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RESEARCH ARTICLE

Synthesis, Cytotoxic Activity and Molecular Docking Studies of 4-Aryl-6-methyl-3,4-Dihydropyrimidin-2(1*H*)-ones/thiones with Eg5 Protein

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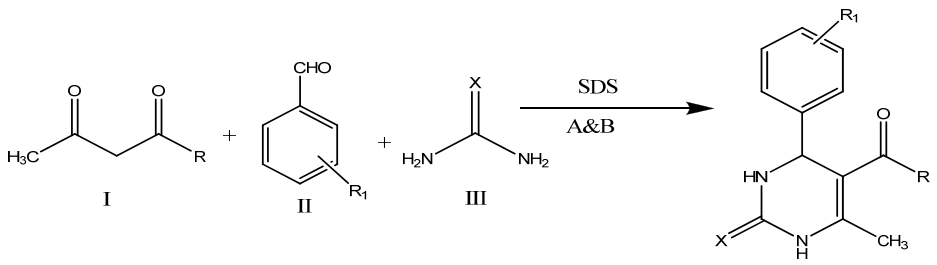
Abstracts: Various Biginelli compounds (dihydropyrimidinones, DHPM) have been synthesized efficiently and in high yields under mild, solvent free and eco-friendly conditions in a one pot reaction of 1, 3-dicarbonyl compounds, aldehydes and urea/thiourea using sodium dodecyl sulphate (SDS) as a novel catalyst under two experimental conditions have been introduced. Synthesized compounds were evaluated for their cytotoxicity screening in lung cancer (A-549) and colon cancer (HT-29) cell lines by the MTT assay method. Molecular docking studies were carried out synthesized DHPM derivatives using GOLD software with the crystal structure of Eg5 protein (1QOB) to gain some structural insights on the binding mode and possible interaction with the active site.

Keywords: Pyrimidine, Cytotoxic activity and Docking studies

Introduction

Dihydropyrimidinones (DHPM) have exhibited important biological and pharmacological properties as the integral backbone of several calcium channel blockers[1], antihypertensive[2], anti-tumor[3], α1-adrenergic antagonist[4], antimycobacterial[5] and anti-inflammatory[6] activities. Several alkaloids isolated from marine sources also exhibit interesting biological activities, molecular structures of which contain the dihydropyrimidinone moiety[7]. Several synthetic strategies have been reported for the synthesis of 3,4-dihydropyrimidin-2(1*H*)-one/thione derivatives. To enhance the efficiency of the Biginelli reaction, various catalysts and reaction conditions have been studied, which allow the preparation of DHPMs in good to high yields but they still have limitations like long reaction time, costly chemicals/catalysts, makes this method environmentally hazardous, therefore, development of simple, efficient, clean and high yielding and environmentally friendly approaches using new catalysts for the synthesis of these compounds is an important table of organic chemists[8-10]. Here, we wish to report the capacity of SDS as potential catalyst for the synthesis of DHPMs and evaluated for their *in vitro* cytotoxic studies and docking studies were conducted.



X=O,S
R= -CH_3, -OC_2H_5, -OCH_3
R_1= -H, 4-OCH_3, 4-OH, 2-OH, 4-Cl, 3-OCH_3-4-OH, -4-NO_2,
A- Micro-wave irradiation method; B-Conventional method

Scheme 1

Materials and methods

The purity of the compounds was checked by TLC using ethyl acetate, benzene (4:6) as solvent system of and iodine vapours for visualization. Melting points were detected in open capillaries using Bachi melting point apparatus and are uncorrected. IR spectra were recorded on Perkin- Elmen RX1-FTIR. ^{1}H NMR spectra on a JEOL 400 spectrometer using TMS as an internal standard and mars spectra in JEOL DX 300 in E1 ionization made at 70ev. MW reactions were carried out in a BPL-SANYO domestic micro-wave oven.

