INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

SYNTHESIS, CHARACTERIZATION AND CYTOTOXICITY STUDIES OF NEW BIGINELLI ADDUCTS

Kiran Manda[1*], AVS. Sastry[2] and V. Girijasastry[1]

[1]A.U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam - 530 003, Andhra Pradesh, India.
[2]Maharajah's College of Pharmacy, Phool Baugh, Vizianagaram - 535 002, Andhra Pradesh, India.

ABSTRACT

Novel dihydrothiopyrimidnone were synthesized by using Biginelli reaction from thiourea, aceylacetone 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 prostate cancer cell lines (D-145) and human lung cancer cell lines (A-549). **C7** has shown potent activity with IC_{50} **28 µg/mL** and **35µg/mL** against prostate cancer cell lines and lung cancer cell lines. Compound **C4** has shown moderate activity against lung cancer cell lines with IC_{50} **34 µg/ mL and 25 µg/ mL** against prostate cancer cell lines and lung cancer cell lines when compare to the standard Methotrexate.

Keywords: Biginelli reaction, Dihydrothiopyrimidnone, Cytotoxicity, Prostate cancer cell lines.

INTRODUCTION

Noncommunicable diseases (NCDs) are a major threat to global health, causing a significant amount of death every year. Second only to cardiovascular disease, cancer is becoming a global burden which lead to an estimation of 8.7 million deaths in 2015 [1]. Moreover, cancer is expected to rank as the leading cause of death and the single most significant barrier for the increase of life expectancy in every country worldwide [2]. Contrary to common misperception, cancer is a major health challenge not only in high-income countries but also in low- and middle-income countries (LMICs), where the number of cancer occurrence is rapidly growing [3]. Unfortunately, almost all anticancer drugs are associated with serious side effects, making the search for novel chemical agents that are cytotoxic to cancer cells with less side effects an urgent need. The dihydropyrimidnones (DHPMs) can be synthesized by a straight forward and well known protocol Biginelli reaction [4], which involves three component condensations of aromatic aldehyde, acetoaceticester and urea in ethanolic solution. In the name of the scientist Biginelli these compounds were named biginelli compounds. DHPMs are increasingly gaining importance due to their biological and pharmacological properties 5 like antiviral activity, antibacterial activity, antitumor, analgesic, cardiovascular activity, anti-inflammatory, bloodplatelet aggregation inhibitor, anti-hypertensive agents and alpha-1-antagonists. The compounds containing DHPM core unit show excellent calcium channel modulation[6-13]. In addition, it was found that there were several isolated marine alkaloids[12] having interesting biological activities which contains the dihydropyrimidinone-5-carboxylate core. In order to improve the yields of dihydropyrimidinones a few other approaches involving modified experimental conditions (change in the catalysts, solvents, temperatures etc.) have been reported [14].

From the literature, it has been clearly emphasized that pyrimidinones having anticancer properties like Monstrol (Figure 1).

Keeping in view, it is worthwhile to synthesize some new dihydropyrimidinone derivatives by using eco-friendly, cheap easily available citric acid as a catalyst and evaluate them for their anticancer properties.

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 the synthesis of dihydropyrimidines[16]

The equimolar mixture of thiourea (I), acetyl acetone (II), aldehyde (III) and catalytic amount of citric acid in ethanol (solvent) were taken in a round bottomed flask and refluxed for 6-7 h at 80 ^{0}C and the reaction was monitored by using TLC. After completion of the reaction, obtained crude product was transferred into a beaker containing crushed ice and then filtered, washed with ice cold water and recrystallized using ethanol to get the pure compound. The structural properties of synthesized compounds were given in **Table1.**

Synthesis and characterization data of 5-acetyl-4-(3'- ethoxy- 4'- hydroxyphenyl)-6-methyl-3, 4-dihydropyrrimidine- 2(1*H*)-thione (C1)

A mixture of acetylacetone (0.01 mole), 3-ethoxy-4-hydroxy benzaldehyde (0.01 mole), thiorea (0.01 mole) and ciric acid (10 mol%) in water (25 mL) was taken into a 250 ml dry beaker and reflux it for 1 hr at 80^o C. The reaction progress was monitored by TLC. After completion of reaction, the mixture was poured into 100 ml of ice cold water, filtered. The obtained solid was washed with ice coled water, dried and purified by recrystallization using absolute ethanol.

