

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

M. AJITHA\*<sup>1</sup>, K. RAJNARAYANA<sup>2</sup>

<sup>1</sup> Center for Pharmaceutical Sciences, IST, JNTU, Kukatpally, Hyderabad- 500085, India, <sup>2</sup> Glukem Pharmaceutical (P) Ltd, IDA, Phase II, Cherlapally, Hyderabad 500052, India. Email: phd03@rediffmail.com

Received: 1 June 2011, Revised and Accepted: 26 July 2011

### 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<sub>31</sub>, AJ<sub>32</sub>, AJ<sub>25</sub>, AJ<sub>22</sub> and AJ<sub>21</sub> 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<sup>1-8</sup>. 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<sup>9-11</sup>. 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<sup>12</sup>. 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 nucleus at the 5-position of 3,4-dihydropyrimidine ring [benzaldehyde, 4-chlorobenzaldehyde, 4-hydroxybenzaldehyde and 4-methoxybenzaldehyde] in one skeleton (B<sub>1</sub> to B<sub>9</sub>, AJ<sub>1</sub> to AJ<sub>45</sub>, 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<sup>13</sup>

#### Materials

RPMI-1640 (Himedia, Mumbai, India), Trypsin 0.25% (Gibco, USA), FBS (fetal bovine serum) (Gibco 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<sup>5</sup> 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<sub>2</sub> 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<sub>1-45</sub> is depicted in the scheme-1. Isatin semicarbazone B<sub>1-9</sub> 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<sup>14</sup>. Compounds AJ<sub>1-45</sub> were synthesized by refluxing B<sub>1-9</sub> with ethylacetoacetate and appropriate aromatic aldehydes (Benzaldehyde, 4-chlorobenzaldehyde, 4-hydroxybenzaldehyde, 4-methoxybenzaldehyde and 2-nitrobenzaldehyde) by multicomponent one pot condensation using named Biginelli'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 capillaries on a galen camp apparatus (Sanyo galen camp, lough, borough, UK), and were uncorrected. IR spectra (KBR, cm<sup>-1</sup>) were recorded on perkin elmer spectrophotometer (577 model). H1 NMR spectra were recorded on a Bruker WM-400 spectrophotometry (in δppm)

#### Isatin semicarbazone (B<sub>1</sub> to B<sub>9</sub>)

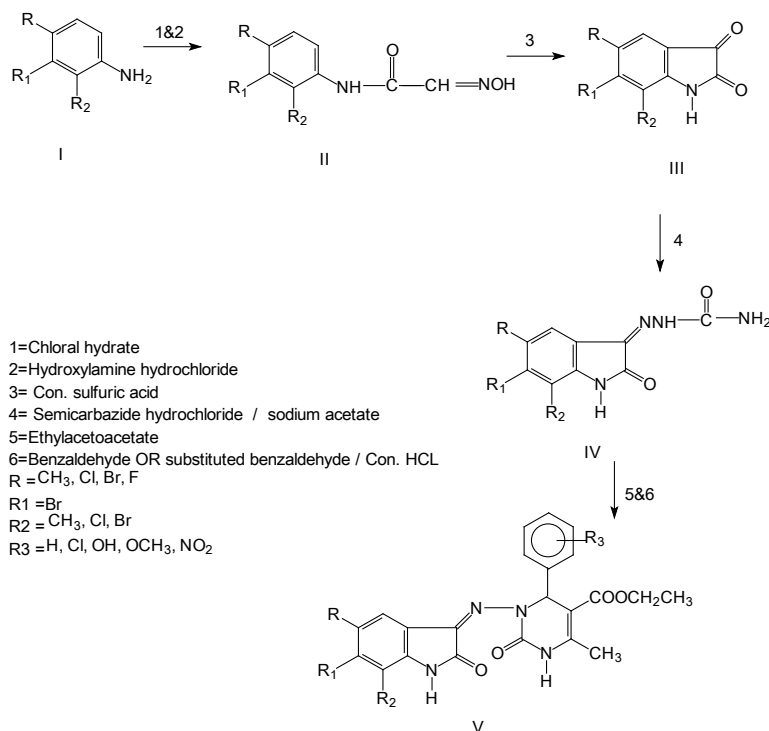
To a stirred solution of an appropriate isatin (A<sub>1</sub> to A<sub>9</sub>) 2gm in 20ml of alcohol at room temperature, semicarbazide hydrochloride, sodium acetate dissolved in water is added to the above solution and refluxed on a water bath for about 1 hour, the resultant yellow crystalline solid was filtered, washed repeatedly with small portions of cold water and finally with small portions of cold methanol and recrystallized with methanol to give pure products (B<sub>1</sub> to B<sub>9</sub>). The data of the compounds produced was compared data available in the literature.