Experimental

Synthesis of 4-(aryl)-1,2,3,4-tetrahydropyrimidine-2-(1H)-ones/thiones (D-1 to D-15)

A) *Micro-wave Irradiation Method:* To a mixture of *β*-ketoester (0.01mol, I), aldehyde (0.01 mol, II), urea or thiourea (0.01 mol, III) and Sodium dodecyl sulphate (10 %w/v in water) was subjected to microwave irradiation at 220W for 5-6 min. The completion of the reaction (Scheme 1) was monitored by TLC. After cooling to room temperature, the reaction mixture was poured into 100 ml of cold water and stirred for 5 min. The separated solid was filtered under suction, washed with cold water and then recrystallized from ethanol to afford the pure product[11].

B) *Conventional Method:* To a mixture of β-ketoester (0.01mol, I), aldehyde (0.01 mol, II), urea or thiourea (0.01 mol, III) and Sodium dodecyl sulphate (10 %w/v in water) was heated under reflux for 4-5 h with magnetic stirring. The completion of the reaction was monitored by TLC. After cooling to room temperature, the reaction mixture was poured into 100 mL of cold water and stirred for 5 min. The separated solid was filtered under suction, washed with cold water and then recrystallized from ethanol to afford the pure product[11]. Spectral and physical data of synthesized compounds are described on Table 1 & 2.

Table 1. Spectral data of synthesized DHPMs

Compd. Code	IR (KBr) cm^{-1}	^{1}H NMR δppm
D-1	3241(N-H), 1713(C=O)	2.1(3H,s,-CH_3),2.29(3H,s,-CH_3), 5.26(1H,s,H of pyrimidine ring),7.24(5H,m,Ar-H) 7.82(1H,s,-NH), 9.17(1H,s,-NH)
D-2	3249(N-H), 1738(C=O)	1.04(3H,t,-$OCH_2\underline{C}H_3$), 2.23(3H,s,-CH_3), 3.95(2H,q,-$O\underline{C}H_2CH_3$), 7.15(5H,m,Ar-H), 7.77 (1H,s,NH), 9.85(1H,s ,-NH)

Contd….

