ^{0}C, well supported by its mass spectra ESI [M+H] at 372.65 m/z. The FT-IR spectrum (cm) of the compound N6 shown the characteristic bands at 3338 (-N-H), 2225 (-CN), 1598 (-C=N), 1325(-C-N-), 1628 (C=C Ar), 1298 (-CF3), 1037 (O-CH3) and 3013 (Ar C-H).1 The H NMR spectrum of N6 Showed two characteristic peaks at 7.66 (1H, s,Pyridine-5H) and 6.80 (2H, s, -NH$_2$) protons of 2-amino-3-cyano pyridine ring and the remaining aromatic proton at 8.10 – 7.32 (4H, Ar-H), 7.35 – 6.92 (4H, Ar-H$_b$) accounting for the eight aromatic protons, apart from methoxy proton at 3.86 (3H, s, Ar-OCH3). The ^{13}C NMR spectrum of compound N6 compounds displayed the carbons signals as follows at δ values: 156.28 (C-6), 111.35 (C-5), 157.21 (C-4), 85.28 (C3), 117.18 (C-31), 169.20 (C-2),137.33 (C-1’’), 114.41 (C-2", 4"), 158.08 (C-3"), 125.36(C-5"), 117.18 (C-5"), 58.21 (C-3"-1,),130 (C-2' and 6'), 120 (C-3' and 5') 138.60 (C-3'), 137.33 (C-4') and 123.73 (C-4'-). The elemental analysis data agreed with calculated data for N6. By adopting the above scheme, 2 amino pyridine 3 carbonitriles were obtained from novel chalcones of 4'-trifluoromethyl acetophenone. (section-I)

Section-3

SYNTHESISANDCHARACTERIZATION OF 2-AMINO-3-CYANOPYRANS (O1-O10)

Scheme-3

Synthesis of 2-amino-4-(1H-indol-3-yl)-6-[4-(trifluoromethyl) phenyl]4H- pyran-3-carbonitrile (O6): Michael addition involving the reaction between 1-(4-(trifluoromethyl) phenyl)-3(3-methoxyphenyl) prop-2-en-1-one and malononitrile in appropriate solvent resulted in the formation of 2-amino-3-cyano-4H-pyran.

Procedure: 0.01 mole of 1-(4-(trifluoromethyl) phenyl)-3-(3 methoxyphenyl) prop-2-en-1-one (**C6)** and 0.01mole of malononitrile were dissolved in ethanol and to this catalytic amount of pyridine was added. The mixture refluxed for 10-15 hrs. The reaction mixture was neutralised. The contents were slowly poured into crushed ice. The precipitate was filtered, rinsed with water and then dried followed by recrystallisation from DMF.

The compound O6 was analysed for a molecular formula $C_{20}H_{15}F_3N_2O_2$, m.p. 340° C, well supported by its mass spectra ESI of $[M+H]^+$ at 373.68 m/z. The FT-IR spectrum (cm^{-1}) of the compound O6 shown the characteristic bands at 3356 (-N-H), 2232 (-CN),1135 (Pyran -C-O-C-), 1323 (-C-N-), 1628 (C=C Ar), 1298 (-CF3), 1037 (-O-CH3) and 3013 (Ar C-H) cm^{-1}. The ^{1}H NMR spectrum of O6 showed three characteristic peaks at 4.99 (1H d, J = 5.6 Hz, Pyran-5H), 3.94 (1H d, J = 5.6 Hz, Pyran-4H) and 6.74

(2H, s, -NH2) of pyran ring and the remaining aromatic protons at 7.94 – 7.27 (4H, Ar-H), 7.15 – 6.74 (4H, Ar-Hb) the spectrum accounting for the eight aromatic protons, apart from methoxy proton at 3.79 of 3-methoxy benzene ring. The^{13}C NMR spectrum of compound **O6** displayed all the carbon signals at δ values: 143.73 (C-6), 91.74 (C-5), 30..85 (C-4), 68.25 (C-3), 117.41 (C-3^{1}), 159.21 (C-2), 137 (C-1''), 113 (C-2'', 4''), 160.08 (C-3''), 57.22 (C-3'''), 125.33 (C-5''), 121.19 (C-6''), 138.61 (C-1'), 130.77 (C-2' and C-4'), 120.47 (C-3' and C-5') 137.37 (C-4') and 123.63 (C-4'') The elemental analysis data agreed with calculated data. By adopting the above scheme, 2- amino pyran- 3- carbonitriles were obtained from novel chalcones (section-I)