#### 3- Substituted-[3,4-dihydro pyrimidinones]-Indolin-2-ones (AJ<sub>1 to 45</sub>)

Compounds B<sub>1</sub> to B<sub>9</sub> (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<sub>1</sub> to AJ<sub>45</sub>). The compounds obtained were characterized by physical and spectral data .for eg, the yield of the compound C<sub>2</sub> [R<sub>1</sub>= H, R<sub>2</sub> = H, R<sub>3</sub> = benzaldehyde] was 2g

[65]M.P246 and spectral data (KBr): 159 [NH, indole], 3330 [NH, pyrimidine], 1720[NH-CO], 1688 [C=O,indole], 1621[C=N,1360-1280[C-N,1300-1000[C-O]. PMR spectra [inDMSO- D<sub>6</sub>, 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<sub>3</sub>] 4.0[q, 2H,O CH<sub>2</sub>] 2.20 [S,3H, CH<sub>3</sub>]. CompoundsAJ<sub>1-45</sub> were prepared similarly.



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

R=H, CH<sub>3</sub>, F, Cl, Br

R<sub>1</sub>= Br

R<sub>2</sub> = Br, Cl, CH<sub>3</sub>

R<sub>3</sub> = Benzaldehyde, 4-Cl benzaldehyde, 4-OH benzaldehyde, 4-OCH<sub>3</sub> benzaldehyde, 2-nitro benzaldehyde.