D-3	3246(N-H), 1709(C=O)	1.04(3H,t,-$OCH_2\underline{C}H_3$), 2.23(3H,s,-CH_3), 3.79 (3H,s,-OCH_3), 3.94(2H,q,-$O\underline{C}H_2CH_3$), 6.97(4H,m,Ar-H), 7.75(1H,s,-NH), 9.73(1H,s,-NH)
D-4	3290(N-H), 1690(C=O)	δ1.03(3H,t, $OCH_2\underline{C}H_3$), 2.22(3H,s,-CH_3), 3.94(2H,q,-$O\underline{C}H_2CH_3$), 6.61(4H,m,Ar-H), 7.73(1H,s,-NH), 8.86(1H,s,-NH), 9.76(1H,s,-OH)
D-4	3224(N-H), 1748(C=O)	1.04(3H,t,-$OCH_2\underline{C}H_3$), 2.23(3H,s,-CH_3), 3.95(2H,q,-$O\underline{C}H_2CH_3$), 7.76(1H,s,-NH), 8.27(1H,s,-NH), 9.73(1H,s,-OH)
D-6	3242(N-H), 1723(C=O)	1.04(3H,s\t,-$OCH_2\underline{C}H_3$),2.24(3H,s,-CH_3),3.21(2H,q,-$\underline{C}H_2CH_3$), 7.16(4H,m,Ar-H),8.51(1H,s,-NH),9.46(1H,s ,-NH)
D-7	3274(N-H), 1758(C=O)	1.06(3H,t,-$OCH_2\underline{C}H_3$), 2.26(3H,s,-CH3), 3.95(2H,q,-$O\underline{C}H_2CH_3$), 7.84(1H,s,-NH), 9.35(1H,s,-NH)
D-8	3265(N-H), 1742(C=O)	1.12(3H,t,-$OCH_2\underline{C}H_3$), 2.31(3H,s,CH_3), 4.01(2H,q,-$O\underline{C}H_2CH_3$), 9.61(1H,s,-NH), 10.27(1H,s,-NH)
D-9	3283(N-H), 1715(C=O)	1.90(3H,s,-CH_3), 2.02(3H,s,-CH_3), 5.07(1H,s,H of pyrimidine ring), 9.51(1H,s,-NH), 10.05(1H,s,-NH)
D-10	3213(N-H), 1715(C=O)	2.06(3H,s,CH_3), 2.27(3H,s,CH_3), 3.71(3H,s,-OCH_3), 6.86(4H,m,Ar-H), 7.7(1H,s,-NH), 9.10(1H,s,-NH)
D-11	3204(N-H), 1698(C=O)	0.97(t,3H,-$OCH_2\underline{C}H_3$), 2.21(3H,s,CH_3), 3.82(2H,q,-$O\underline{C}H_2CH_3$), 7.96(1H,s,-NH), 8.81(1H,s,-NH)
D-12	3422(O-H) 1672(C=O)	1.14(t,3H,-$OCH_2\underline{C}H_3$), 2.28(3H,s,-CH_3), 3.97(2H,q,-$O\underline{C}H_2CH_3$), 6.84(4H,m,Ar-H), 7.67(1H,s,-NH), 9.18(1H,s,-NH), 9.87(1H,s,OH)
D-13	3401(-OH), 1673(C=O)	1.17(3H,t,-$OCH_2\underline{C}H_3$),2.35(3H,s,-CH3), 3.86(3H,s,-OCH_3), 4.09(2H,q,-$O\underline{C}H_2CH_3$7.676(1H,s,-NH), 9.738(1H,s,OH)
D-14	3397(O-H), 1689(C=O)	1.14(t,3H,-$OCH_2\underline{C}H_3$), 2.28(3H,s,-CH_3), 3.97(2H,q,-$OCH_2CH_3$7.67(1H,s,-NH), 9.18(1H,s,-NH), 10.02(1H,s,OH)
D-15	3321(N-H), 1709(C=O)	2.27(3H, s,-CH_3), 3.82(3H, s,-OCH_3), 7.19(4H,m, Ar-H), 9.61(1H,s,-NH), 10.12(1H,s,-NH)

Table 2. Physical Properties of synthesized DHPMs

S.No.	Comp. Code	R^1	R_1	X	Mol. Formula	M.P °C	% Yield	
							Conventional	MWI
1	D-1	C_6H_5	CH_3	O	$C_{13}H_{14}N_2O_2$	200-02	93	95
2	D-2	C_6H_5	OC_2H_5	O	$C_{14}H_{16}N_2O_3$	208-210	94	96
3	D-3	4-OCH_3 C_6H_4	OC_2H_5	O	$C_{15}H_{18}N_2O_4$	199-201	87	89
4	D-4	4-OHC_6H_4	OC_2H_5	O	$C_{14}H_{16}N_2O_4$	226-229	90	92
5	D-5	2-OH-C_6H_4	OC_2H_5	O	$C_{14}H_{16}N_2O_4$	199-200	92	94
6	D-6	4-ClC_6H_4	OC_2H_5	O	$C_{14}H_{15}N_2O_3Cl$	209-211	95	95
7	D-7	4-$NO_2C_6H_4$	OC_2H_5	O	$C_{14}H_{15}N_3O_5$	206-08	90	94
8	D-8	C_6H_5	OC_2H_5	S	$C_{14}H_{16}N_2O_2S$	208-210	94	96
9	D-9	C_6H_5	CH_3	S	$C_{13}H_{14}N_2OS$	210-211	93	95
10	D-10	4-(OCH_3)-C_6H_4	CH_3	O	$C_{14}H_{13}N_2O_3$	190-191	92	96
11	D-11	4-ClC_6H_4	OC_2H_5	S	$C_{14}H_{15}N_2O_2SCl$	209-211	95	95
12	D-12	4-OH C_6H_4	OC_2H_5	S	$C_{14}H_{16}N_2O_3S$	227-228	88	89
13	D-13	4-OH,3-OCH_3	OC_2H_5	O	$C_{15}H_{18}N_2O_5$	233-235	82	84
14	D-14	2-OH C_6H_4	OC_2H_5	S	$C_{14}H_{16}N_2O_3$	220-223	85	85
15	D-15	4-Cl C_6H_4	OCH_3	O	$C_{13}H_{13}N_2O_3Cl$	203-205	90	90