In vitro Anti-Cancer activity

I EXPERIMENTAL PROTOCOL [17]:

There are numerous *in-vitro* assays to measure cell viability and proliferation. The 3(4,5-dimethylthiazol- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reagent passed into cells of mitochondria where it is reduced to succinate dehydrogenase which is measured at 570 nm. The anti-cancer activity in Lung cancer cell lines (A549), breast cancer cell lines (MCF 7) and Cytotoxicity (HEK 293) was measured by MTT Assay.MTT Assay is a colorimetric assay used to estimate the reduction of yellow MTT by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, dark purple colored formazan crystal. The cells are then solubilized in DMSO and solubilized formazan reagent is measured spectrophotometrically at 570 nm.

MATERIALS AND METHODS: DMEM (Dulbecco's modified Eagles medium), MTT [3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide], trypsin, EDTA Phosphate Buffered Saline (PBS) were purchased from Sigma Chemicals Co. (St. Louis, MO) and Fetal Bovine Serum (FBS) was purchased from Gibco. 25 cm ^{2} and 75 cm^{2} flask and 96 well plates purchased from Eppendorf India.

Maintenance of Cell lines: The Cancer cell lines were purchased from NCCS, Pune and the cells were maintained in DMEM supplemented with 10 % FBS and the antibiotics penicillin/streptomycin (0.5 mL^{-1}) in an atmosphere of 5% CO $_{2}$/ 95% air at 37 ^{0}C.

Test Compound Preparation:

For MTT assay, Each Test compound was weighed separately and dissolved in DMSO. With media, made up final concentration to1 mg/ ml and the cells were treated with a series of concentrations from 10 to 100 µg/ ml.

MTT ASSAY PROCEDURE:

Cell viability was evaluated by the MTT Assay with three independent experiments with six concentrations of compounds in triplicates and MIC values were given in below table **13.** Cells were trypsinized and performed the Trypan blue assay to know viable cells in cell suspension. Cells were counted by haemocytometer and seeded at density of 5.0 X 10 ^{3} cells / well in 100 µl media in 96 well plate culture medium and incubated overnight at 37^{0} C. After incubation, take off the old media and add fresh media 100 µl with different concentrations of test compound in represented wells in 96 plates. After 48 hrs, Discard the drug solution and add the fresh media with MTT solution (0.5 mg / mL$^{-1)}$ to each well and plates were incubated at 37^{0}C for 3 hrs. At the end of incubation time, precipitates are formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 570 nm on a microplate reader.

The percentage growth inhibition was calculated using the following formula.