The chemical structure was confirmed through physical and spectral data. The IR spectra of the compound **C1** showed the absorption band at around 3306, 3172 and 1705 cm^{-1} regions, resulting from the –NH, Ar-H and C=O functions respectively. Further, in the ^{1}H NMR spectra, singlet at 1.33 and 211 accounted for the methyl group of –OCH_2CH_3 and methyl group protons of pyrimidine ring at 6th position. Another singlet at 2.29 integrated for methyl group of acetyl group at 5th position of pyrimidine ring. The singlet at 5.26 accounted for proton of chiral carbon at 4th position, which confirming the cyclization results in formation of dihydropyrimidinone. The aromatic protons are shown in multiplets around δ 7.24 to 7.37. The two broad singlets with the chemical shift values 6.81 and 8.94 were due to –NH protons. The singlet at 9.64, due to OH group at 4th position of phenyl ring on dihydropyrimidnone.

The ES-MS (Fig 21) showed its molecular ion peak at 231 (M+1), which is in accordance with its molecular formula $C_{13}H_{14}N_2O_2S$.

By adopting the above synthetic procedure, compounds **(C2 to C10)** were also synthesized. The characteristic physicochemical properties were given in table no. l and spectral data was presented separately in detail.

Spectral data of synthesized new dihydrothiopyrimidnones (C2-C10)

5-acetyl-4-(3',4'-dimethoxyphenyl)-6-methyl-3,4-dihydropyrimidine-2(1H)-thione (C2)

Yeild 87 %, m.p.: 208, m.w.:321, FTIR (KBR, $V_{max,}$ cm^{-1}): 3257 (NH), 3131 (Ar—H), 2961 (C-H), 1697 (C=O), 1329(C-O-C).; ^{1}H NMR ($CDCl_3$): 2.06 (s, 3H, -CH_3), 2.27 (s, 3H, -$COCH_3$), 5.15 (s, 1H, H of pyrimidine ring), 6.69 (d, 3H, Ar-H), 7.04 (d, 2H, Ar-H), 7.70 (s, 1H, NH), 9.10 (s, 1H, NH), 9.37 (s, 1H, OH);

5-acetyl-4-(furfuryl)-6-methyl-3,4-dihydropyrimidine-2(1H)-thione (C3)

Yeild 81 %, m.p.: 218, m.w.:307, FTIR (KBR, $V_{max,}$ cm^{-1}): 3269 (NH), 3121 (Ar-CH), 2949 (CH), 1696 (C=O), 1379(C-O-C); ^{1}H NMR ($CDCl_3$): 2.06 (s, 3H, -CH_3), 2.27 (s, 3H, -$COCH_3$), 5.15 (s, 1H, H of pyrimidine ring), 6.69 (d, 2H, Ar-H), 7.04(d, 2H, Ar-H), 7.70 (s, 1H, NH), 9.10 (s, 1H, NH).

5-acetyl-4-(4'-chlorophenyl)-6-methyl-3,4-dihydropyrimidine-2(1H)-thione (C4)

Yeild 83 %, m.p.: 243, m.w.: 322, FTIR (KBR, $V_{max,}$ cm^{-1}): 3316 (NH), 3152 (Ar-CH), 2975 (C-H), 1705 (C=O), 1609(C=C); ^{1}H NMR ($CDCl_3$): 1.31 (s, 3H, -CH2CH_3), 2.11 (s, 3H, -CH_3), 2.31 (s, 3H, -$COCH_3$), 3.96 (q, 2H, -OCH_2), 5.18 (s, 1H, H of pyrimidine ring), 6.58(d, 2H, Ar-H), 6.72 (d, 2H, Ar-H), 6.82 (s, 1H, Ar-H), 8.94 (s, 1H, N ^{1}H), 9.64 (1, 1H, N^{3}H), 10.18 (1, 1H, SH).