Cytotoxicity studies

In vitro cytotoxicity was assessed by MTT assay method on human lung carcinoma (A-549) and colon carcinoma (HT-29) cell lines was performed[12].

Docking

The x-ray crystal structure of Eg5 obtained from the protein data bank (PDB ID:1QOB)[13]. The 3D structures of the derivatives were constructed with the ChemBioDraw Ultra11.0 and hydrogen was added in all the ligand structure. Docking studies were performed by GOLD 3.0.1(Genetic Optimization for Ligand Docking) software, the final corrector PDB file of the protein and synthesized analogous were submitted to GOLD 3.0.1 software tools in order to run docking process and all the parameters set as default .At the final stage through the docked structures of all analogous, best conformation was selected and prepare figures and running protein ligand interactions.

Results and Discussion

Chemistry

The 4-(substituted phenyl-)-3,4-dihydropyrimidine-2-(1*H*)-ones/thiones (D-1 to D-15) were prepared using one pot Biginelli reaction using Sodium doceyl sulphate as catalyst and water as solvent as depicted in scheme. The IR spectra of the compound **D-1** showed the absorption bands at 3241, 2985 and 1713 cm^{-1} due to presence of -NH, Ar-H and C=O groups respectively. ^1H NMR spectra shows signals at δ2.29 (s,-$COCH_3$), 7.24 (m,Ar-H), 7.82 & 9.12 (br, -NH). The MS spectra showed M+1 peak at 231 with its molecular formula $C_{13}H_{14}N_2O_2$.

Cytotoxic activity

Based on docking studies the compounds were selected for their anticancer activity against colon cancer **(HT-29)** and lung cancer **(A-549)** cell lines by MTT assay method. The percent inhibition and IC_{50} values for the tested compounds were calculated. The compound **D-12** showed significant activity against colon cancer **(HT-29)** and lung cancer **(A-549)** cell lines with IC_{50} values at **30.10** and **28.36** µg/mL respectively which may be due to the presence of 4-hydroxy phenyl substituent at C-4 position of DHPM pharmacophore while the compounds **D-5**, **D-13** and **D-14** showed moderate activity and other compounds did not show cytotoxic activity against colon **(HT-29)** and lung cancer **(A-549)** cell lines. Sulphur substituted compounds **(D-4, D-12)** at C-2 position of DHPM nucleus were found to be more potent than oxygen substituted DHPM. The results demonstrated in Table 3 that the pyrimidine nucleus posses the cytotoxic activity and can be used as anticancer agents.

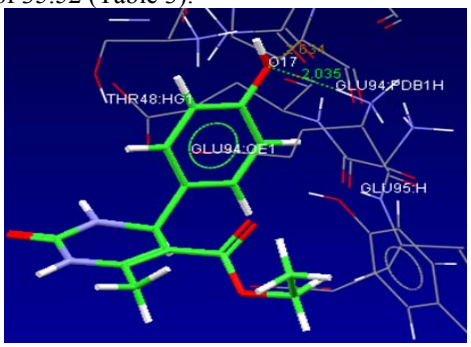
Table 3. Cytotoxic activity results and docking scores of DHPMs

