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DIHYDROPYRIMIDINONE DERIVED CHALCONES AS EMERGING ANTICANCER SCAFFOLDS AGAINST A549 LUNG CANCER CELL LINES

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ABSTRACT
Novel dihydropyrimidnone chalcones were synthesized by using Clasein Schmidth condensation from 5-acetyl, 6-methyl, 4- (3- ethoxy, 4-hydroxy phenyl) dihyro pyrimidine-2-one by reacting with various substituted aldehydes. Their structural characterizations were evaluated by FT-IR, 1H NMR, 13C NMR, mass spectroscopy. They were screened for *in vitro* anticancer activity by using MTT assay against human lung cancercell lines (A-549). Dihydropyrimidnone chalcones **CH4 (21± 2), CH13 (23± 2)** and **CH15 (18± 2)** showed nearly equal potent with the standard drug Methothrexate (**11± 2).** Docking was done with crystalline protein structure of SAMDH1 which has rapid gene expression in lung cancer cell than other cancer cells. Amino acid residues GLY-159, ARG-160, ASP-94, TYR-39, GLY-40, HIS-53, of SAMHD1 were found to be directly interacting with the synthesized compounds. The most active compounds with dock scores (Kcal/mol) are as CH4= - 8.3, CH13 = -8.7, CH15 compared to Methothrexate (-6.10 kcal/mol) and these results have correlated with in vitro MIC values. Hence, these novel dihydropyrimidinone chalcones were considered as lead anti-cancer agents against lung cancer.

KEYWORDS: Claiesn-Schmidt condensation, Chalcones, cytotoxicity, (A-549) lung cancer cell lines, SAMDH1.

INTRODUCTION

Lung cancer has one of the highest death rates among different types cancer, where only 15.1% of patients diagnosed with non-small-cell-lung cancer, representing 85% of the total lung cancer, reach the 5 year survivor rate [1]. Unfortunately, the survival rate is drastically reduced by three thirds, that is to 4.2%, in those patients with metastasized lung cancer, and every year more than 1.5 million of new cases are diagnosed. Thus, the dramatic low survivor rate and the increase number of patients with this type of cancer encourage development of new therapeutic compounds. Therefore, the discovery of new molecules and their in vitro evaluation represents an important step towards the discovery of new drug candidates for the treatment of this and other types of cancer. Among the myriads of new molecules tested in vitro as chemotherapeutic agents, we focused our investigation in the synthesis of dihydropyrimidnone chalcones.

Chalcone is a generic term given to compounds bearing the 1, 3-diphenyl-2 propen-1-one framework and belong to the flavonoid family [2,3]. Chemically they are open-chain flavonoids in which the two aromatic rings are joined by a three carbon a,b-unsaturated carbonyl system. Chalcones are abundantly present in nature starting from ferns to higher plants [4] and a number of them are polyhydroxylated in the aryl rings. In plants, chalcones are converted to the corresponding (2S)-flavanones in a stereospecific reaction catalyzed by the enzyme chalcone isomerase. This close structural and biogenetic relationship between chalcones and flavanones explains why they often co-occur as natural products. Pharmacologically chalcones were known to display wide range of biological activities such as antimicrobial[4], anti-inflammatory[5], antiplasmodial[6], immunosuppresion[7], antioxidant[8], antihyperglycemic activity[9] and anti-tumor [10-11], antiviral [12] activities. From the literature, it has been clearly emphasized that pyrimidinones and chalcones having anticancer properties. By synthesizing pyrimidinone chancone derivatives may from either chalcones or modified chalcones continued to exhibit a spectrum of biological activities

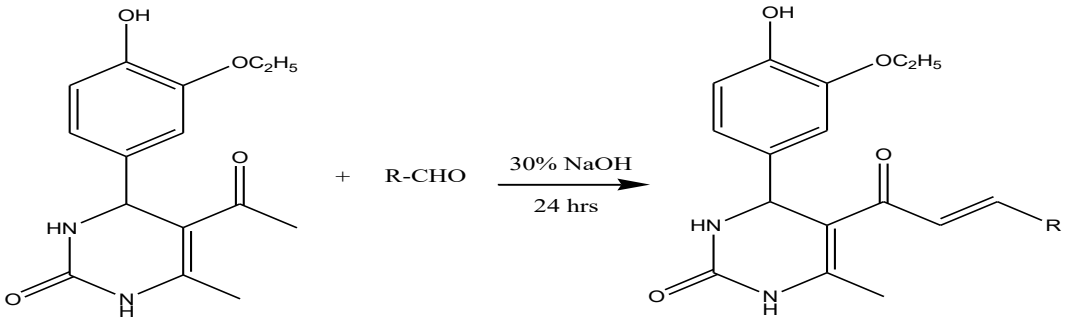
MATERIALS AND METHODS:

All the chemicals used in the synthesis were procured from Sigma Aldrich. TLC plates for monitoring of reactions and silica gel (100-200 mesh) for column chromatography obtained from Merck. The Melting points were determined by using EZ-Melt automated melting point apparatus. The FT-IR spectra were recorded on BRUKER ALPHA-T FT.IR spectrophotometer using KBr pellet method. The Mass spectra were recorded on Agilent 6320 Ion Trap LC-MS (Positive/Negative ion electro spray ionization method). The 1H NMR spectra of the compounds were recorded on BRUKER 400 MHz NMR Spectophotometer.