$$\% \text{ Inhibition} = \frac{100(\text{Control-treatment})}{\text{Control}}$$

The IC$_{50}$ value was determined by using linear regression equation i.e. $y = mx+c$. Where, $y = 50$, m and c values were derived from the viability graph. The MTT reagent enters the cells, penetrates into mitochondria contained SDH where it is reduced to insoluble purple colored formazan crystals. This reduction function cannot be seen in dead cells. The cells are then solubilized in DMSO and the released, solubilized formazan reagent in microplate reader used to evaluate spectrophotometrically at 570 nm, whereas optical density (OD) values indirectly reflected the number of cells that survived. A good activity is indicated when OD value is greater, the more living cells present in the microplate surface well. Test compounds C110, N1-10, O1-10 treated against MCF 7, A549 and HEK 293 celllines.The compounds were tested against all the three cell lines i.e., HEK 293, A549 and MCF-7 at different concentrations. The growth inhibition of test compounds on cell lines was concentration dependent and is displayed in below tables 01&02. All the 30 compounds were evaluated for their anticancer potency against the A549 and MCF-7, employing colorimetric tetrazolium dye (MTT) assay. In the chalcone monosubstituted series C1-7, 9-10; O1-7, 9-10 for anticancer activity; among the disubstituted series, C8 produced the best activity IC$_{50}$ 72.70±4.16 in MCF7 but lower than the chalcones series. The compounds having an electron withdrawing (-F, -Br) and -donating groups (-OH, -OCH3, -CH3) at position 2, 3, and 4 (compound 10) resulted among chalcone series possessed somewhat better activity. This suggests that electron-withdrawing and -donating groups on the aromatic ring were required for improving the potency among the chalcone series. In the caseof the pyridine series, same phenomena can be seen. In the monosubstituted series N1-7, 910, substituting position 2, 3, or 4 yielded improvement in the activity better than the chalcones series. In the disubstituted series, compound N8 having electron-donating substitution at 2 and 4 was found to be potent pyridine, was equipotent to the standard Cisplatin at IC50 16.12±0.19 µg/mL in MCF-7 cell lines. Among Compounds N3, N4, and N6; the most potency of pyridine is at IC50 13.27±5.21µg/mL, 09.21±2.07 and 13.32±4.90 respectively. In A549 cell lines, compound N4 has good potency at IC$_{50}$ value 10.25±3.45; superior activity than standard 14.38±0.37. Similar phenomena can be seen among the pyrans series. In the monosubstituted series O1-7, 9-10, substituting position 2, 3, or 4 did not yield any improvement in the activity. In the disubstituted series, O8 compound having electron-donating substitution at 2 and 4 was found to be the most potent, with IC$_{50}$12.36±3.55µg/mL. Compound O4 was equipotent to the standard Cisplatin in MCF-7 cell lines. It can be seen that the pyridine derivatives exhibited superior activity than the pyrans and chalcones. Compounds exhibited better affinity towards MCF-7 but lower affinity towards A549 cell lines. The SAR features indicated that heterocyclic pyridine ring was more essential than the propenone and pyrans motif found in chalcones. The compounds were also tested against the normal cells HEK 293 and were found to be nontoxic to these cells.

Table 01: Cytotoxic activity (MIC of % Viability IC$_{50}$ (µg)) * of Chalcones (C1-10), Pyridines (N1-10), Pyrans (O1-10)

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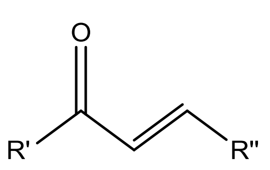
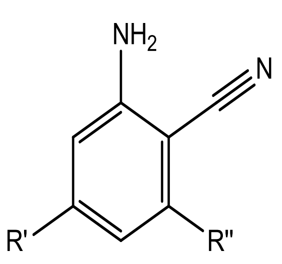
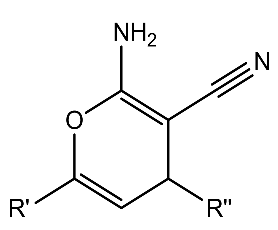
<tr><td colspan="2"></td><td colspan="2"></td><td colspan="3"></td></tr>
<tr><td>R’’</td><td>Compound</td><td></td><td>Compound code</td><td></td><td>Compound code</td><td></td></tr>
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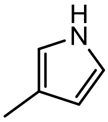
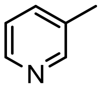
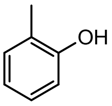
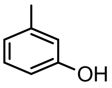
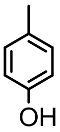
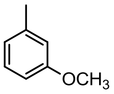
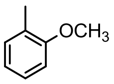
	**C1**	701.10±1.02	**N1**	750.46±1.52	**O1**	770.34±1.27
	**C2**	1123.01±2.85	**N2**	1152.71±1.24	**O2**	1105.01±2.08
	**C3**	628.23±2.23	**N3**	552.03±3.03	**O3**	721.73±1.21
	**C4**	2251.21±2.29	**N4**	2297.25±2.23	**O4**	3218.21±2.20
	**C5**	500.25±1.62	**N5**	667.21±3.21	**O5**	521.84±1.91
	**C6**	1521.07±5.31	**N6**	1240.22±4.37	**O6**	1131.52±3.55
	**C7**	1248.51±3.11	**N7**	1125.25±3.20	**O7**	1247.76±3.73