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

Com	R	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	Mol. formula	M.P (°C)	Mass spectra/H <sup>1</sup> NMR	Yield (%)
AJ <sub>1</sub>	H	H	H	H	C <sub>22</sub> H <sub>20</sub> N <sub>4</sub> O <sub>4</sub>	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 <sub>3</sub> ], 2.25[S,3H,CH <sub>3</sub> ], 4[q,2H,0CH <sub>2</sub> ]	68
AJ <sub>2</sub>	CH <sub>3</sub>	H	H	H	C <sub>23</sub> H <sub>22</sub> N <sub>4</sub> O <sub>4</sub>	246	12.03[S,1H,NH indole]11.73[S,1H,NH pyrimidine] 6.0-7.0[m,8H,2Ar-H]	65
AJ <sub>3</sub>	F	H	H	H	C <sub>22</sub> H <sub>19</sub> N <sub>4</sub> O <sub>4</sub> F	248	11.75[S,1H,NH indole]11.50[S,1H,NH pyrimidine] 6.1-7.2[m,8H,2Ar-H]	70
AJ <sub>4</sub>	Cl	H	H	H	C <sub>22</sub> H <sub>19</sub> N <sub>4</sub> O <sub>4</sub> Cl	251	0.9[t,CH <sub>3</sub> ], 2.20[S,3H,CH <sub>3</sub> ], 4[q,2H,0CH <sub>2</sub> ]	72
AJ <sub>5</sub>	Br	H	H	H	C <sub>22</sub> H <sub>19</sub> N <sub>4</sub> O <sub>4</sub> Br	252	11.75[S,1H,NH indole]11.50[S,1H,NH pyrimidine] 6.1-7.2[m,8H,2Ar-H]	68
AJ <sub>6</sub>	H	Br	H	H	C <sub>22</sub> H <sub>19</sub> N <sub>4</sub> O <sub>4</sub> Br	253	0.9[t,CH <sub>3</sub> ], 2.20[S,3H,CH <sub>3</sub> ], 4[q,2H,0CH <sub>2</sub> ]	65
AJ <sub>7</sub>	Br	H	Br	H	C <sub>22</sub> H <sub>18</sub> N <sub>4</sub> O <sub>4</sub> Br <sub>2</sub>	255	11.75[S,1H,NH indole]11.50[S,1H,NH pyrimidine] 6.0-7.2[m,8H,2Ar-H]	72
AJ <sub>8</sub>	H	H	CH <sub>3</sub>	H	C <sub>23</sub> H <sub>22</sub> N <sub>4</sub> O <sub>4</sub>	244	11.50[S,1H,NH indole]11.25[S,1H,NH pyrimidine] 6.0-7.2[m,6H,2Ar-H]	65
AJ <sub>9</sub>	H	H	Cl	H	C <sub>22</sub> H <sub>19</sub> N <sub>4</sub> O <sub>4</sub> Cl	245	0.9[t,CH <sub>3</sub> ], 2.25[S,3H,CH <sub>3</sub> ], 3.9[q,2H,0CH <sub>2</sub> ]	67
AJ <sub>10</sub>	H	H	H	Cl	C <sub>22</sub> H <sub>19</sub> N <sub>4</sub> O <sub>4</sub> Cl	244	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 <sub>3</sub> ], 2.19[S,3H,CH <sub>3</sub> ], 4.2[q,2H,0CH <sub>2</sub> ]	67
AJ <sub>11</sub>	CH <sub>3</sub>	H	H	Cl	C <sub>23</sub> H <sub>20</sub> N <sub>4</sub> O <sub>4</sub> Cl	246	7.2[m,7H,2Ar-H] 0.9[t,CH <sub>3</sub> ], 2.50[S,3H,CH <sub>3</sub> ], 3.9[q,2H,0CH <sub>2</sub> ]	66
AJ <sub>12</sub>	F	H	H	Cl	C <sub>22</sub> H <sub>18</sub> N <sub>4</sub> O <sub>4</sub> ClF	248	11.18[S,1H,NH indole]10.88[S,1H,NH pyrimidine] 6.8-7.8[m,6H,2Ar-H]	65
AJ <sub>13</sub>	Cl	H	H	Cl	C <sub>22</sub> H <sub>18</sub> N <sub>4</sub> O <sub>4</sub> Cl <sub>2</sub>	254	1.9[t,CH <sub>3</sub> ], 2.6[S,3H,CH <sub>3</sub> ], 3.8[q,2H,0CH <sub>2</sub> ]	66
AJ <sub>14</sub>	Br	H	H	Cl	C <sub>22</sub> H <sub>18</sub> N <sub>4</sub> O <sub>4</sub> ClBr	256	10.99 [S,1H,NH indole]10.80[S,1H,NH pyrimidine] 6.6-7.6[m,6H,2Ar-H]	65
							0.8[t,CH <sub>3</sub> ], 2.53[S,3H,CH <sub>3</sub> ], 3.8[q,2H,0CH <sub>2</sub> ]	66
							10.99[S,1H,NH indole]10.80[S,1H,NH pyrimidine] 6.6-7.6[m,9H,2Ar-H]	66
							2.53[S,3H,CH <sub>3</sub> ], 0.9[t,CH <sub>3</sub> ],3.9[q,2H,0CH <sub>2</sub> ]	67
							10.99[S,1H,NH indole]10.88[S,1H,NH pyrimidine] 6.6-7.6[m,9H,2Ar-H]	67