EXPERIMENTAL

General procedure for synthesis of dihydropyrimidnone chalcones:

A mixture of 5-acetyl, 6-methyl, 4- (3- ethoxy, 4-hydroxy phenyl) dihyro pyrimidine-2-one (1mmol) and substituted benzaldehydes (1mmol) in ethanol (10ml), were taken in a round bottomed flask. To that mixture catalytic amount of aqueous 30% NaOH (10ml) was added. The mixture was stirred at room temperature for 24 hrs and reaction was monitored by TLC. After completion of reaction, the reaction mixture was acidified with 1.1N, resulted into the formation of precipitate. The precipitate was filtered under vacuum and the product was washed with water. The obtained solid was purified by using column chromatography from a mixture of ethyl acetate and hexane (1:1).



SPECTRAL DATA OF SYNTHESIZED COMPOUNDS:

Characterization of 1-(6'- methyl-4'- (3''- ethoxy, 4''- hydroxyphenyl)-2'-oxo-3', 4'-dihydro pyrimidinyl)-3-phenyl)-2-propene- 1-one (CH1).

The chemical structure was confirmed through physical and spectral data. The IR (cm^{-1}) spectrum showed the characteristic absorption bands at 3432 (OH), 3256 (NH), 1679 (C=O), 1595 (C=C) and 1324 (C-O-C), resulting that confirmation of respective functional groups. The ^1H NMR spectrum (400 MHz, $CDCl_3$) of compound **CH1** showed the characteristic signals of CO-CH= and =CH-Ar at δ 6.62 and 7.05 as doublets with coupling coanstant ***J* =16.1 Hz** respectively confirming the *trans* geometry at the ethylenic double bond of the chalcone. The peaks in between 6.55-7.31 integrated for ten protons, of which two were already accounted for the ethylenic protons and the other eight must be the aromatic protons, the singlets at 7.41 and 8.21 due to –NH protons and another singlet at 9.32 confirming the phenolic proton (-OH).

Compound **CH1**, analyzed for $C_{19}H_{19}ClO$, m.p. 136-138 ºC, exhibited a $[M+H]^+$ at *m/z* 300.1 in its positive ion mode electron spray ionization mass spectrum. 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 **CH1** was confirmed as **1-(6'- methyl-4'- (3''- ethoxy, 4''- hydroxyphenyl)-2'-oxo-3', 4'-dihydro pyrimidinyl)-3-phenyl)-2-propene- 1-one.** 1

By adopting the above synthetic procedure, compounds **(CH2 to CH15)** were also synthesized. The characteristic physicochemical properties were given in table no. l and spectral data was presented separately in detail.

1-(6'-methyl-4'-(3''-ethoxy, 4''-hydroxyphenyl)-2'-oxo-3', 4'-dihydropyrimidinyl)-3 (4'''-methyl phenyl)-2-propene-1-one (CH2).

Yeild 87 %, m.p.: 208, m.w.:321, FTIR (KBR, $V_{max,}$ cm^{-1}): 3336 (-OH), 3255 (NH), 1701 (C=O), 1595 (C=C), 1321(C-O-C); ^1H NMR ($CDCl_3$): 1.29 (s, 3H, - CH_3), 2.19 (s, 3H, -CH_3), 3.8 (q, 2H,O CH_2 CH_3), 5.11 (s, 1H, H of pyrimidine ring), 6.31 (dd, 2H, Ar-H), 6.71 (d, 1H, J=15.8Hz, -CH=CH-), 6.91 (d, 1H, Ar-H), 7.13(d, 1H, J=16.2Hz, -CH=CH-), 7.24(m, 7H, Ar-H), 7.91(s, 1H, -NH), 9.32 (s, 1H, -NH), 10.09 (s, 1H, -OH), 10.31 (s, 1H, -OH).

