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Research Article

SYNTHESIS AND EVALUATION OF NEW 3- SUBSTITUTED-[3, 4-DIHYDROPYRIMIDINONES]-INDOLIN-2-ONES FOR CYTOTOXIC ACTIVITY

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ABSTRACT

Forty five New 3-substituted [3,4-dihydropyrimidinones]-Indolin-2-ones have been synthesized and tested for in vitro cytotoxic activity against CHO cell lines and MCF-7 cell lines by MTT method.Of them compounds AJ_{31}, AJ_{32}, AJ_{25}, AJ_{22} and AJ_{21} exhibited greater cytotoxic activity.The compounds were more sensitive to CHO cell lines compared to MCF-7 cell lines However,some of the compounds exhibited similar cytotoxic activities to that of standard cisplatin.

Keywords: Dihydropyrimidinones, Indolin-2-ones, Cytotoxic activity, Cell lines

INTRODUCTION

Heterocyclic systems possessing an indole moiety exhibit a number of interesting biological activities such as antiviral, antibacterial, anti fungal, anti-inflammatory, analgesic, diuretic and anticonvulsant activities [1-8]. A lot of work has been carried out on indole derivatives and no work has been carried on 3- substituted [3,4-dihydro pyrimidinones]-Indolin-2-ones. It is also evident from the literature that dihydro pyrimidinones are equally important interms of pharmacological activities such as Calcium channel blockers, antifungal, and antihypertensive agents [9-11]. Therefore, it seemed promising to synthesize some new 3- substituted [3,4-dihydro pyrimidinones]-Indolin-2-ones using the multi component one pot condensation of biginelli's synthesis using Isatin semicarbazone, ethylacetoacetate and aromatic aldehyde [12]. We present here our results on the design of new 3- substituted [3,4-dihydro pyrimidinones]-Indolin-2-ones emphasizing in particular the presence of aromatic nucleas at the 5-postion of 3,4-dihydropyrimidine ring [benzaldehyde,4-chlorobenzaldehyde,4-hydroxybenzaldehyde and 4-methoxybenzaldehyde] in one skeleton (B_1 to B_9, AJ_1 to AJ_{45}, Scheme-1). All the compounds presented here were assayed for antioxidant activity by DPPH method using UV double beam spectrophotometer and Reverse phase HPLC methods.

MATERIALS AND METHODS

Cytotoxic activity

Microculture tetrazolium (MTT) assay[13]

Materials

RPMI-1640 (Himedia, Mumbai, India), Trypsin 0.25% (Gibcous, USA), FBS (fetal bovine serium) (Gibcous USA), MTT 4 mg/ml (Himedia), DMSO (Merck, India), Lysis buffer (15% SLS in 1:1 DMF and water), Composition of RPMI: 9.54 gm/lit, 10% FBS, 2000 mg sodium bicarbonate, 250 µl each of penicillin (60 mg/ml), streptomycin (100 mg/ml), amphotericin (200 mg/ml).

Principle

Microculture tetrazolium assay (MTT) is based on the metabolic reduction of 3-(4,5-dimethylthiazol-2,5-diphenyl)tetrazolium bromide (MTT) to water insoluble formazan crystals with mitochondrial dehydrogenase enzyme, which gives direct correlation of viable cells.

Method

Cell suspension (0.1 ml containing 5 x 10^5 cells / 100 µl), 0.1 ml of the compound solution (10, 20, 50, 100, 150 and 200 µg in DMSO such that the final concentration of DMSO in media is less than 1%) was added to the 96 well plates and kept in carbon dioxide incubator with 5% CO_2 at 37°C for 72 hours. Blank contains only cell suspension and control wells contain 1% DMSO and cell suspension. After 72 hours, 20 µl of MTT was added and kept in carbon dioxide incubator for 2 hours followed by 80 µl of lysis buffer (15% SLS in 1:1 DMF and water). The plate was covered with aluminium foil to protect from light, and then the 96 well plate was kept on rotary shaker for 8 hours. After 8 hours the 96 well plates were processed on ELISA reader for absorption at 562 nm. The readings were averaged and viability of the test samples was compared with DMSO control.