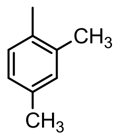
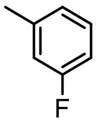
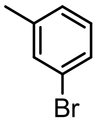
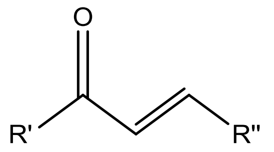
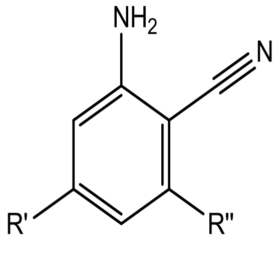
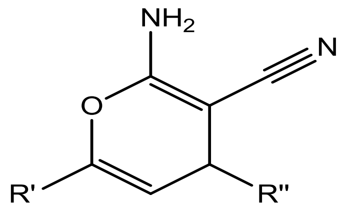
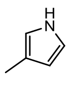
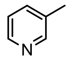
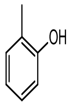
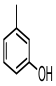
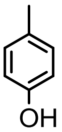
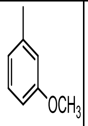
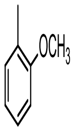
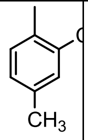
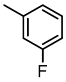
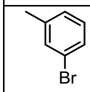
	**C8**	1230.71±3.10	**N8**	1430.15±1.82	**O8**	1317.20±5.01
	**C9**	1512.28±3.10	**N9**	1721.61±2.82	**O9**	1400.20±6.62
	**C10**	1350.71±3.10	**N10**	1521.20±1.32	**O10**	1317.25±3.11

Table 02 Anticancer activity (MIC of % Viability IC_{50} (µg/mL) * of Chalcones (C1-10), Pyridines (N1-10), Pyrans (O1-10)

									
R''	**Compound code**	**A549**	**MCF 7**	**Compound code**	**A549**	**MCF 7**	**Compound code**	**A549**	**MCF 7**
	**C1**	101.03±5.26	**86.27** ±4.32	**N1**	48.25 ±7.62	**53.00** ±7.19	**O1**	62.28±8.2 1	72.41±5.64
	**C2**	141.21±4.13	**151.23**	**N2**	36.21	**25.21**	**O2**		

			±4.16		±5.28	±4.10		13.47±3.2 6	52.12±3.54
	**C3**	153.2±7.16	**130.21** ±1.26	**N3**	22.52 ±2.69	13.27 ±5.21	**O3**	25.21±4.3 6	38.71±5.41
	**C4**	150.72±1.57	**128.03** ±4.46	**N4**	10.25 ±3.45	09.21 ±2.07	**O4**	28.69±3.14	16.27±3.8
	**C5**	147.24±6.35	**121.21** ±5.72	**N5**	20.21 ±4.14	26.31 ±7.30	**O5**	51.21±7.1 4	65.14±5.76
	**C6**	126.25±5.32	**101.54 ±7.32**	**N6**	20.32±3 .16	13.32± 4.90	**O6**	11.01±3.25	23.65±310
	**C7**	129.51±9.58	**101.2± 7.34**	**N7**	28.13±6 .54	21.80	**O7**	58.57±4.63	72.54±451
	**C8**	131.58±7.21	**72.70± 4.16**	**N8**	30.25±7 .67	16.21± 3.38	**O8**	12.36±3.55	30.14±5.7
	**C9**	121.42±5.32	**108.26**	**N9**	54.25±8 .05	58.35± 5.01	**O9**	69.47±5.85	71.21±2.83
	**C10**	128.15±6.37	**112.62 ±1.69**	**N10**	52.69±5 .21	60.11± 4.00	**O10**	68.51±7.52	84.73±8.57
Cisplatin		14.38±0.37	**16.12±** 0.19						

ANTIMICROBIAL ACTIVITY

Development of novel antimicrobial agents is always a topic of interest to medicinal chemists. This is due to the limitations with the existing drug molecules with antimicrobial resistance (AMR), narrow antimicrobial spectrum of activity, unwanted side effects and longer periods of treatment. As there is a rich scope for the design and development of novel antimicrobial drugs, in the present study we have tested the synthesized compounds against selected bacterial and fungal strains.