AJ15	H	Br	H	Cl	C <sub>22</sub> H <sub>18</sub> N <sub>4</sub> O <sub>4</sub> C1Br	257	0.9[t,CH <sub>3</sub> ], 2.25[S,3H,CH <sub>3</sub> ], 3.8[q,2H,0CH <sub>2</sub> ] 10.99[S,1H,NH indole]10.88[S,1H,NH pyrimidine] 6.6-7.6[m,9H,2Ar-H]	66
AJ16	Br	H	Br	Cl	C <sub>21</sub> H <sub>17</sub> N <sub>4</sub> O <sub>4</sub> C1Br <sub>2</sub>	259	0.9[t,CH <sub>3</sub> ], 2.25[S,3H,CH <sub>3</sub> ], 3.8[q,2H,0CH <sub>2</sub> ] 11.00[S,1H,NH indole]10.92[S,1H,NH pyrimidine] 6.4-7.1[m,5H,2Ar-H]	67
AJ17	H	H	CH <sub>3</sub>	C1	C <sub>23</sub> H <sub>20</sub> N <sub>4</sub> O <sub>4</sub> C1	246	0.9[t,CH <sub>3</sub> ], 2.25[S,3H,CH <sub>3</sub> ], 4[q,2H,0CH <sub>2</sub> ] 11.00[S,1H,NH indole]10.92[S,1H,NH pyrimidine] 6.4-7.1[m,5H,2Ar-H]	65
AJ18	H	H	Cl	Cl	C <sub>22</sub> H <sub>18</sub> N <sub>4</sub> O <sub>4</sub> C1 <sub>2</sub>	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 <sub>3</sub> ], 2.25[S,3H,CH <sub>3</sub> ], 4[q,2H,0CH <sub>2</sub> ]	65
AJ19	H	H	H	OH	C <sub>22</sub> H <sub>19</sub> N <sub>4</sub> O <sub>5</sub>	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 <sub>3</sub> ], 2.3[S,3H,CH <sub>3</sub> ], 4[q,2H,0CH <sub>2</sub> ]	68
AJ20	CH <sub>3</sub>	H	H	OH	C <sub>23</sub> H <sub>21</sub> N <sub>4</sub> O <sub>5</sub>	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 <sub>3</sub> ], 2.6[S,3H,CH <sub>3</sub> ], 3.8[q,2H,0CH <sub>2</sub> ]	67
AJ21	F	H	H	OH	C <sub>22</sub> H <sub>18</sub> N <sub>4</sub> O <sub>5</sub> F	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 <sub>3</sub> ], 2.25[S,3H,CH <sub>3</sub> ], 4[q,2H,0CH <sub>2</sub> ]	66
AJ22	Cl	H	H	OH	C <sub>22</sub> H <sub>18</sub> N <sub>4</sub> O <sub>5</sub> C1	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 <sub>3</sub> ], 2.25[S,3H,CH <sub>3</sub> ], 4[q,2H,0CH <sub>2</sub> ]	67
AJ23	Br	H	H	OH	C <sub>22</sub> H <sub>18</sub> N <sub>4</sub> O <sub>5</sub> Br	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 <sub>3</sub> ], 2.25[S,3H,CH <sub>3</sub> ], 4[q,2H,0CH <sub>2</sub> ]	68
AJ24	H	Br	H	OH	C <sub>22</sub> H <sub>18</sub> N <sub>4</sub> O <sub>5</sub> Br	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 <sub>3</sub> ], 2.25[S,3H,CH <sub>3</sub> ], 4[q,2H,0CH <sub>2</sub> ]	66
AJ25	Br	H	Br	OH	C <sub>22</sub> H <sub>17</sub> N <sub>4</sub> O <sub>5</sub> Br <sub>2</sub>	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 <sub>3</sub> ], 2.20[S,3H,CH <sub>3</sub> ], 3.9[q,2H,0CH <sub>2</sub> ]	68
AJ26	H	H	CH <sub>3</sub>	OH	C <sub>23</sub> H <sub>21</sub> N <sub>4</sub> O <sub>5</sub>	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 <sub>3</sub> ], 2.6[S,3H,CH <sub>3</sub> ], 3.8[q,2H,0CH <sub>2</sub> ], 3.8[S,3H,0CH <sub>3</sub> ]	65
AJ27	H	H	Cl	OH	C <sub>23</sub> H <sub>22</sub> N <sub>4</sub> O <sub>5</sub>	263	11.88[S,1H,NH indole]11.75[S,1H,NH pyrimidine] 6.2-7.4[m,6H,2Ar-H] 0.9[t,CH <sub>3</sub> ], 2.6[HS,3H,CH <sub>3</sub> ], 3.8[q,2H,0CH <sub>2</sub> ]	66
AJ28	H	H	H	OCH <sub>3</sub>	C <sub>23</sub> H <sub>22</sub> N <sub>4</sub> O <sub>5</sub>	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 <sub>3</sub> ], 2.6[S,3H,CH <sub>3</sub> ], 3.8[q,2H,0CH <sub>2</sub> ], 3.8[S,3H,CH <sub>3</sub> ]	64
AJ29	CH <sub>3</sub>	H	H	OCH <sub>3</sub>	C <sub>24</sub> H <sub>23</sub> N <sub>4</sub> O <sub>5</sub>	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 <sub>3</sub> ], 2.5[S,3H,CH <sub>3</sub> ], 4[q,2H,0CH <sub>2</sub> ], 6.4[S,3H,CH <sub>3</sub> ], 3.8[S,3H,0CH <sub>3</sub> ]	63