Synthesis of the compounds

The reaction sequence used in the synthesis of the target compounds AJ_{1-45} is depicted in the scheme-1. Isatin semicarbazone B_{1-9} were obtained from appropriate isatin in alcohol with addition of semicarbazide hydrochloride and sodium acetate in water and refluxed on waterbath for about 1 hour [14]. Compounds AJ_{1-45} were synthesized by refluxing B_{1-9} with ethylacetoacetate and appropriate aromatic aldehydes (Benzal dehyde, 4-chlorobenzaldehyde, 4-hydroxybenzaldehyde, 4-methoxy-benzaldehyde and 2-nitro benzaldehyde) by multicomponent one pot condensation using named Bigineli's reaction in presence of catalytic amount of concentrated hydrochloric acid for 10-12 hours. All the newly synthesized compounds were characterized by physical, spectral (IR, Mass, NMR) and Elemental analysis.

Experimental

All reagents used were purchased from Sd fine chemical company, Mumbai, india. Melting points were determined in an open capilries on a galen camp apparatus (Sanyo gallen camp, lough, borough, UK), and were un corrected. IR spectra (KBR, cm^{-1}) were recorded on perkin elmer spectrophotometer(577 model). H1 NMR spectra were recorded on a brukar WM-400 spectrophotometry (in δppm)

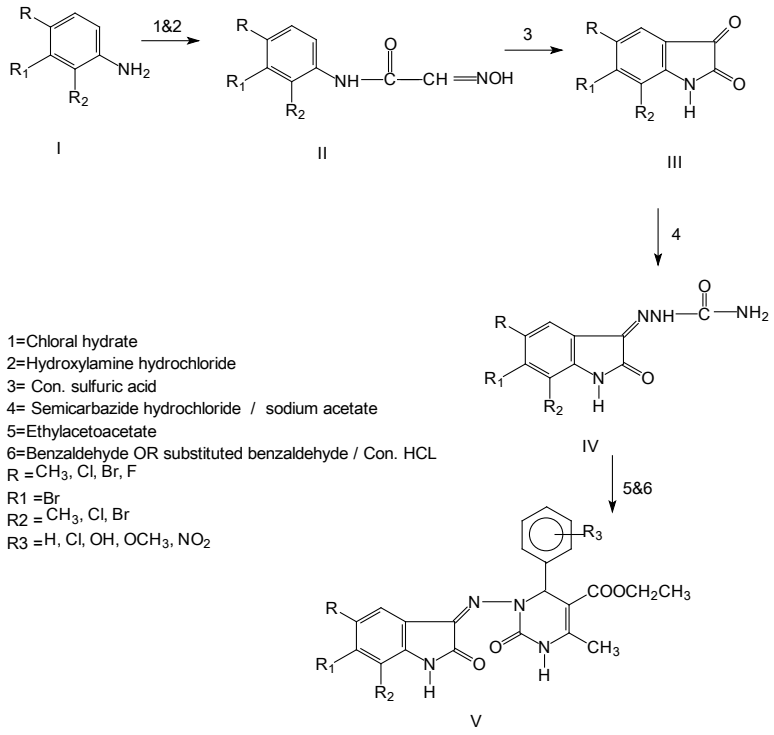
Isatin semicarbazone (B_1 to B_9)

To a stirred solution of an appropriate isatin(A_1 to A_9) 2gm in 20ml of alcohol at room temperature, semicarbazide hydrochloride, sodium acetate dissolved in water is added to the above solution and refluxed on a water bath for about 1hour, the resultant yellow crystalline solid was filtered ,washed repeatedly with small portions of cold water and finally with small portions of cold methanol and recrystllized with methanol to give pure products (B_1 to B_9). The data of the compounds produced was compared data available in the literature.