1-(6'-methyl-4'-(3''-ethoxy,4''-hydroxyphenyl)-2'-oxo-3',4'-dihydropyrimidinyl)-3 (2'''-chlorophenyl)-2-propene-1-one (CH3)

Yeild 81 %, m.p.: 218, m.w.:307, FTIR (KBR, $V_{max,}$ cm^{-1}): 3412(OH), 3284(NH), 3121 (Ar-H), 1689 (C=O), 1319(C-O-C), 1136(C-Cl); ^1H NMR ($CDCl_3$): 1.36 (s, 3H, - CH_3), 2.39 (s, 3H, -CH_3), 3.95 (q, 2H,O CH_2 CH_3), 5.31(s, 1H, H of pyrimidine ring),6.41 (dd, 2H, Ar-H), 6.64 (d, 1H, J=16.4Hz, -CH=CH-), 7.11(s, 1H, Ar-H), 7.32 (d, 1H, J=16.4Hz, CH=CH-), 7.53 (m, 4H, Ar-H), 7.731(s, 1H, -NH), 8.62 (s, 1H, -NH), 9.82 (s, 1H, Ar-OH)

1-(6'-methyl-4'-(3''-ethoxy,4''-hydroxyphenyl)-2'-oxo-3',4'-dihydropyrimidinyl)-3(4'''-fluorophenyl)-2-propene-1-one (CH4).

Yeild 83 %, m.p.: 243, m.w.: 322, FTIR (KBR, $V_{max,}$ cm^{-1}): 3481 (OH), 3319 (NH), 1679(C=O), 1559 (C=C), 1339 (C-O-C), 1201 (C-F); ^1H NMR ($CDCl_3$)1.35 (s 3H, - CH_3), 2.28 (s, 3H, -CH_3), 3.9 (q, 2H,O CH_2 CH_3), 5.41(s, 1H, H of pyrimidine ring), 6.62 (dd, 2H, Ar-H), 6.83 (d, 1H, J=17.2Hz, -CH=CH-),7.32 (d, 1H, J=16.5Hz, -CH=CH-),7.83 (s, 1H, -NH), 8.122 (m, 9H,Ar-H), 7.83 (s, 1H, -NH), 8.92 (s, 1H, -NH) , 9.32 (s, 1H, Ar-OH)

1-(6'-methyl-4'-(3''-ethoxy,4''-hydroxyphenyl)-2'-oxo-3',4'-dihydropyrimidinyl)-3(2'''-bromophenyl)-2-propene-1-one (CH5).

Yeild 81 %, m.p.: 206, m.w.: 314, FTIR (KBR, $V_{max,}$ cm^{-1}): 3432(OH), 3390 (NH), 3270 (Ar-CH), 1695 (C=O), 1324(C-O-C), 1091 (C-Br) ; ^{1}H NMR ($CDCl_3$)1.33 (s, 3H, - CH_3), 2.39 (s, 3H, -CH_3), 3.9 (q, 2H,O CH_2 CH_3), 5.25 (s, 1H, H of pyrimidine ring), 6.61 (dd, 2H, Ar-H), 6.81 (d, 1H, J=16Hz, -CH=CH-),7.12 (d, 1H, Ar-H), 7.32 (d, 1H, J=16Hz,-CH=CH-),7.41 (d, 2H, J=8.8 Hz), 7.68 (d, 2H, J=8.8 Hz), 8.21 (s,1H, -NH), 9.34(s, 1H, -NH), 10.02 (s, 1H, Ar-OH)

1-(6'-methyl-4'-(3''-ethoxy,4''-hydroxyphenyl)-2'-oxo-3',4'-dihydropyrimidinyl)-3(2''',4'''-dichlorophenyl)-2-propene-1-one (CH6).

Yeild 65 %, m.p.: 239, m.w.: 309, FTIR (KBR, $V_{max,}$ cm^{-1}): 3462(OH), 3359 (NH), 1696 (C=O), 1612 (C=C), 1333(C-O-C), 1197 (C-Cl); ^{1}H NMR ($CDCl_3$)1.38 (s, 3H, - CH_3), 2.27 (s, 3H, -CH_3), 3.89 (q, 2H, -OCH_2CH_3), 5.30 (s, 1H, H of pyrimidine ring), 6.55 (dd, 2H, Ar-H), 6.85 (d, 1H, J=17.8Hz, -CH=CH-), 7.31 (d, 1H, J=7.8Hz, CH=CH-), 7.52 (m, 8H, Ar-H), 8.79 (s, 1H, -NH), 9.29 (s, 1H, -NH), 9.73 (s, 1H, Ar-OH)

1-(6'-methyl-4'-(3''-ethoxy, 4''-hydroxyphenyl)-2'-oxo-3',4'-dihydropyrimidinyl)-3(4'''-nitrophenyl)-2-propene-1-one (CH7).