5-acetyl-4-phenyl-6-methyl-3,4-dihydropyrimidine-2(1H)-thione (C5)
Yeild 81 %, m.p.: 206, m.w.: 314, FTIR (KBR, $V_{max,}$ cm^{-1}): 3241 (NH), 3102 (Ar-CH), 1695 (C=O), ^{1}H NMR ($CDCl_3$): 2.12 (s, 3H, $-CH_3$), 2.22 (s, 3H, $-COCH_3$), 5.18 (s, 1H, H of pyrimidine ring), 6.58 (t, 1H, Ar-H), 7.25 (d, 2H, Ar-H), 7.45 (d, 1H, Ar-H), 7.81 (s, 1H, NH), 9.17 (s, 1H, NH), , 10.23 (s, 1H, OH).

5-acetyl-4-(4'-isopropylphenyl)-6-methyl-3,4-dihydropyrimidine-2(1H)-thione (C6)
Yeild 65 %, m.p.: 239, m.w.: 309, FTIR (KBR, $V_{max,}$ cm^{-1}): 3239 (NH), 3093 (Ar-CH), 2957 (C-H), 1701 (C=O), 1325 (C-O-C); ^{1}H NMR ($CDCl_3$): 1.17(m, 6H, $-2CH_3$), 2.15 (s, 3H, $-CH_3$), 2.33 (s, 3H, $-COCH_3$), 2.93 (q, 1H, -CH), 5.25 (s, 1H, H of pyrimidine ring), 7.13 (d, 2H, Ar-H), 7.20 (d, 2H, Ar-H), 7.79 (s, 1H, NH), 9.70 (s, 1H, NH), 10.24 (s, 1H, SH).

5- acetyl-4-(2',4'-dichlorophenyl)-6-methyl-3,4-dihydropyrimidine-2(1H)-thione (C7)
Yeild 61 %, m.p.: 241, m.w.: 325, FTIR (KBR, $V_{max,}$ cm^{-1}): 3359 (NH), 3162 (Ar—H), 2979 (C-H), 1719 (C=O), 1I92(C-Cl); ^{1}H NMR ($CDCl_3$): 2.06 (s, 3H, $-COCH_3$), 2.27 (s, 3H, CH_3), 5.15 (s, 1H, H of pyrimidine ring), 6.99 (s, 1H, Ar-H), 7.11 (s, 1H, Ar-H), 7.23 (s, 1H, Ar-H), 7.69 (s, 1H, NH), 8.85 (s, 1H, NH), 10.18 (s, 1H, SH).

5-acetyl-4-cinnamyl-6-methyl-3,4-dihydropyrimidine-2(1H)-thione (C8)
Yeild 61 %, m.p.: 241, m.w.: 325, FTIR (KBR, $V_{max,}$ cm^{-1}): 3267 (NH), 3149 (Ar-CH), 2989(C-H), 1707 (C=O), 1612 (C=C); ^{1}H NMR ($CDCl_3$): 2.72 (s, 6H, $-2CH_3$), 4.85 (s, 1H, H of pyrimidine ring), 6.11(d, 1H, -CH=CH-), 6.11(d, 1H, -CH=CH-), 7.24 (d, 2H, Ar-H), 7.30 (t, 1H, Ar-H), 7.39 (d, 2H, Ar-H), 7.81 (m, 1H, N^1H), 9.26 (s, 1H, N^3H)

5- acetyl-4-(4'-methoxyphenyl)-6-methyl-3,4-dihydropyrimidine-2(1H)-thione (C9)
Yeild 77 %, m.p.: 214, m.w.: 314, FTIR (KBR, $V_{max,}$ cm^{-1}): 3236 (NH), 3115 (Ar-CH), 1704 (C=O), 1329(C-O-C); ^{1}H NMR ($CDCl_3$): 2.12 (s, 3H, $-CH_3$), 2.32 (s, 3H, $-COCH_3$), 3.73 (s, 3H, $-OCH_3$), 5.23 (s, 1H, H of pyrimidine ring), 6.89 (d, 1H, Ar-H), 7.14 (d, 2H, Ar-H), 6.82 (d, 1H, Ar-H), 9.55 (s, 1H, N^1H), 10.27 (s, 1H, N^3H).