3- Substituted-[3,4-dihydro pyrimidinones]-Indolin-2-ones ($AJ_{1\ to\ 45}$)

Compounds B_1 to B_9 (2.04gm, 0.01mol), ethylacetoacetate and aromatic aldehyde (0.01 mol), in drymethanol and a few drops of concentrated hydrochloric acid as a catalyst was condensed by multicomponent one pot condensation by named Biginelli's

reaction for 10 to 12 hours on a water bath. The solvent was evaporated, the precipitated solid was poured on to crushed ice, filtered, dried and recrystallized from methanol to give pure products (AJ_1 to AJ_{45}). The compounds obtained were characterized by physical and spectral data .for eg, the yield of the compound C_2 [R_1= H, R_2 = H, R_3 = benzaldehyde] was 2g [65]M.P246 and spectral data (KBr): 159 [NH, indole], 3330 [NH, pyramidine], 1720[NH-CO], 1688 [C=O,indole], 1621[C=N,1360-1280[C-N,1300-1000[C-O]. PMR spectra [inDMSO- D6, ppm] 12.03 [S, 1H, NH indole], 11.73 [S,1H,NH pyrimidine] 6.0-7.0[m, 8H, 2Ar-H], 0.9[t-CH_3] 4.0[q, 2H,O CH_2] 2.20 [S,3H, CH_3]. CompoundsAJ_{1-45}were prepared similarly.



Scheme 1: Schematic diagram of 3- substituted-[3,4-dihydro pyrimidinones]-Indolin-2-ones

R=H, CH_3, F, Cl, Br
R_1= Br
R_2 = Br, CI, CH_3
R_3 = Benzaldehyde, 4-Cl benzaldehyde, 4-OH benzaldehyde, 4-OCH_3 benzaldehyde, 2-nitro benzaldehyde.

Table 1: Physical and spectral data for 3-substituted [3,4-dihydropyrimidinones]-Indolin-2-ones