AJ30	F	H	H	OCH <sub>3</sub>	C <sub>23</sub> H <sub>21</sub> N <sub>4</sub> O <sub>5</sub> F	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 <sub>3</sub> ], 2.5[S,3H,CH <sub>3</sub> ], 4[q,2H,0CH <sub>2</sub> ], 3.8[S,3H,0CH <sub>3</sub> -Ar]	64
AJ31	Cl	H	H	OCH <sub>3</sub>	C <sub>23</sub> H <sub>21</sub> N <sub>4</sub> O <sub>5</sub> C1	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 <sub>3</sub> ], 2.5[S,3H,CH <sub>3</sub> ], 4[q,2H,0CH <sub>2</sub> ], 3.8[S,3H,0CH <sub>3</sub> ]	64
AJ32	Br	H	H	OCH <sub>3</sub>	C <sub>23</sub> H <sub>21</sub> N <sub>4</sub> O <sub>5</sub> Br	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 <sub>3</sub> ], 2.5[S,3H,CH <sub>3</sub> ], 4[q,2H,	65
AJ33	H	Br	H	OCH <sub>3</sub>	C <sub>23</sub> H <sub>21</sub> N <sub>4</sub> O <sub>5</sub> Br	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 <sub>3</sub> ], 2.25[S,3H,CH <sub>3</sub> ], 4[q,2H,0CH <sub>2</sub> ]	63
AJ34	Br	H	Br	OCH <sub>3</sub>	C <sub>23</sub> H <sub>20</sub> N <sub>4</sub> O <sub>5</sub> Br <sub>2</sub>	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 <sub>3</sub> ], 2.25[S,3H,CH <sub>3</sub> ], 4[q,2H,0CH <sub>2</sub> ]	64
AJ35	H	H	CH <sub>3</sub>	OCH <sub>3</sub>	C <sub>24</sub> H <sub>23</sub> N <sub>4</sub> O <sub>5</sub>	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 <sub>3</sub> ], 2.25[S,3H,CH <sub>3</sub> ], 4[q,2H,0CH <sub>2</sub> ]	62
AJ36	H	H	Cl	OCH <sub>3</sub>	C <sub>23</sub> H <sub>21</sub> N <sub>4</sub> O <sub>5</sub> C1	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 <sub>3</sub> ], 2.25[S,3H,CH <sub>3</sub> ], 4[q,2H,0CH <sub>2</sub> ]	63
AJ37	H	H	H	NO <sub>2</sub>	C <sub>23</sub> H <sub>21</sub> N <sub>4</sub> O <sub>5</sub> C1	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 <sub>3</sub> ], 2.25[S,3H,CH <sub>3</sub> ], 4[q,2H,0CH <sub>2</sub> ]	64
AJ38	CH <sub>3</sub>	H	H	NO <sub>2</sub>	C <sub>23</sub> H <sub>21</sub> N <sub>4</sub> O <sub>5</sub> C1	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 <sub>3</sub> ], 2.25[S,3H,CH <sub>3</sub> ], 4[q,2H,0CH <sub>2</sub> ]	63
AJ39	F	H	H	NO <sub>2</sub>	C <sub>23</sub> H <sub>21</sub> N <sub>4</sub> O <sub>5</sub> C1	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 <sub>3</sub> ], 2.25[S,3H,CH <sub>3</sub> ], 4[q,2H,0CH <sub>2</sub> ]	64
AJ40	Cl	H	H	NO <sub>2</sub>	C <sub>23</sub> H <sub>21</sub> N <sub>4</sub> O <sub>5</sub> C1	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 <sub>3</sub> ], 2.25[S,3H,CH <sub>3</sub> ], 4[q,2H,0CH <sub>2</sub> ]	65
AJ41	Br	H	H	NO <sub>2</sub>	C <sub>23</sub> H <sub>21</sub> N <sub>4</sub> O <sub>5</sub> C1	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 <sub>3</sub> ], 2.25[S,3H,CH <sub>3</sub> ], 4[q,2H,0CH <sub>2</sub> ]	64
AJ42	H	Br	H	NO <sub>2</sub>	C <sub>23</sub> H <sub>21</sub> N <sub>4</sub> O <sub>5</sub> C1	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 <sub>3</sub> ], 2.25[S,3H,CH <sub>3</sub> ], 4[q,2H,0CH <sub>2</sub> ]	63
AJ43	Br	H	Br	NO <sub>2</sub>	C <sub>23</sub> H <sub>21</sub> N <sub>4</sub> O <sub>5</sub> C1	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 <sub>3</sub> ], 2.25[S,3H,CH <sub>3</sub> ], 4[q,2H,0CH <sub>2</sub> ]	64
AJ44	H	H	CH <sub>3</sub>	NO <sub>2</sub>	C <sub>23</sub> H <sub>22</sub> N <sub>5</sub> O <sub>6</sub>	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 <sub>3</sub> ], 2.25[S,3H,CH <sub>3</sub> ], 4[q,2H,0CH <sub>2</sub> ]	62
AJ45	H	H	Cl	NO <sub>2</sub>	C <sub>22</sub> H <sub>18</sub> N <sub>4</sub> O <sub>6</sub> C1	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 <sub>3</sub> ], 2.25[S,3H,CH <sub>3</sub> ], 4[q,2H,0CH <sub>2</sub> ]	63