5- acetyl-4-(4'-hydroxyphenyl)-6-methyl-3,4-dihydropyrimidine-2(1H)-thione (C10)
Yeild 70 %, m.p.: 243, m.w.: 323, FTIR (KBR, $V_{max,}$ cm^{-1}): 3449 (OH), 3256 (NH), 3132 (Ar-CH), 2959 (C-H), 1691(C=O), 1329 (C-O-C); ^{1}H NMR ($CDCl_3$): 2.15 (s, 3H, $-CH_3$), 2.29 (s, 3H, $-COCH_3$), 5.28 (s, 1H, H of pyrimidine ring), 6.58 (t, 1H, Ar-H), 6.72 (d, 2H, Ar-H), 6.82 (d, 1H, Ar-H), 9.64 (s, 1H, N^1H), 10.16 (s, 1H, N^3H), 10.36 (s, 1H, SH).

CYTOTOXICITY STUDIES

The *in vitro* cytotoxicity of the test compounds (**C1 to C10**) was evaluated by the MTT assay proposed by Mosmann in 1983. This is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product **(Figure 4)**. The cells are then solubilized with DMSO and the released, solubilized formazan reagent is measured spectrophotometrically at 570 nm. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells. When the amount of dark purple formazan produced by the cells is treated with an agent compared with the amount of formazan produced by untreated control cells, the effectiveness of the agent in causing death of cells can be deduced through the production of a dose-response curve.

MATERIALS

DU-145 (Prostate cancer) and A-549 (Lung cancer) cell line were obtained from National Centre for Cell Science (NCCS), Pune, India. DMEM (Dulbeccos Modified Eagels Medium), MEM (Minimum Essential Media Eagle), 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.

METHOD

a) Maintenance of cell lines

DU-145 cell lines and A-549 cell lines were grown as adherent in DMEM media and the culture was maintained in a humidified atmosphere with 5% CO_2.

b) Preparation of samples for cytotoxicity

Stock solutions of test compounds (**C1 to C10)** were prepared (10 mg/mL) in DMSO and from them various dilutions were made with sterile water to get the final drug concentrations of 10, 50, 100 and 200 µg/mL.

c) Cytotoxicity evaluation[17,18]

The cells were seeded in 96 well plates at a density of $1x10^4$ (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^{0}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 37^{0}C 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 37^{0}C for 10 min. The absorbance at 570 nm was measured on a spectrophotometer.

Methotrexate was used as reference drug for comparison. Assay was performed in triplicate for 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 tumor cells by 50% as compared to the control untreated cells. IC_{50} values were determined from the plot: % inhibition versus concentration.

% inhibition at the given concentration =

$$\frac{\text{1- (Absorbance average)}}{\text{(Control absorbance average)}} \times 100$$

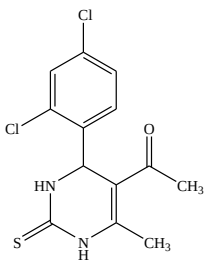
IC_{50}=Inv.log (50-c) / m; c and m derived from y= mx+c of plot of % inhibition Vs log C. The results of the compounds are shown in **Table 2**.