Materials and methods: The antimicrobial activity was performed against six different antimicrobial strains. The organisms selected are listed below in Table 03

Experimental Protocol [18]: Antimicrobial screening can be performed by either cup plate method or by the serial tube dilution method. However, in the present study, cup plate method [59, 393-395] was employed for the antimicrobial evaluation. The composition of the nutrient media for both the activities is summarized in the Tables 04 and05 below.

All the synthesized compounds were tested for their antimicrobial activity at 0.1% concentration. Benzyl penicillin was used as a standard for the antibacterial activity, whereas fluconazole was used for antifungal testing.

Firstly, the glassware was cleaned and kept in a hot air oven at 160 °C for 2 h. The media was sterilized and the solutions of standard drugs and all the test compounds were kept ready. In the meantime, nutrient agar medium was prepared. The weighed quantities of peptone, meat extract, and sodium chloride were dissolved in 1 L of distilled water and the pH of the medium was adjusted to 7.2. After the dissolution of agar, the medium was distributed into conical flasks each containing 25 mL. The media and sterile water were sterilized by autoclaving at 121 °C temperature and 15 lbs/sq. inch pressure for 20 min. Petri plates, test tubes, pipettes, and borer required for experiment were sterilized by dry heat sterilization using hot air oven. Cultures of respective organisms (18 h old) were taken and suspension of these microorganisms was made using sterile water. Later, 0.5 mL of this suspension was used as inoculum and pour plate technique was used for estimation of bacterial load in each sample. The inoculated agar medium was poured into sterile 10 cm-diameter petri dishes and the medium in the plates was allowed to solidify. The solutions of the test compounds in concentrations of 0.1 µg/mL were prepared in DMSO. The cups of 5 mm diameter were prepared using a borer in the corresponding medium. In each plate, 5 wells were prepared. Three wells were for test compounds, one for standard compound and another one was used as control. In each well, samples were poured and then plates were left for 45 min in a refrigerator for diffusion. After incubation for 18 h at 37 °C, the plates were examined for inhibition zones. The experiments were done in triplicate on the same day with the same conditions in order to minimize the experimental errors. The zone of inhibition values was calculated using vernier caliper and represented as a mean of three values and standard deviation was applied. The results of the antimicrobial screening data are summarized in Tables 06 &07.

Table 03: List of bacterial and fungal strains employed in the present study

Gram-positive bacteria	*Bacillus subtilis* (NCIM-2079), *Staphylococcus aureus* (NCIM2079)
Gram-negative bacteria	*Escherichia coli* (NCIM-2065) ,*Proteus vulgaris* (NCIM-2027)
Fungi	*Candida albicans* (MDCC-227), *Aspergillus niger* (MTCC 5889)

Table 04: Composition of the nutrient media for antibacterial activity

S.No	Composition	Quantities
1	Beef extract	0.15 g
2	Sodium chloride	0.4 g
3	Peptone	0.25 g
4	Agar	0.75 g
5	pH	7.2-7.4
6	Water	50 ml

Table 05: Composition of the nutrient media for antifungal activity

S.No	Composition	Quantities
1	Potato starch	0.2 g
2	Dextrose	1 g
3	Agar	0.75 g
4	pH	5.0±0.2
5	Distilled water	50 ml

Table 06 Antibacterial activity (MIC in µg/ml) * of Chalcones (C1-10), Pyridines (N1-10), Pyrans (O1-10)