Com	R	R_1	R_2	R_3	Mol. formula	M.P (°C)	Mass spectra/H¹NMR	Yield (%)
AJ1	H	H	H	H	$C_{22}H_{20}N_4O_4$	243	405,11.70[S,1H,NH indole]11.25[S,1H,NH pyrimidine] 6.6-7.6[m,9H,2Ar-H] 0.9[t,CH_3], 2.25[S,3H,CH_3], 4[q,2H,0CH_2]	68
AJ2	CH_3	H	H	H	$C_{23}H_{22}N_4O_4$	246	12.03[S,1H,NH indole]11.73[S,1H,NH pyrimidine] 6.0-7.0[m,8H,2Ar-H] 0.9[t,CH_3], 2.25[S,3H,CH_3], 4[q,2H,0CH_2]	65
AJ3	F	H	H	H	$C_{22}H_{19}N_4O_4F$	248	11.75[S,1H,NH indole]11.50[S,1H,NH pyrimidine] 6.1-7.2[m,8H,2Ar-H] 0.9[t,CH_3], 2.20[S,3H,CH_3], 4[q,2H,0CH_2]	70
AJ4	Cl	H	H	H	$C_{22}H_{19}N_4O_4C1$	251	11.75[S,1H,NH indole]11.50[S,1H,NH pyrimidine] 6.1-7.2[m,8H,2Ar-H] 0.9[t,CH_3], 2.20[S,3H,CH_3], 4[q,2H,0CH_2]	72
AJ5	Br	H	H	H	$C_{22}H_{19}N_4O_4Br$	252	11.75[S,1H,NH indole]11.50[S,1H,NH pyrimidine] 6.1-7.2[m,8H,2Ar-H] 0.9[t,CH_3], 2.20[S,3H,CH_3], 4[q,2H,0CH2]	68
AJ6	H	Br	H	H	$C_{22}H_{19}N_4O_4Br$	253	11.75[S,1H,NH indole]11.50[S,1H,NH pyrimidine] 6.0-7.2[m,8H,2Ar-H] 0.9[t,CH_3], 2.20[S,3H,CH_3], 4[q,2H,0CH_2]	65
AJ7	Br	H	Br	H	$C_{22}H_{18}N_4O_4Br_2$	255	11.50[S,1H,NH indole]11.25[S,1H,NH pyrimidine] 6.0-7.2[m,6H,2Ar-H] 0.9[t,CH_3], 2.20[S,3H,CH_3], 4[q,2H,0CH_2]	72
AJ8	H	H	CH_3	H	$C_{23}H_{22}N_4O_4$	244	12.0[S,1H,NH indole]11.70[S,1H,NH pyrimidine] 6.0-7.0[m,8H,2Ar-H] 0.9[t,CH_3], 2.25[S,3H,CH3], 3.9[q,2H,0CH_2]	65
AJ9	H	H	C1	H	$C_{22}H_{19}N_4O_4C1$	245	439.5, 11.70[S,1H,NH indole]11.55[S,1H,NH pyrimidine] 6.6-7.6[m,8H,2Ar-H] 0.9[t,CH_3], 2.19[S,3H,CH3], 4.2[q,2H,0CH_2]	67
AJ10	H	H	H	C1	$C_{22}H_{19}N_4O_4C1$	244	439.5, 11.20[S,1H,NH indole]10.80[S,1H,NH pyrimidine] 6.3-7.2[m,7H,2Ar-H] 0.9[t,CH_3], 2.50[S,3H,CH_3], 3.9[q,2H,0CH_2]	67
AJ11	CH_3	H	H	C1	$C_{23}H_{20}N_4O_4C1$	246	11.18[S,1H,NH indole]10.88[S,1H,NH pyrimidine] 6.8-7.8[m,6H,2Ar-H] 1.9[t,CH_3], 2.6[S,3H,CH_3], 3.8[q,2H,0CH2]	66
AJ12	F	H	H	C1	$C_{22}H_{18}N_4O_4C1F$	248	10.99 [S,1H,NH indole]10.80[S,1H,NH pyrimidine] 6.6-7.6[m,6H,2Ar-H] 0.8[t,CH_3], 2.53[S,3H,CH_3], 3.8[q,2H,0CH_2]	65
AJ13	Cl	H	H	C1	$C_{22}H_{18}N_{4254}O_4C1_2$	254	10.99[S,1H,NH indole]10.80[S,1H,NH pyrimidine] 6.6-7.6[m,9H,2Ar-H],2.53[S,3H,CH_3], 0.9[t,CH3],3.9[q ,2H,OCH_2]	66
AJ14	Br	H	H	C1	$C_{22}H_{18}N_4O_4C1Br$	256	10.99[S,1H,NH indole]10.88[S,1H,NH pyrimidine] 6.6-7.6[m,9H,2Ar-H]	67