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

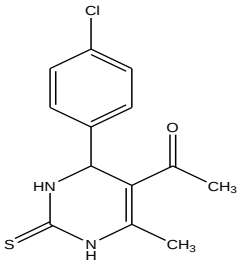
RESULTS AND DISCUSSIONS

In our proposed investigation, it was extended to synthesize some new dihydropyrimidinone (C1-C10) synthesized by condensing thiourea, acetylacetone with different aldehydes in the presence of citric acid at 60^{0}C. The spectral characterizations were carried out on the synthesized dihydro pyrimidinone chalcones using suitable IR, 1H NMR, mass spectra and elemental analysis data.

The compounds **C1-C10** have shown considerable cytotoxic activity against the prostate cancer cell line (DU-145) and lung cancer cell line (A-549). The compounds bearing electron withdrawing substituents had shown remarkable activity. The most potent compound was **C7** with IC_{50} **28 µg/mL** against prostate cancer cell lines. It has also shown activity against lung cancer cell lines at **35µg/mL**. Compound **C4** has shown moderate activity against lung cancer cell lines with IC_{50} **34 µg/ mL and 25 µg/ mL** against prostate cancer cell lines and lung cancer cell lines. The molecules containing electron releasing substituents showed poor cytotoxic activity. These results indicate that the compounds bearing halogenated substituents possess promising cytotoxic activity when compared to the compounds containing methoxyl, phenyloxy, hydroxy, isopropyl and dimethylamino groups.



C7



C4

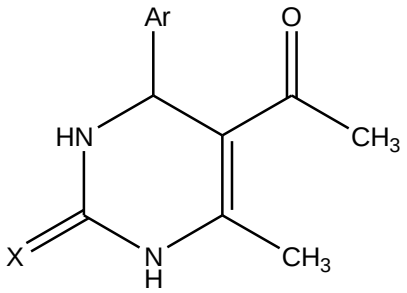
CONCLUSION

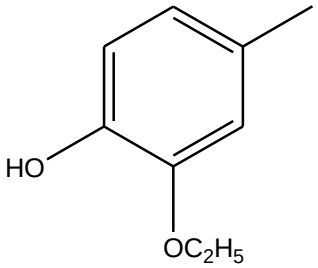
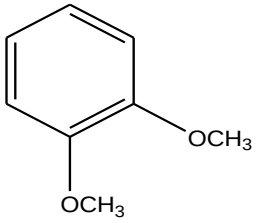
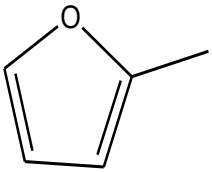
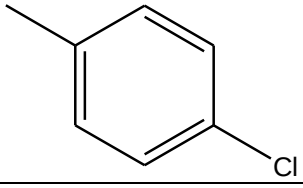
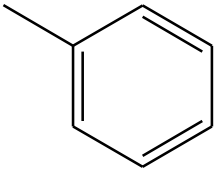
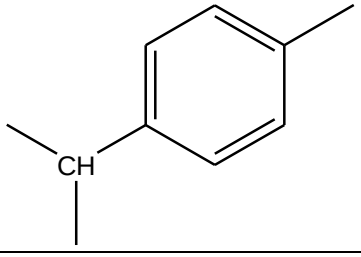
In this present work we have synthesized ten new dihydrothiopyrimidinones and screened their anti-cancer activity against the prostate cancer cell line (DU-145) and lung cancer cell line (A-549) by MTT assay. From *in vitro* assay results indicate that the compounds bearing halogenated substituents possess promising cytotoxic activity when compared to the compounds containing methoxyl, phenyloxy, hydroxy, isopropyl and dimethylamino groups.

AKNOWLEDGEMENTS

The authors are thankful to UGC for providing financial assistance throughout our research work.