Yeild 61 %, m.p.: 241, m.w.: 325, FTIR (KBR, $V_{max,}$ cm^{-1}): 3412(OH), 3238 (NH), 1647 (C=O), 1552 (C=C), 1309(C-O-C) ; ^{1}H NMR ($CDCl_3$)1.35 (s, 3H, - CH_3),2.23(s, 3H, -CH_3), 2.77(s, 3H, -CH_3), 3.9 (q, 2H,OCH_2CH_3), 5.18(s, 1H, H of pyrimidine ring), 6.53(d, 1H, J=17.6Hz, -CH=CH-), 6.61 (dd, 2H, Ar-H), 6.92 (d, 1H, J=16.4Hz, -CH=CH-), 7.18(d, 1H, Ar-H), 7.56 (d, 2H, J=6Hz), 7.67 (d, 2H, J=6Hz), 8.77 (s, 1H, -NH), 9.78 (s, 1H, -NH), 10.32 (s, 1H, Ar-OH).

1-(6'-methyl-4'-(3''-ethoxy,4''-hydroxyphenyl)-2'-oxo-3',4'-dihydropyrimidinyl)-3(4'''-methoxyphenyl)-2-propene-1-one (CH8).

^{1}H NMR ($CDCl_3$)1.25 (s, 3H, - CH_3), 2.27(s, 3H, -CH_3),3.09 (s, 3H, OCH_3), 3.91 (q, 2H, OCH_2CH_3), 5.09 (s, 1H, Hof pyrimidine ring), 6.31 (dd, 1H, Ar-H), 7.01 (d, 1H, J=16.8Hz,-CH=CH-), 719 (d, 1H, J=16.8Hz, CH=CH-), 7.36 (m, 9H, Ar-H), 7.97 (s, 1H, -NH), 9.06 (s, 1H, -NH), 9.57 (s, 1H, Ar-OH)

1-6'-methyl-4'-(3''-ethoxy,4''-hydroxyphenyl-2'-oxo-3',4'-dihydropyrimidinyl)-3(4'''-dimethylaminophenyl)-2-propene-1-one (CH9).

Yeild 77 %, m.p.: 214, m.w.: 314, FTIR (KBR, $V_{max,}$ cm^{-1}): 3432(OH), 3199(NH), 1679(C=O), 1420(C=C),1311(C-O-C) ; ^{1}H NMR ($CDCl_3$)1.13 (s, 3H, - CH_3), 1.24 (t, 6H, N-CH_3)$_2$, 3.9 (q, 2H,OCH_2CH_3), 5.21 (s, 1H, H of pyrimidine ring), 6.51 (dd, 1H, Ar-H), 6.75(d, 1H, J=15.4Hz, CH=CH-),7.12 (d, 1H, Ar-H) 7.35 (d, 1H, J=15.4Hz, CH=CH-), 7.59 (d, 2H, J=8.4Hz), 7.717 (d, 2H, J=8.4Hz), 8.32(s, 1H, -NH), 9.712 (s, 1H, -NH), 10.11 (s, 1H, Ar-OH)

1-(6'-methyl-4'-(3''-ethoxy, 4''-hydroxyphenyl)-2'-oxo-3', 4'-dihydropyrimidinyl)-3(3''', 4'''-dimethoxyphenyl)-2-propene-1-one (CH11).

Yeild 70 %, m.p.: 243, m.w.: 323, FTIR (KBR, $V_{max,}$ cm^{-1}): 3124 (N-(CH3)$_2$), 1698(C=O), 1591 (C=C), 1344(C-O-C); ^{1}H NMR ($CDCl_3$)1.13 (t, 3H, - $\underline{CH_3}$), 1.24 (t, 6H, N-$CH_2\underline{CH_3}$)$_2$, 3.9 (q, 2H,O$\underline{CH_2}CH_3$), 5.21 (s, 1H, Hof pyrimidine ring), 6.51 (dd, 1H, Ar-H), 6.75(d, 1H, J=15.4Hz, CH=$\underline{CH}$-),7.12 (d, 1H, Ar-H) 7.35 (d, 1H, J=15.4Hz, CH=$\underline{CH}$-), 7.59 (d, 2H, J=8.4Hz), 7.717 (d, 2H, J=8.4Hz), 8.32(s, 1H, -NH), 9.712 (s, 1H, -NH), 10.11 (s, 1H, Ar-OH)

1-(6'-methyl-4'-(3''-ethoxy,4''-hydroxyphenyl)-2'-oxo-3',4'-dihydropyrimidinyl)-3(3''',4''',5'''-trimethoxyphenyl)-2-propene-1-one (CH12).

Yeild 77 %, m.p.: 204, m.w.: 341, FTIR (KBR, $V_{max,}$ cm^{-1}): 1.33 (s, 3H, - CH_3), 2.33 (s, 3H, -CH_3), 3.71 (s, 9H, -OCH_3), 4.21 (q, 2H,OCH_2CH_3), 5.23 (s, 1H, H of pyrimidine ring), 6.31 (dd, 2H, Ar-H), 6.55(d, 1H, J=15.6Hz, -CH=CH-), 6.74(d, 1H, Ar-H), 6.94 (d, 1H, Ar-H), 7.12(d, 1H, J=16.8Hz, -CH=CH-), 7.91 (m, 3H, Ar-H), 8.63 (s, 1H, -NH), 8.81 (s, 1H, -NH), 9.41 (s, 1H, Ar-OH)

1-(6'-methyl-4'-(3''-ethoxy,4''-hydroxyphenyl)-2'-oxo-3',4'-dihydropyrimidinyl)-3(4'''-hydroxyphenyl)-2-propene-1-one (CH13).