Compound	Percent inhibition, µg/mL		
	IC_{50} HT-29	IC_{50} A-549	Docking score
D-2	189.15	156.62	28.65
D-4	36.74	48.26	29.96
D-5	49.68	56.00	29.09
D-6	137.86	128.23	26.97
D-7	117.12	93.75	28.72
D-11	125.12	132.43	27.88
D-12	30.10	28.36	35.52
D-13	53.75	66.26	29.07
D-14	37.36	42.18	29.53

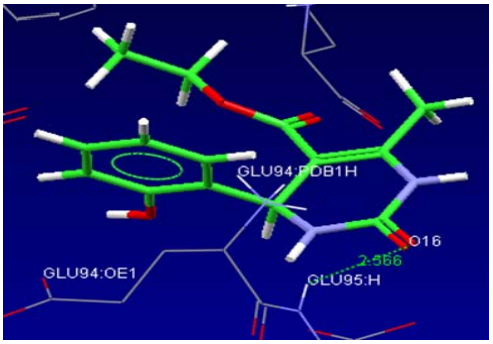
HT-29, human colon carcinoma; A-549, human lung carcinoma

Docking studies

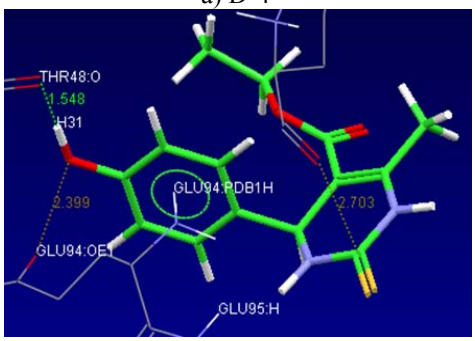
Docking analysis revealed that hydrogen bond interactions were the crucial factors affecting inhibitory action of the compounds. Amino acids Glu-95, Thr-48, His-92, Glu-94 and Asn-9 of Eg5 protein were found to be directly interacting with the DHPMs in the form of hydrogen bond interactions. Most of the synthesized compounds showed hydrogen bonding interaction with His-92. Bioisosteric replacement of thiourea 'S' with urea 'O' in the synthesized compounds (D-4, D-5, D-12 and D-14) appeared to be oriented in similar fashion and retained (Figure 1). The most active compound D-12 (against A-549 and HT-29 cancer cell lines) fitted best in the active site of Eg5 inhibitor protein and attained the score of 35.52 (Table 3).



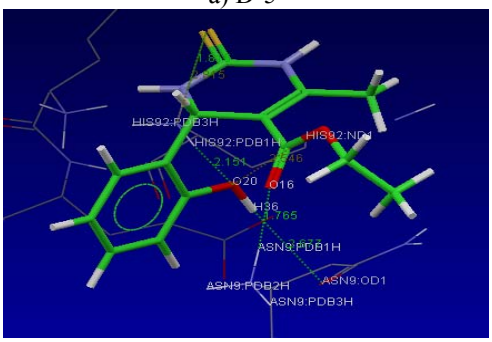
a) D-4



a) D-5



c) D-12



c) D-12

Figure 1. (a) H-bond interactions (green) between compound D-4 and 1QOB **(b)** compound D-5 and 1QOB **(c)** compound D-12 and 1QOB **(d)** compound D-14 and 1QOB

Conclusion

The DHPMs (D-1 to D-15) were synthesized using SDS as novel catalyst under two experimental conditions. The synthesized compounds were characterized by FT-IR, ^{1}H NMR and LC-MS. The synthesized compounds docking studies were carried out on the crystal structure of Eg5 (1QOB) to gain some structural insights on the binding mode and possible interaction with the active site. The top ranked molecule were selectively evaluated, experimentally for their cytotoxic activity using MTT assay method. Among the tested compounds **D-12** shows signifint activity may due to the presence of –OH group at C-4 phenyl ring and sulphur in dihydropyrimidine ring.These studies shows that DHPM's scaffold can be utilized for designing of novel cytotoxic agents.

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