Table 1: Structural properties of dihydropyrimidnones



Compound	Ar	X
C1		S
C2		S
C3		S
C4		S
C5		S
C6		S

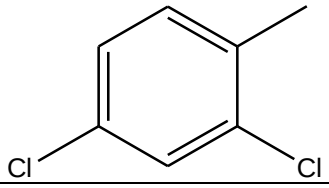
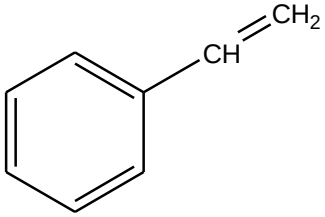
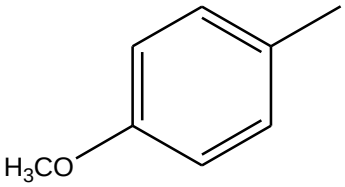
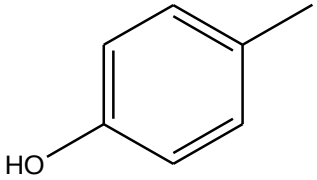
C7		S
C8		S
C9		S
C10		S

Table I.5: IC 50 (µg/mL) values of the dihydropyrimidines (C1 to C20)

Compound	IC_{50} values (µg/mL)	
	DU-145	A-549
C1	99 ± 1	85 ± 1
C2	133 ± 2	95 ± 2
C3	66 ± 2	66 ± 2
C4	**34 ± 2**	**25 ± 2**
C5	79 ± 2	95± 2
C6	32 ± 2	56± 2
C7	**28 ± 2**	**35 ± 2**
C8	42 ± 2	65 ± 2
C9	58 ± 2	46 ± 2
C10	85 ± 1	64 ± 1
Methotrexate	**11 ± 1**	**8 ± 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.

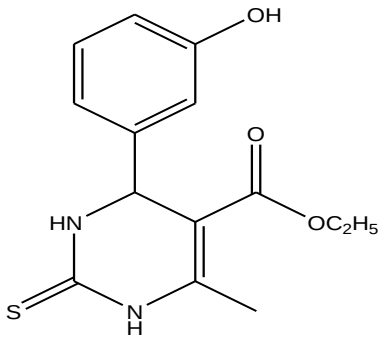
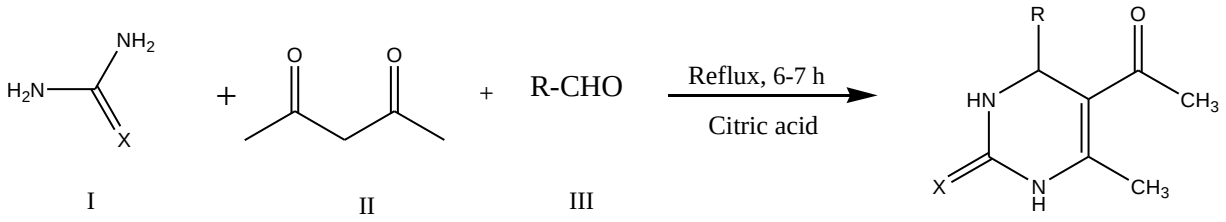