Yeild 71 %, m.p.: 238, m.w.: 319, FTIR (KBR, $V_{max,}$ cm^{-1}): 3263 (NH), 1711 (C=O), 1591 (C=C), 1330(C-O-C); ^{1}H NMR ($CDCl_3$)1.25 (t, 3H, - $\underline{CH_3}$), 2.27(s, 3H, -$\underline{CH_3}$),3.09 (s, 3H, OCH_3), 3.91 (q, 2H,O $\underline{CH_2}$ CH_3), 5.09 (s, 1H, Hof pyrimidine ring), 6.31 (dd, 1H, Ar-H), 7.01 (d, 1H, J=16.8Hz,-$\underline{CH}$=CH-), 719 (d, 1H, J=16.8Hz, CH=$\underline{CH}$-), 7.36 (m, 9H, Ar-H), 7.97 (s, 1H, -NH), 9.06 (s, 1H, -NH), 9.57 (s, 1H, Ar-OH)

1-6'-methyl-4'-(3''-ethoxy,4''-hydroxyphenyl-2'-oxo-3',4'-dihydropyrimidinyl)-3(2'''-fyryl)-2-propene-1-one (CH14).

Yeild 77 %, m.p.: 237, m.w.: 298, FTIR (KBR, $V_{max,}$ cm^{-1}): 3449(OH), 3238(NH), 3110(Ar-H), 1727 (C=O), 1610 (C=C),1329 C-O-C) ; ^{1}H NMR ($CDCl_3$)1.29 (, 3H, - CH_3), 2.19(s, 3H, -CH_3), 5.29 (s, 1H, H of pyrimidine ring), 6.51 (dd, 2H, Ar-H), 6.68 (d, 1H, J=16.6Hz,-CH=CH-),7.05(s, 1H, Ar-H),7.15(d, 2H, Ar-H), 7.24(d, 1H, J=16.6Hz, CH=CH-), 7.35(d, 2H, Ar-H), 7.792 (s, 1H, -NH), 8.62 (s, 1H, -NH), 9.52 (s, 1H, Ar-OH)

1-6'-methyl-4'-(3''-ethoxy,4''-hydroxyphenyl-2'-oxo-3',4'-dihydropyrimidinyl)-3-cinnamyl-2-propene-1-one (CH15).

Yeild 57 %, m.p.: 228, m.w.: 329, FTIR (KBR, $V_{max,}$ cm^{-1}): 3502(OH), 3263 (NH), 1711 (C=O), 1591 (C=C), 1330(C-O-C) ; ^{1}H NMR ($CDCl_3$)1.25 (s, 3H, - CH_3), 2.27(s, 3H, -CH_3),3.09 (s, 3H, OCH_3), 3.91 (q, 2H, OCH_2CH_3), 5.09 (s, 1H, H

of pyrimidine ring), 6.31 (dd, 1H, Ar-H), 7.01 (d, 1H, J=16.8Hz,-CH=CH-), 719 (d, 1H, J=16.8Hz, CH=CH-), 7.36 (m, 9H, Ar-H), 7.97 (s, 1H, -NH), 9.06 (s, 1H, -NH), 9.57 (s, 1H, Ar-OH)

Anti-cancer Activity:
Anti-cancer activity of all the synthesized dihydropyrimidinone chalcones was determined by an *in vitro* MTT Assay method against human lung cancer cells (A-549). MTT assay is a colorimetric assay which measures the reduction of yellow 3- (4, 5-dimethylthiazol – 2 - yl)- 2, 5-diphenyl tetrazoliumbromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, dark purple coloured formazan product. The cells are then solubilized with DMSO and the solubilized formazan complex is measured spectrophotometrically at 570 nm. Since the reduction of MTT can only occur in metabolically active cells, the level of the activity is a measure of the viability of the cells. The amount of the dark purple formazan complex produced by the cells treated with an agent is compared with the amount of formazan produced by the untreated control cells.

Molecular Docking studies of dihydropyrimidnone chalcones- Target protein SAMHD1 bound to Cytarabine-TP in the catalytic pocket.
Molecular docking is an important tool in structural molecular biology and computer aided drug design. The main objective ligand-protein docking is to predict and interpret the predominant and most reliable binding mode(s) with a known protein three-dimentional structure.