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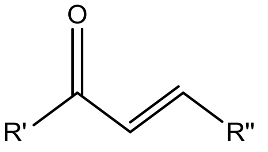
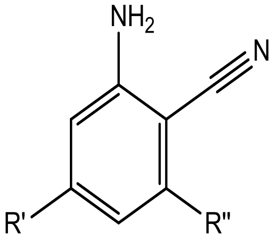
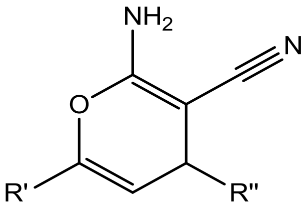
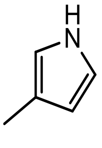
<tr><td colspan="6"> </td><td colspan="5"> </td><td colspan="5"> </td></tr>
<tr><td>R''</td><td>Compound code</td><td><i>S. aureus</i></td><td><i>B. subtilis</i></td><td><i>E. coli</i></td><td><i>P. vulgaris</i></td><td>Compound code</td><td><i>S. aureus</i></td><td><i>B. subtilis</i></td><td><i>E. coli</i></td><td><i>P. vulgaris</i></td><td>Compound code</td><td><i>S. aureus</i></td><td><i>B. subtilis</i></td><td><i>E. coli</i></td><td><i>P.vulgaris</i></td></tr>
<tr><td> </td><td>C1</td><td>64</td><td>64</td><td>64</td><td>64</td><td>N1</td><td>65</td><td>65</td><td>64</td><td>61</td><td>O1</td><td>55</td><td>55</td><td>59</td><td>59</td></tr>
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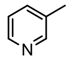
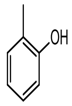
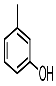
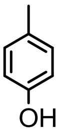
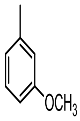
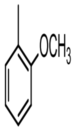
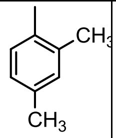
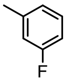
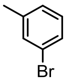
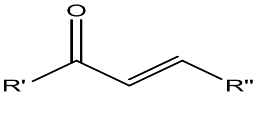
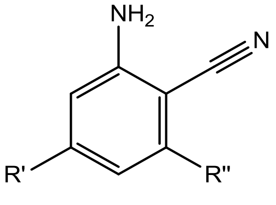
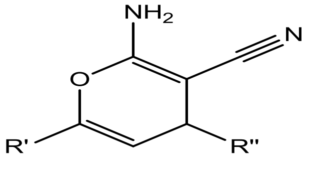
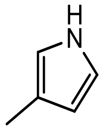
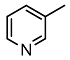
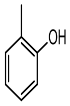
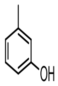
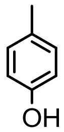
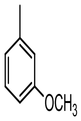
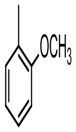
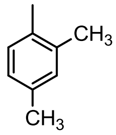
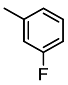
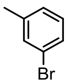
	**C2**	15	15	20	20	**N2**	15	15	19	10	**O2**	19	19	19	19
	**C3**	2	2	2	2	**N3**	2	2	2	2	**O3**	2	2	2	2
	**C4**	1.5	1.5	1.5	1.5	**N4**	1.5	1.5	1.5	1.5	**O4**	1	1	1	1
	**C5**	2	2	2	2	**N5**	1.5	1.5	1.5	1.5	**O5**	1	1	1	1
	**C6**	2	2	1.5	1.5	**N6**	0.5	0.5	1.5	1.5	**O6**	1	1	1	1
	**C7**	5	5	7	7	**N7**	05	03	03	03	**O7**	5	9	9	6
	**C8**	10	10	11	11	**N8**	09	08	05	05	**O8**	5	5	5	5
	**C9**	30	30	28	28	**N9**	32	32	30	30	**O9**	31	31	28	28
	**C10**	32	32	30	30	**N10**	30	30	28	28	**O10**	30	30	28	28
Benzyl penicillin		2	2	2	2						* R’-$C_7H_4F_3$				

Table 07 Antifungal activity (MIC in µg/ml)* of Chalcones (C1-10), Pyridines (N1-10), Pyrans (O1-10)

									
R”	**Compound code**	***Candida albicans***	***Aspergillus niger***	**Compound code**	***Candida albicans***	***Aspergillus niger***	**Compound code**	***Candida albicans***	***Aspergillus niger***