Fig. 1: Structure of Monastrol



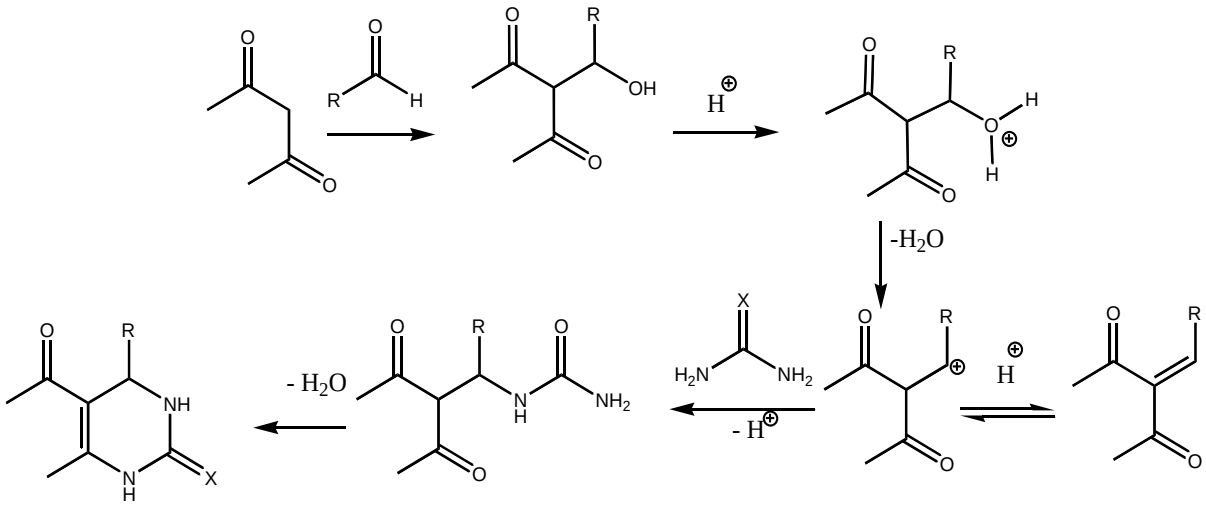
I II III

dihydropyrimidinones (C1-C20)