							0.9[t,CH_3], 2.25[S,3H,CH3], 3.8[q,2H,0CH_2]	
AJ15	H	Br	H	Cl	$C_{22}H_{18}N_4O_4C1Br$	257	10.99[S,1H,NH indole]10.88[S,1H,NH pyrimidine] 6.6-7.6[m,9H,2Ar-H] 0.9[t,CH3], 2.25[S,3H,CH_3], 3.8[q,2H,0CH_2]	66
AJ16	Br	H	Br	Cl	$C_{21}H_{17}N_4O_4C1Br_2$	259	11.00[S,1H,NH indole]10.92[S,1H,NH pyrimidine] 6.4-7.1[m,5H,2Ar-H] 0.9[t,CH_3], 2.25[S,3H,CH_3], 4[q,2H,0CH_2]	67
AJ17	H	H	CH_3	C1	$C_{23}H_{20}N_4O_4C1$	246	11.00[S,1H,NH indole]10.92[S,1H,NH pyrimidine] 6.4-7.1[m,5H,2Ar-H] 0.9[t,CH3], 2.25[S,3H,CH_3], 4[q,2H,0CH_2]	65
AJ18	H	H	Cl	Cl	$C_{22}H_{18}N_4O_4C1_2$	255	11.00[S,1H,NH indole]10.92[S,1H,NH pyrimidine] 6.6-7.6[m,5H,2Ar-H] 0.9[t,CH_3], 2.25[S,3H,CH_3], 4[q,2H,0CH_2]	65
AJ19	H	H	H	OH	$C_{22}H_{19}N_4O_5$	258	420, 11.90[S,1H,NH indole]11.85[S,1H,NH pyrimidine] 6.4-7.4[m,7H,2Ar-H] 0.9[t,CH_3], 2.3[S,3H,CH_3], 4[q,2H,0CH_2]	68
AJ20	CH_3	H	H	OH	$C_{23}H_{21}N_4O_5$	259	11.88[S,1H,NH indole]11.75[S,1H,NH pyrimidine] 6.2-7.4[m,6H,2Ar-H] 1.1[t,,CH_3], 2.6[S,3H,CH_3], 3.8[q,2H,0CH_2]	67
AJ21	F	H	H	OH	$C_{22}H_{18}N_4O_5F$	261	11.92[S,1H,NH indole]11.80[S,1H,NH pyrimidine] 6.6-7.6[m,6H,2Ar-H] 0.9[t,CH_3], 2.25[S,3H,CH_3], 4[q,2H,0CH_2]	66
AJ22	Cl	H	H	OH	$C_{22}H_{18}N_4O_5C1$	263	11.92[S,1H,NH indole]11.80[S,1H,NH pyrimidine] 6.6-7.6[m,6H,2Ar-H] 0.9[t,CH_3], 2.25[S,3H,CH_3], 4[q,2H,0CH_2]	67
AJ23	Br	H	H	OH	$C_{22}H_{18}N_4O_5Br$	266	11.92[S,1H,NH indole]11.80[S,1H,NH pyrimidine] 6.6-7.6[m,6H,2Ar-H] 0.9[t,CH_3], 2.25[S,3H,CH_3], 4[q,2H,0CH_2]	68
AJ24	H	Br	H	OH	$C_{22}H_{18}N_4O_5Br$	267	11.92[S,1H,NH indole]11.80[S,1H,NH pyrimidine] 6.6-7.6[m,6H,2Ar-H] 0.9[t,CH_3], 2.25[S,3H,CH_3], 4[q,2H,0CH_2]	66
AJ25	Br	H	Br	OH	$C_{22}H_{17}N_4O_5Br_2$	270	11.80[S,1H,NH indole]11.00[S,1H,NH pyrimidine] 6.6-7.6[m,5H,2Ar-H] 1.0[t,CH_3], 2.20[S,3H,CH_3], 3.9[q,2H,0CH_2]	68
AJ26	H	H	CH_3	OH	$C_{23}H_{21}N_4O_5$	258	11.88[S,1H,NH indole]11.75[S,1H,NH pyrimidine] 6.2-7.4[m,6H,2Ar-H] 0.9[t,CH_3], 2.6[S,3H,CH_3],3.8[q,2H,0CH_2],3.8[S,3H,0CH_3]	65
AJ27	H	H	Cl	OH	$C_{23}H_{22}N_4O_5$	263	11.88[S,1H,NH indole]11.75[S,1H,NH pyrimidine] 6.2-7.4[m,6H,2Ar-H] 0.9[t,CH3], 2.6[HS,3H,CH3], 3.8[q,2H,0CH_2]	66
AJ28	H	H	H	OCH_3	$C_{23}H_{22}N_4O_5$	245	43, 11.99[S,1H,NH indole]12.0[S,1H,NH pyrimidine] 6.5-7.6[m,7H,2Ar-H] 0.8[t,CH_3], 2.6[S,3H,CH_3], 3.8[q,2H,0CH_2],3.8[S,3H,CH_3]	64
AJ29	CH_3	H	H	OCH_3	$C_{24}H_{23}N_4O_5$	246	11.90[S,1H,NH indole]11.75[S,1H,NH pyrimidine] 6.6-7.6[m,7H,2Ar-H] 0.9[t,CH_3, 2.5[S,3H,CH_3], 4[q,2H,0CH_2],6.4[S,3H,CH_3],3.8[S,3H,0CH_3]	63