	**C1**	15	19	**N1**	13	11	**O1**	12	17
	**C2**	15	10	**N2**	16	13	**O2**	10	10
	**C3**	14	12	**N3**	12	10	**O3**	11	10
	**C4**	13	16	**N4**	16	13	**O4**	12	12
	**C5**	13	13	**N5**	16	13	**O5**	14	12
	**C6**	18	12	**N6**	15	16	**O6**	14	12
	**C7**	10	17	**N7**	14	13	**O7**	14	12
	**C8**	11	18	**N8**	13	12	**O8**	14	12
	**C9**	12	17	**N9**	15	14	**O9**	14	15
	**C10**	18	10	**N10**	14	14	**N10**	13	15
Fluconazole		1	1	* * R'-$C_7H_4F_3$					

RESULTS AND DISCUSSIONS

Pyrans exhibited excellent antibacterial potency whereas pyridines showed tremendous antifungal activity compared to the reference drugs Benzyl penicillin and Fluconazole. In chalcones, pyridines and pyrans, the nature and position of the substituents on the aromatic ring played a vital function for the antimicrobial activity. Compounds C3-8, N3-8, and O3-8 had an electron-donating group (-OH, -OCH3, CH_3) substituted at different positions on the aromatic ring and compounds C9-10, N9-10, and O9-10 had electron-withdrawing groups (-F, -Br) substituted at different positions on the aromatic ring. Compounds C3, 4, 5, 6, 7, 9, 10; N3, 4, 5, 6, 7, 9, 10 and O3, 4, 5, 6, 7, 9, 10 represent monosubstituted chalcone, pyridine, and pyranderivatives; C8, N8, and O8 represent disubstituted chalcone, pyridine, pyran derivatives. In the monosubstituted chalcone series C1-7, 9-10, the methoxy group at position 3 and the hydroxyl group at position 4 showed potent antibacterial activity in *E. coli*and *P. vulgaris*with MIC value of 1.5 µg/mL This is lower than the antibacterial activity of the reference drugs. Among the disubstituted pyrans series C8, substitution at positions 2 and 4 showed better antibacterial activity *(S. aureus, B. subtilis, E. coli*and *P. vulgaris)* with MIC value of 5 µg/mL than chalcones and pyridines series. Compounds C4, N4, N6, O4, O5, O6 had lower activity than the reference drugs. Its antibacterial activity was found to be greater than benzyl penicillin. In contrast, the antifungal potency of C10, O2, O3 was found to be 10 µg/mL, which was less compared to fluconazole (1 µg/mL). On comparing compounds 6 and 7 it can be observed that the methoxy substituent is favourable to structure pyridines with MIC value of 0.5 µg/mL and >3 µg/mL *(S.aureus and B.subtilis),* structure pyrans with MIC value of 1 µg/mL and >5 µg/mL *(S.aureus, B.subtilis, E.coli* and *P.vulgaris),* structure chalcones with MIC value of <2 µg/mL and >5 µg/mL *(S.aureus, B.subtilis, E.coli* and *P.vulgaris)*. On comparing compounds C9, C10, N9, N10, O9 and O10 it can be seen that substituting the electron-donating group instead of the electron withdrawing group at position 3 was favorable for their antibacterial and antifungal activities. Most of the chalcones demonstrated better antibacterial and antifungal activity, with the minimum inhibitory concentration (MIC) ranging from 1.5–10 µg/mL. Some of the compounds viz., C1, C2, N1, N2, O1 and O2, showed poor activity, with the MIC ranging between 10 and 64 µg/mL. It can be concludedfrom the structure–activity relationship studies that the presence of additional methoxy groups at the ortho positions (C7, N7, and O7) was deleterious whereas substitution of methoxy groups in meta position (C6, N6, and O6) was critical for the antimicrobial activity. Structure activity relationship studies (SAR) studies suggested that substituents on the phenyl ring exhibited brilliant antibacterial activity compared to reference and improved their antifungal activity by 10-fold.

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