X=S, Thiourea

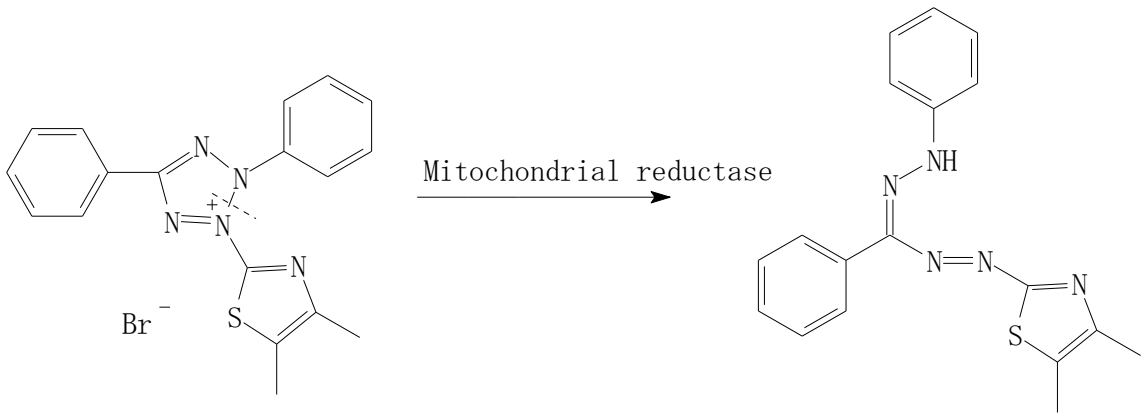
Scheme I

Fig. 2: Protocol for the synthesis of target compounds



R= Different aromatic aldehydes, X= S

Fig. 3: Proposed mechanism for construction of dihydropyridine derivatives



MTT (80) **Purple coloured complex (81)**

Fig. 4: Reduction of MTT

REFERENCES

1. Fitzmaurice C. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: A systematic analysis for the global burden of disease study. JAMA Oncol. 2017; 3:524–548.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394–424.
3. Vineis P and Wild CP. Global cancer patterns: Causes and prevention. Lancet. 2014; 383:549–557.
4. De Fatima A, Braga TC, Neto LDS, Terra BS, Oliveira BG, da Silva DL and Modolo LV. A mini-review on Biginelli adducts with notable pharmacological properties. J Adv Res. 2015;6:363–373.
5. Nagarajaiah H, Mukhopadhyay A and Moorthy JN. Biginelli reaction: An overview. Tetrahedron Lett. 2016;57:5135–5149.
6. Pagano N, Teriete P, Mattmann ME, Yang L, Snyder BA, Cai Z, Heli ML and Cosford NDP. An integrated chemical biology approach reveals the mechanism of action of HIV replication inhibitors. Bioorg Med Chem. 2017;25:6248–6265.
7. Zhu X, Zhao G, Zhou X, Xu X, Xia G, Zheng Z, Wang L and Yang X. Song L. 2,4-Diaryl-4,6,7,8-tetrahydroquinazolin-5(1*H*)-one derivatives as anti-HBV agents targeting at capsid assembly. Bioorg Med Chem Lett. 2010;20:299–301
8. Matias M, Campos G, Santos AO and Falcão A. Silvestre S., Alves G. Potential antitumoral 3,4-dihydropyrimidin-2-(1*H*)-ones: Synthesis, in vitro biological evaluation and QSAR studies. RSC Adv. 2016;6:84943–84958.
9. Lauro G, Strocchia M, Terracciano S, Bruno I, Fischer K, Pergola C, Werz O, Riccio R and Bifulco G. Exploration of the dihydropyrimidine scaffold for the development of new potential anti-inflammatory agents blocking prostaglandin E_2 synthase-1 enzyme (mPGES-1). Eur J Med Chem. 2014;80:407–415.
10. Dhumaskar KL, Meena SN, Ghadi SC and Tilve SG. Graphite catalyzed solvent free synthesis of dihydropyrimidin-2(1*H*)-ones/thiones and their antidiabetic activity. Bioorg Med. Chem Lett. 2014;24:2897–2899.
11. Akhaja TN and Raval JP. 1,3-Dihydro-2H-indol-2-ones derivatives: Design, synthesis, in vitro antibacterial, antifungal and antitubercular study. Eur J Med Chem. 2011;46:5573–5579.
12. Wani MY, Ahmad A, Kumar S and Sobral AJFN. Flucytosine analogues obtained through Biginelli reaction as efficient combinative antifungal agents. Microb Pathog. 2017;105:57–62
13. Lewis RW, Mabry J, Polisar JG, Eagen KP, Ganem B and Hess GP. Dihydropyrimidinone positive modulation of delta-subunit-containing gamma-aminobutyric acid type A receptors, including an epilepsy-linked mutant variant. Biochemistry. 2010;49:4841–4851.
14. Bhatt JD, Chudasama CJ and Patel KD. Diarylpyrazole Ligated Dihydropyrimidine Hybrids as Potent Non-Classical Antifolates and Their Efficacy Against Plasmodium falciparum. Arch Pharm. 2017;350:1700088.
15. Rashid U, Sultana R, Shaheen N, Hassan SF, Yaqoob F, Ahmad MJ, Iftikhar F, Sultana N, Asghar S and Yasinzai M. Structure based medicinal chemistry-driven strategy to design substituted dihydropyrimidines as potential antileishmanial agents. Eur. J. Med. Chem. 2016;115:230–244.
16. Rodina A, Vilenchik M, Moulick K, Aguirre J, Kim J, Chiang A, Litz J, Clement CC, Kang Y and She Y. Selective compounds define Hsp90 as a major inhibitor of apoptosis in small-cell lung cancer. Nat Chem Biol. 2007;3:498.
17. Teleb M, Zhang FX, Farghaly AM, Wafa OMA, Fronczek FR, Zamponi GW and Fahmy H. Synthesis of new N3-substituted dihydropyrimidine derivatives as L-/T-type calcium channel blockers. Eur J Med Chem. 2017;134:52–61.
18. Putatunda S, Chakraborty S, Ghosh S, Nandi P, Chakraborty S, Sen PC and Chakraborty A. Regioselective N^1-alkylation of 3,4-dihydropyrimidine-2(1*H*)-ones: Screening of their

biological activities against Ca^{2+}-ATPase. Eur J Med Chem. 2012;54:223–231.
19. Gangwar N and Kasana VK. 3,4-Dihydropyrimidin-2(1*H*)-one derivatives: Organocatalysed microwave assisted synthesis and evaluation of their antioxidant activity. Med Chem Res. 2012;21:4506–4511.
20. Kumar BRP, Sankar G, Baig RBN and Chandrashekaran S. Novel Biginelli dihydropyrimidines with potential anticancer activity: A parallel synthesis and CoMSIA study. Eur J Med Chem. 2009;44:4192–4198.