Materials and Methods
Computational details
An Intel Core2 Duo Processor with memory of 4GB RAM running with the Windows7, 64 bit operating system was used to carry out all the computations. All the molecular docking studies were performed using the Auto Dock Tools-1.5.6 molecular docking program. Molecular structures of Proteins and ligand molecules were prepared and energetically minimized to avoid the critical problems before performing the docking calculations. Chemical structures of ligands and their derivatives were drawn with the help of Chemdraw Ultra 8.0 application and saved in an appropriate format for the usage.

Protein preparation
SAMHD1 bound to Cytarabine-TP in the catalytic pocket {6DW3} was taken from the RCSB PDB (rcsb.org) Protein Data Bank (PDB ID: 6DW3)[75]for protein preparation using Auto Dock Tools-1.5.6. This performs the following steps: Downloading the protein in 'pdb'format, assigning of bond orders, addition of hydrogens and optimization of hydrogen bonds by flipping amino side chains, correction of charges, deleting co-crystallised ligand , followed by adding charges and minimization of the protein complex. All the bound water molecules, ligands and cofactors were removed (preprocess) from the proteins which were taken in `pdb' format. The tool neutralized the side chains that are not close to the binding cavity and do not participate in salt bridges. Further, restrained minimization of co-crystallized complex which reorients side chain hydroxyl groups and alleviates potential steric clashes.

Ligand preparation
The LigPrep was performed by using Auto Dock Tools-1.5.6., in various steps. Different ligands were drawn by using ChemDraw Ultra 8.0 and were saved as MDL (mol) format, followed by minimizing the energy using MM2 option in Chem3D Ultra 8.0 and saved as pdb.format. Then they were opened in Auto Dock Tools-1.5.6.and inserted from ligand option; suitable charges were added and checked for torsion tree and then finally saved as pdbqt.format.

AU DOCKER
Flexible docking method with the application AU DOCKER was employed to carry out all the docking calculations. AU DOCKER is a ligand-docking application that utilizes a genetic algorithm (GA) to explore ligand conformation flexibility and orientation with partial flexibility of the protein, and satisfy ligand-binding requirements. Advantage involved in AU docking over many other docking algorithms is that it allows for both unconstrained ligand flexibility and partial flexibility of the binding pocket thus affording a more realistic environment for ligand-receptor association. This standard set parameters were used in all the calculations.
Dock score recorded on each binding mode using a fitness function that accounts for the frequency of interactions between the ligand and receptor atoms[77]. DOCK score is the atom-atom potential derived from a data base of protein-ligand complexes. Traditional scoring functions are based on force field or on regression, where parameters are derived from a set of experimental binding affinities and structures. DOCK score uses a different approach; information about the frequency of interaction between ligand and receptor atoms is gathered by analyzing existing ligand-protein structures in the PDB and this information is used to generate statistical potentials. The empirical parameters used in the scoring function are hydrogen bond energies, atom radii, polarisabilities, torsion potentials and hydrogen bond directionalities. The top 10 ranked solutions of the ligands were taken for further observation of binding orientation and H-bond interactions.
All the designed ligands and one single protein 1G3U (without co-crystallised ligand) were kept in single folder for easy work. Then, the protein was kept in the receptor area and ligands were kept in ligand area in the AU DOCKER software. No.of. poses and exhaustiveness were limited upto 10 and we set for 8 each. Grid parameters were to be entered (sizes

and centers as x, y, z). Then those are set for run and the dock scores for each ligand were recorded individually as well as one single out file containing all ligands dock score and hydrogen bond interaction listed table no:3

RESULTS AND DISCUSSION

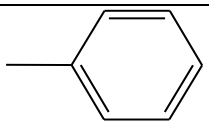
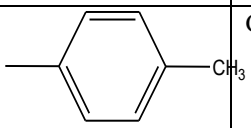
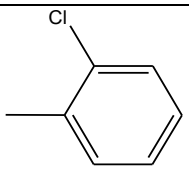
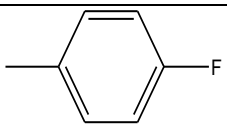
In our proposed investigation, it was extended to synthesize some novel dihydropyrimidinone chalcones (CH1-CH15) synthesized by condensing 5-acetyl **dihydropyrimidnone** with different aldehydes in the presence of 30% ethanolic NaOH at room temperature and stirred for 24 hr. The spectral characterizations were carried out on the synthesized dihydro pyrimidinone chalcones using suitable IR, 1H NMR, mass spectra and elemental analysis data. All the dihydropyrimidnone chalcones derivatives (**CH1-CH15**) were exhibited characteristic absorption bands in the IR spectra (cm-1) in between 3400-3500 (OH), 3100-3300 (NH), 1600-1700 (C=O), 1500-1600 (C=C) and 1200-1400 (C-O-C) and at other regions of the spectrum depending upon the specific substituent present in each compound. 1H NMR spectra of the dihydropyrimidinone chalcones was showed characteristic resonance signal for –Ar-CO-CH =CH-Ar at δ 6.00 - 7. 50 as doublets with coupling constant ***J* =16.1 Hz,** respectively confirming the *trans* geometry at the ethylenic double bond of the chalcone. Peaks were clearly shown in the spectra accounting for the aromatic protons for the different substituent protons in between the corresponding regions of the spectrum.