AJ30	F	H	H	OCH_3	$C_{23}H_{21}N_4O_5F$	248	12.70[S,1H,NH indole]12.0[S,1H,NH pyrimidine] 6.2-7.6[m,7H,2Ar-H] 0.9[t,CH_3], 2.5[S,3H,CH_3], 4[q,2H,0CH_2],3.8[S,3H,OCH_3-Ar]	64
AJ31	Cl	H	H	OCH_3	$C_{23}H_{21}N_4O_5C1$	250	12.70[S,1H,NH indole]12.5[S,1H,NH pyrimidine] 6.6-7.6[m,7,2Ar-H] 0.9[t,CH_3], 2.5[S,3H,CH_3], 4[q,2H,0CH_2],3.8[S,3H,0CH_3]	64
AJ32	Br	H	H	OCH_3	$C_{23}H_{21}N_4O_5Br$	252	12.70[S,1H,NH indole]12.20[S,1H,NH pyrimidine] 6.6-7.6[m,7H,2Ar-H] 0.9[t,CH_3], 2.5[S,3H,CH_3], 4[q,2H,	65
AJ33	H	Br	H	OCH_3	$C_{23}H_{21}N_4O_5Br$	251	12.70[S,1H,NH indole]11.75[S,1H,NH pyrimidine] 6.6-7.6[m,7H,2Ar-H] 0.9[t,CH_3], 2.25[S,3H,CH_3], 4[q,2H,0CH_2]	63
AJ34	Br	H	Br	OCH_3	$C_{23}H_{20}N_4O_5Br_2$	254	12.70[S,1H,NH indole]11.75[S,1H,NH pyrimidine] 6.6-7.6[m,7H,2Ar-H] 0.9[t,CH_3], 2.25[S,3H,CH_3], 4[q,2H,0CH_2]	64
AJ35	H	H	CH_3	OCH_3	$C_{24}H_{23}N_4O_5$	246	12.70[S,1H,NH indole]11.75[S,1H,NH pyrimidine] 6.6-7.6[m,7H,2Ar-H] 0.9[t,CH_3], 2.25[S,3H,CH_3], 4[q,2H,0CH_2]	62
AJ36	H	H	Cl	OCH_3	$C_{23}H_{21}N_4O_5C1$	251	12.70[S,1H,NH indole]11.75[S,1H,NH pyrimidine] 6.6-7.6[m,7H,2Ar-H] 0.9[t,CH_3], 2.25[S,3H,CH_3], 4[q,2H,0CH_2]	63
AJ37	H	H	H	NO_2	$C_{23}H_{21}N_4O_5C1$	251	12.70[S,1H,NH indole]11.75[S,1H,NH pyrimidine] 6.6-7.6[m,7H,2Ar-H] 0.9[t,CH_3], 2.25[S,3H,CH_3], 4[q,2H,0CH_2]	64
AJ38	CH3	H	H	NO_2	$C_{23}H_{21}N_4O_5C1$	251	12.70[S,1H,NH indole]11.75[S,1H,NH pyrimidine] 6.6-7.6[m,7H,2Ar-H] 0.9[t,CH_3], 2.25[S,3H,CH_3], 4[q,2H,0CH_2]	63
AJ39	F	H	H	NO_2	$C_{23}H_{21}N_4O_5C1$	251	12.70[S,1H,NH indole]11.75[S,1H,NH pyrimidine] 6.6-7.6[m,7H,2Ar-H] 0.9[t,CH_3], 2.25[S,3H,CH_3], 4[q,2H,0CH_2]	64
AJ40	CI	H	H	NO_2	$C_{23}H_{21}N_4O_5C1$	251	12.70[S,1H,NH indole]11.75[S,1H,NH pyrimidine] 6.6-7.6[m,7H,2Ar-H] 0.9[t,CH_3], 2.25[S,3H,CH_3], 4[q,2H,0CH_2]	65
AJ41	Br	H	H	NO_2	$C_{23}H_{21}N_4O_5C1$	251	12.70[S,1H,NH indole]11.75[S,1H,NH pyrimidine] 6.6-7.6[m,7H,2Ar-H] 0.9[t,CH_3], 2.25[S,3H,CH_3], 4[q,2H,0CH_2]	64
AJ42	H	Br	H	NO_2	$C_{23}H_{21}N_4O_5C1$	251	12.70[S,1H,NH indole]11.75[S,1H,NH pyrimidine] 6.6-7.6[m,7H,2Ar-H] 0.9[t,CH_3], 2.25[S,3H,CH_3], 4[q,2H,0CH_2]	63
AJ43	Br	H	Br	NO_2	$C_{23}H_{21}N_4O_5C1$	251	12.70[S,1H,NH indole]11.75[S,1H,NH pyrimidine] 6.6-7.6[m,7H,2Ar-H] 0.9[t,CH_3], 2.25[S,3H,CH_3], 4[q,2H,0CH_2]	64
AJ44	H	H	CH3	NO_2	$C_{23}H_{22}N_5O_6$	271	12.70[S,1H,NH indole]11.75[S,1H,NH pyrimidine] 6.6-7.6[m,7H,2Ar-H] 0.9[t,CH_3], 2.25[S,3H,CH_3], 4[q,2H,0CH_2]	62
AJ45	H	H	CI	NO_2	$C_{22}H_{18}N_4O_6C1$	277	12.70[S,1H,NH indole]11.75[S,1H,NH pyrimidine] 6.6-7.6[m,7H,2Ar-H] 0.9[t,CH_3], 2.25[S,3H,CH_3], 4[q,2H,0CH_2]	63