## RESULTS

Compounds AJ<sub>1</sub> to AJ<sub>45</sub> 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<sub>1</sub>-3[(4-methoxyphenyl-5-carboethoxy-6-methyl-3, 4-dihydropyrimidin[1H]-2-one) indolin-2-ones and Y<sub>1</sub>[2-nitro-phenyl-5carboethoxy-6-methyl-3,4-dihydropyrimidin[1H]-2-one) indolin-2-ones] exhibited 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 IC<sub>50</sub> values in Micro moles (μM). Among all the series, compounds AJ31 (R=Cl, R<sub>1</sub>=R<sub>2</sub>=H), AJ32 (R=Br, R<sub>1</sub>=R<sub>2</sub>=H) and AJ25 (R=R<sub>2</sub>=Br, R<sub>1</sub>=H), showed more Cytotoxic activity with IC<sub>50</sub> values of 14.0 and 120.2, 17.6 and 98.2 and 120.2 μM and 25.9 and 95 μM respectively against CHO and MCF-7 cell lines.

Compounds AJ23 (R=Br, R<sub>1</sub>=R<sub>2</sub>=H), AJ22 (R=Cl, R<sub>1</sub>=R<sub>2</sub>=H) and AJ21 (R=F, R<sub>1</sub>=R<sub>2</sub>=H) were next in the order of Cytotoxic activity with IC<sub>50</sub> values of 31.85, 32.75 and 36.58 μM 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	CH <sub>3</sub>	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 <sub>3</sub>	296	284
9	AJ9	H	H	Cl	210	194

Cisplatin at all concentration ranges tested showed an IC<sub>50</sub> 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 <sub>3</sub>	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 <sub>3</sub>	94.63	183.5
9	AJ18	H	H	Cl	52.01	125.4

Cisplatin at all concentration ranges tested showed an IC<sub>50</sub> 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 <sub>3</sub>	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 <sub>3</sub>	89.06	168.8
9	AJ27	H	H	Cl	69.54	120.4

Cisplatin t all concentration ranges tested showed an IC<sub>50</sub> 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 <sub>3</sub>	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 <sub>3</sub>	135.6	154.5
9	AJ36	H	H	Cl	68.7	110.9

Cisplatin at all concentration ranges tested showed an IC<sub>50</sub> 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 <sub>3</sub>	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 <sub>3</sub>	320.4	302
9	AJ45	H	H	Cl	154	168.4

Cisplatin at all concentration ranges tested showed an IC<sub>50</sub> of 25um

## CONCLUSION

Hence we conclude that Z series of Compounds having 4-hydroxy substituent and X<sub>1</sub> Series having 4-methoxy substituent at 5-position of pyrimidine ring exhibited more cytotoxic activity [A]<sub>31</sub> > A]<sub>32</sub> > A]<sub>25</sub> > A]<sub>23</sub> > A]<sub>22</sub>] and A]<sub>21</sub> followed by X, Y and Y1 series. Among Isatins, 5,7-disubstituted halogens are more active than mono substituted halogens against cytotoxic activity followed by Br, Cl, F. The rest of the compounds showed moderate Cytotoxic activities. The IC<sub>50</sub> value of cisplatin was found to be 25 μm.