Anticancer activity: All dihydropyrimidinone chalcone derivatives **(CH1-CH15)** were evaluated for their *in vitro* cytotoxicity activity (A-549) human lung cancer cell line [17] by MTT cell proliferation assay [23,24]. Methotrexate is one of the most effective anticancer agents used as reference drug. The half maximal inhibitory concentration (IC50) was estimated and results are summarized in Table-2. Compounds CH**1, CH2, CH3 and CH11** were displayed less anticancer activity (IC50 □□90 ± 1 μg/mL) and compounds CH**5, CH6, CH7, CH8, CH9, CH10, CH12 and CH14** were exhibited moderate anticancer activity (30 μg/mL □to □IC50 □70 μg/mL). Compounds **4, 13** and **15** were demonstrated with potent anti lung cancer activity (IC50 < 30μg/mL).

Docking analysis of dihydropyrimidinone chalcones was performed against SAMDH1 which has more gene expression in lung cancer cells than other cancer cells. Docking analysis reveals that hydrogen bonding interactions with the catalytic pocket of the target protein were crucial factors, affecting inhibitory action of the compounds. Aminoacid residues **TYR-315, HIS-275, ARG-220, GLY-219, ASP-218, GLY-375** of SAMHD1 protein were found to be directly interacting with the synthesized molecules in the form of hydrogen bond interactions. The synthesized compounds and Cytarabine showed most of the Hydrogen bonding interactions with aminoacid residues HIS-275, GLY-375 present in the catalytic pocket of the SAMHD1. The highest docking scores were showed by the compounds **CH4= -8.6, CH7= -8.7, CH13= -9.7, CH16= -9.0** and these results were clearly correlated with their *in vitro* MIC values

CONCLUSSION:

In this present work we have synthesized fifteen novel dihydropyrimidinone chalcones and screened their anti-cancer activity against the A549 human lung adenocarcinoma cell line by MTT assay. From *in vitro* assay results, we observed that compounds having strong electron withdrawing groups, polar groups and heterocyclic ring substitution on aromatic ring of dyhydropyrimidnone chalcone were showed moderate to potent activity when compared with standard Methothrexate. Docking results of synthesized compounds with the target protein SAMDH1 also correlating the *in-vitro* assay results. These results indicated that compounds **CH4, CH13, CH15** could be used as potential lead compounds for anticancer drug development

Compound	R	Molecular Formula	Relative Molecular Mass (RMM)	Melting Point (°C)	Elemental Analysis	Yield %
CH1		$C_{22}H_{22}N_2O_4$	380	250.05°C	C-69.83 H-5.86 N-7.40 O-16.91	92
CH2		$C_{23}H_{24}N_2O_4$	390	270.84°C	C-70.39 H-6.16 N-7.14 O-16.31	87
CH3		$C_{22}H_{21}ClN_2O_4$	415	295.49°C	C-64.00 H-5.13 Cl-8.59 N-6.79 O-15.50	65
CH4		$C_{22}H_{21}FN_2O_4$	395	271.16°C	C-66.66 H-5.34 F-4.79 N-7.07 O-16.14	85