RESULTS

Compounds AJ_1 to AJ_{45} consisting of five series, X-3 [(4-phenyl-5-carboethoxy-6-methyl-3,4-dihydropyrimidin[1H]-2-one) indolin-2-ones], Y-3 [(4-chlorophenyl-5- carboethoxy-6-methyl-3, 4-dihydro pyrimidin [1H]-2-one) indolin-2-ones], Z-3[(4-hydroxyphenyl-5 carboethoxy -6-methyl-3,4-dihydropyrimidin [1H]-2-one)indolin-2-ones] X_1-3[(4-methoxyphenyl-5-carboethoxy-6-methyl-3, 4-dihydropyrimidin[1H]-2-one) indolin-2-ones and Y_1[2-nitro-phenyl-5carboethoxy-6-methyl-3,4-dihydropyrimidin[1H]-2-one) indolin-2-ones] exibited in vitro cytotoxic activity against CHO cell lines and MCF-7 cell lines. The activity was represented by percent protection. All the results are depicted in table 2 to 6.

DISCUSSION

The activity was represented by IC50 values in Micro moles (µM). Among all the series,compounds AJ31(R=CI, R_1=R_2=H), AJ32 (R=Br,R_1=R_2=H)and AJ25 (R=R_2=Br,R_1=H), showed more Cytotoxic activity with IC50 values of 14.0and120.2,17.6 and 98.2 and 120.2µM and 25.9 and 95 µM respectively against CHO and MCF-7 cell lines.

Compounds AJ23 (R=Br,R_1=R_2=H), AJ22 (R=Cl,R_1=R_2=H) and AJ21 (R=F,R_1=R2=H) were next in the order of Cytotoxic activity with IC_{50} values of31.85, 32.75 and 36.58 uM against CHO cell lines. Compounds showed more Cytotoxic activity against CHO cell lines compared to MCF-7 cell lines.

Table 2: Cytotoxic activity *of* 3[(4-phenyl-5-carboethoxy-6-methyl-3, 4- Dihydropyrimidin [1H]-2-one) Indolin-2-ones] by MTT method

S. No.	Compound	R	R1	R2	CHO cell lines	MCF-7 cell lines
1	AJ1	H	H	H	352	368
2	AJ2	CH3	H	H	320	301
3	AJ3	F	H	H	322	258
4	AJ4	Cl	H	H	248	201
5	AJ5	Br	H	H	256	240
6	AJ6	H	Br	H	198	296
7	AJ7	Br	H	Br	131	160
8	AJ8	H	H	CH_3	296	284
9	AJ9	H	H	Cl	210	194

Cisplatin at all concentration ranges tested showed an IC_{50} of 25um

Table 3: Cytotoxic activity *of* 3[(4-Chlorophenyl-5-carboethoxy-6-methyl-3,4- Dihydropyrimidin [1H]-2-one)Indolin-2-ones] by MTT method