## ACKNOWLEDGEMENT

The authors are thankful to U.G.C., New Delhi for the financial assistance. Authors are also grateful to Dr. Reddy's Research Foundation, Hyderabad. for providing I.R and PMR spectral analysis. The Author grateful to Professor. Vijay gopal Madam, from CCMB for her support for carrying cytotoxic screening of compounds.

## REFERENCES

- O. A. Sharaf, Some pharmacological activities of new substituted pyrrolo indoles, indolothiazepine, andazole derivatives, Bull. Fac. Pharm. 35: 79–82, (1997).
- S. K. Srivastava, S. Srivastava and S. D. Srivastava, Synthesis of new carbazolyl-thiadiazolyl- 2-oxa-azetidines, antimicrobial, anticonvulsant and anti-inflammatory agents, Indian J. Chem. 38B: 183–187, (1999).
- M. Kupini, M. Medi-ari, M. Movrin and D. Maysinger, Antibacterial and antifungal activities of isatin N-Mannich bases, J. Pharm. Sci. 68: 459–462, (1979).
- S. K. Bhattacharya and A. Chakraborti, Dose related proconvulsant and anticonvulsant activity of isatin, a putative biological factor in rats, Indian. J. Exp. Biol. 36 : 118–121, (1998).
- Gamal-Eldeen A, Kawashty S, Ibrahim L, Shabana M, El-Negoumy S: Evaluation of antioxidant, antiinflammatory, and antinociceptive properties of aerial parts of *Vicia sativa* and its flavonoids, J Nat Remedies. 4 : 81–96, (2004).
- Tozkoparan B, Ertan M, Kelicen P, Demirdamar R: Synthesis and anti-inflammatory activities of some thiazolo[ 3,2-a]pyrimidine derivatives. Farmaco. 54: 588–593, (1999).
- Kappe CO, Fabian WMF, Semons MA: Conformational analysis of 4-aryl-dihydropyrimidine calcium channel modulators. A comparison of ab initio, semiempirical and X-ray crystallographic studies. Tetrahedron. 53: 2803–2807, (1997)
- Ranise A, Bruno O, Bondavalli F, Schenone S, D'Amico M, Falciani M, Filippelli W, Rossi F. 5-Substituted 2,3-dihydro-6-mercapto-1,3-diphenyl-2-thioxo-4(3H)-pyrimidinones and their 6-(acylthio) derivatives with platelet antiaggregating, antiinflammatory, antiarrhythmic, antihyperlipidemic and other activities. Farmaco. 49(9): 551-558, (1994).
- Kappe CO: Biologically active dihydropyrimidones of the Biginelli-type – a literature survey. Eur J Med Chem, 35: 1043–1052, (2000).
- S. N. Pandeya, A. Senthil Raja and J. P. Stables, Synthesis of isatin semicarbazones as novel anticonvulsants – role of hydrogen bonding, J. Pharm. Pharm. Sci. 5: 266–271, (2002).
- Maruyama H, Sakamoto T, Araki Y, Hara H. Anti-inflammatory effect of bee pollen ethanol extract from *Cistus* sp. of Spanish on carrageenan-induced rat hind paw edema. BMC Complement Altern Med. 23: 10-30, (2010).
- Kappe CO: 100 Years of the Biginelli dihydropyrimidine synthesis. Tetrahedron, 49: 6937–6942, (1993).
- Alley M C, Scudiero D A; Feasibility of drug screening with panels of human tumor cell lines using micro culture tetrazolium assay. *Cancer Res.* 48: 589-601, (1998).
- Singh OM, Singh SJ, Devi MB, Devi LN, Singh NI, Lee SG. Synthesis and in vitro evaluation of the antifungal activities of dihydropyrimidinones. *Bioorg Med Chem Lett.* 18(24): 6462-6467, (2008).