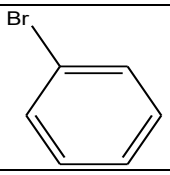
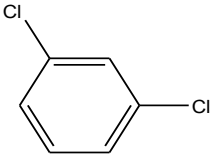
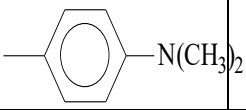
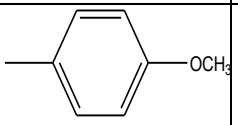
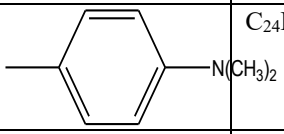
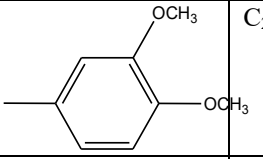
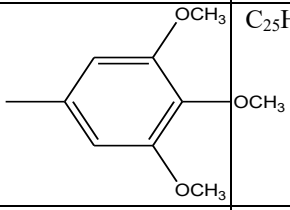
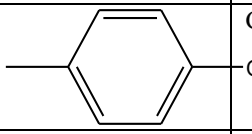
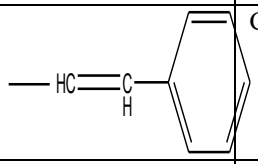
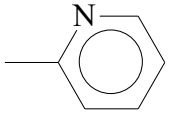
CH5		$C_{22}H_{22}N_2O_4$	376	256.05° **C**	C- 69.83 H-5.86 N-7.40 O-16.91	85
CH6		$C_{22}H_{21}ClN_2O_4$	341	296.49° **C**	C-64.00 H- 5.13 Cl-8.59 N-6.79 O-15.50	79
CH7		$C_{24}H_{27}N_3O_4$	420	220.58° **C**	C- 68.39 H-6.46 N-9.97 O-15.18	82
CH8		$C_{23}H_{24}N_2O_5$	405	195.07° **C**	C-67.63 H-5.92 N-6.86 O-19.59	80
CH9		$C_{24}H_{27}N_3O_4$	423	226.58° **C**	C- 68.39 H-6.46 N-9.97 O-15.18	90
CH10		$C_{24}H_{26}N_2O_6$	436	245.06° **C**	C-65.74 H-5.98 N-6.39 O-21.89	65
CH11		$C_{25}H_{28}N_2O_7$	466	190.55° **C**	C-64.09 H-6.02 N-5.98 O-23.91	95
CH12		$C_{22}H_{22}N_2O_5$	392	264.27° **C**	C-66.99 H-5.62 N-7.10 O-20.28	79
CH13		$C_{24}H_{24}N_2O_4$	406	270.51° **C**	C-71.27 H-5.98 N-6.93 O-15.82	70
CH15		$C_{21}H_{21}N_3O_4$	377	210.32° **C**	C-66.48 H-5.58 N-11.08 O-16.87	76

Table 1: Physicochemical properties of compounds (CH1-CH15)

Compound	**Cell line**
	HT-29
CH1	132 ± 2
CH2	NA
CH3	182 ± 1
CH4	**21± 2**
CH5	68 ± 2
CH6	65 ± 2
CH7	NA
CH8	58 ± 2

CH9	**36 ± 2**
CH10	49 ± 2
CH11	123 ± 2
CH12	128 ± 2
CH13	**23 ± 2**
CH14	75 ± 2
CH15	19±2
Methotrexate	**11 ± 1**

Activity criteria for screening compounds based on their IC50 values Cytotoxicity activity criteria IC50 values (µg/mL) Low > 90; Moderate 30-70; Potent < 30; Highly potent < 10

Table 2: Cytotoxicity results for synthesiszed compound (CH1-CH14) against HT29 Cell lines

CODE	DOCK SCORE	INTERACTIONS
CH 1	-6.3	GLN 149(3.3), TYR 315(3.5), ASP 501(3.0), GLY 508(3.4)
CH 2	-7.2	ASN 504(3.5), GLN 375(3.1), ASP 319(3.0), HIS 215(3.4)
CH 3	-7.4	ASP 218(3.2), GLY 219(3.4), ARG 220(3.5), HIS 215(3.1)
CH 4	**-8.6**	ASN 504(3.4), GLN 375(3.4), ASP 319(3.5), HIS 215(3.4), ASP 218(3.3), GLY 219(3.0)
CH 5	-8.1	ARG 366(3.0), HIS 215(3.4), ASP 218(3.2)
CH 6	-8.2	AS 319(3.2), ASP 311(3.5), GLU 234(3.4), HIS 215(3.2), GLY 219(3.3)
CH 7	**-8.7**	GLN 375(3.4), ARG 220(3.5), ASN 504(3.4), VAL 502(3.2) ASP 501(3.4)
CH 8	-8.4	GLN 375(3.2), ASN 504(3.3), ASP 218(3.3), HIS 215(3.4)
CH 9	-7.7	GLN 149(3.3), TYR 315(3.5), ASP 501(3.0), GLY 508(3.4)
CH 10	-8.3	ASN 504(3.4), HIS 370(3.5), GLN 375(3.4), ARG 220(2.8)
CH 11	-7.0	HIS 215(3.5), GLN 375(3.4), ARG 220 (3.5), ASN 504(3.4), VAL 502(3.2), ASP 501(3.4)
CH 12	-8.2	ASN 504(3.5), GLN 375(3.1), ASP 319(3.0), HIS 215(3.4)
CH 13	**-9.7**	GLY 219(3.0), GLN 375(3.4), HIS 370(2.9), ASN 504(3.9),ASP218(3.3)
CH 14	-8.3	GLU 234(3.4), ASN 504(3.5), GLN 375(3.3), HIS 370(3.5) ASP 218(3.3)
CH 15	-7.1	ASP 218(3.3), GLY 219(3.4), ARG 220(3.3), MET 216(3.2), HIS 215(2.0)
CH 16	**-9.0**	HIS 215(3.5), GLN 375(3.4), ARG 220(3.5), ASN 504(3.4) VAL 502(3.2), ASP 501(3.4)

Table:3 Molecular docking results against SAMDH

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