S. No.	Compound	R	R1	R2	CHO cell lines	MCF-7 cell lines
1	AJ10	H	H	H	141.20	209.0
2	AJ11	CH_3	H	H	82.92	180.6
3	AJ12	F	H	H	37.07	152.2
4	AJ13	Cl	H	H	33.94	120.4
5	AJ14	Br	H	H	31.02	110.1
6	AJ15	H	Br	H	42.50	168.8
7	AJ16	Br	H	Br	74.20	63.9
8	AJ17	H	H	CH_3	94.63	183.5
9	AJ18	H	H	Cl	52.01	125.4

Cisplatin at all concentration ranges tested showed an IC_{50} of 25uM

Table 4: Cytotoxic activity of 3[(4-Hydroxyphenyl-5-carboethoxy-6-methyl-3,4-Dihydropyrimidin [1H]-2-one)Indolin-2-ones] by MTT method

S. No.	Compound	R	R1	R2	CHO cell lines	MCF-7 cell lines
1	AJ19	H	H	H	120.8	180.8
2	AJ20	CH_3	H	H	56.4	160.7
3	AJ21	F	H	H	36.58	124.4
4	AJ22	Cl	H	H	32.75	102.2
5	AJ23	Br	H	H	25.08	74.3
6	AJ24	H	Br	H	77.9	121.6
7	AJ25	Br	H	Br	15.9	95.0
8	AJ26	H	H	CH_3	89.06	168.8
9	AJ27	H	H	Cl	69.54	120.4

Cisplatin t all concentration ranges tested showed an IC_{50} of 25um

Table 5: Cytotoxic activity *of* 3[(4-Methoxyphenyl-5-carboethoxy-6-methyl-3,4-dihydropyrimidin[1H]-2-one)Indolin-2-ones] by MTT method

S. No.	Compound	R	R1	R2	CHO cell lines	MCF-7 cell lines
1	AJ28	H	H	H	130.45	186.6
2	AJ29	CH_3	H	H	125.7	172.6
3	AJ30	F	H	H	120.4	160.0
4	AJ31	Cl	H	H	14.10	120.2
5	AJ32	Br	H	H	17.26	98.2
6	AJ33	H	Br	H	84.03	94.6
7	AJ34	Br	H	Br	123.0	210.0
8	AJ35	H	H	CH_3	135.6	154.5
9	AJ36	H	H	Cl	68.7	110.9

Cisplatin at all concentration ranges tested showed an IC_{50} of 25um

Table 6: Cytotoxic activity of 3[(3-Nitrophenyl-5-carboethoxy-6-methyl-3,4-dihydropyrimidin[1H]-2-one)Indolin-2-ones] by MTT method

S. No.	Compound	R	R1	R2	CHO cell lines	MCF-7 cell lines
1	AJ37	H	H	H	325.4	306.8
2	AJ38	CH_3	H	H	309.4	254
3	AJ39	F	H	H	302.9	233.8
4	AJ40	Cl	H	H	118	131.7
5	AJ41	Br	H	H	204	220
6	AJ42	H	Br	H	282	294.2
7	AJ43	Br	H	Br	33	112.9
8	AJ44	H	H	CH_3	320.4	302
9	AJ45	H	H	Cl	154	168.4

Cisplatin at all concentration ranges tested showed an IC_{50} of 25um

CONCLUSION

Hence we conclude that Z series of Compounds having 4-hydroxy substituent and X_1 Series having 4-methoxy substituent at 5-position of pyrimidine ring exhibited more cytotoxic activity[AJ_{31}>AJ_{32}> AJ_{25}> AJ_{23} > AJ_{22}] and AJ_{21}followed by X, Y and Y1 series. Among Isatins, 5,7-disubstituted halogens are more active than mono subsituted halogens against cytotoxic activity followed by Br,C1,F. The rest of the compounds showed moderate Cytotoxic activities.The IC50 value of cisplatin was found to be 25um.

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