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Synthetic Novel Flavanoid derivatives act as Potential Antineoplastic Agent

Asish Bhaumik *[1], B. Ramu[2], Sk. Basheer[3], Prasenjit Das[4], J.Mastanaiah[5]

[1,3]Department of Pharmaceutical Chemistry, Teja College of Pharmacy, Kodad, Nalgonda-508206, Telangana State, India.
[2]Department of Biochemistry, MITS College of Pharmacy, Kodad, Nalgonda-508206, Telangana State, India.
[4]Department of Pharmaceutical Chemistry, Regional Institute of Pharmaceutical Science & Technology, Abhoynagar, Agartala-799005, Tripura, India.
[5]Department of Pharmacology, GATE School of Pharmacy, Kodad, Nalgonda-508206, Telangana, State, India.

A B S T R A C T

Neoplasm or tumour is a mass of tissue formed as a result of abnormal, excessive, uncoordinated, autonomas and purposeless proliferation of cells. The main objective of the present work was the synthesis of 2- (2, 3, 4, & 5 substituted phenyl) 3-hydroxy-4H-Chromen-4-one and evaluation of *in-vitro* antineoplastic activity. The synthesized compounds were characterized by IR, NMR, and Mass spectroscopy. The *in-vitro* antineoplastic activities were carried out against Human Prostate cancer cell line DU-145 and Human Leukemia Cancer Cell line MOLT- 4 and SRB assay was used to analyze the cell growth inhibition of the both cancer cell lines. The results had been showed that compound 4A- 4J were possessed an excellent antineoplastic activity (at 25 µg/ml) against both cancer cell lines. 5-Fluro uracil (5-FU, at 15µg/ml) was used as a standard drug for DU-145 cell line and doxorubicin (at 15µg/ml) was used as a standard drug for MOLT-4 cell. IC_{50} values of the synthesized compounds were found to be 4A (IC_{50} of 2.2 µg/ml), 4B (IC_{50} of 2.3 µg/ml), 4C (IC_{50} of 2.6 µg/ml), 4D (IC_{50} of 2.8 µg/ml), 4E (IC_{50} of 3.1 µg/ml), 4F (IC_{50} of 2.9 µg/ml), 4G (IC_{50} of 2.3 µg/ml), 4H (IC_{50} of 3.4 µg/ml), 4I (IC_{50} of 3.8 µg/ml) and 4J (IC_{50} of 2.0 µg/ml) against DU-145 cell line and 4A (IC_{50} of 2.3 µg/ml), 4B (IC_{50} of 2.4 µg/ml), 4C (IC_{50} of 2.5 µg/ml), 4D (IC_{50} of 2.7 µg/ml), 4E (IC_{50} of 2.9 µg/ml), 4F (IC_{50} of 3.1 µg/ml), 4G (IC_{50} of 3.3 µg/ml), 4H (IC_{50} of 4.3 µg/ml), 4I (IC_{50} of 3.9 µg/ml) and 4J (IC_{50} of 2.2 µg/ml) against MOLT-4 cell line. The IC_{50} values of standard drugs 5-FU and doxorubicin were found to be 1.88 µg/ml and 1.91 µg/ml.

Keywords: Chromen, Neoplasia, Antineoplastic activity DU-145, MOLT-4, SRB assay, IC_{50}

A R T I C L E I N F O

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***Corresponding Author**
Asish Bhaumik
Dept. of Pharmaceutical Chemistry,
Teja College of Pharmacy, Kodad,
Nalgonda-508206, Telangana, India.
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1. Introduction

Flavanoids belong to a large group of abundant plant secondary metabolites, which can be found in vascular plants like ferns, conifers and flowering plants[1-3]. These natural compounds are generally divided into various classes on the basis of their molecular structures including chalcones, flavones, flavanones, flavanols, and anthocyanidins. Approxymately, 4000 varieties of flavanoids have been identified and many of these are intense pigments, providing a spectrum of yellow, red and blue colours in flowers, fruits and leaves[3-6]. Besides their contribution to plant colour, flavanoids have several pharmacological benefits such as anticancer, anti inflammatory, anti-allergic and are known as effective anti oxidants, metal chelators and free radical scavengers [3, 7, 8, 9, 10] Natural and synthetic flavanoids are therefore of considerable interest in the development of novel therapeutic agents for various diseases and are generally believed to be non-toxic compounds since they are widely distributed in the human diet[2,5].

Chalcones:

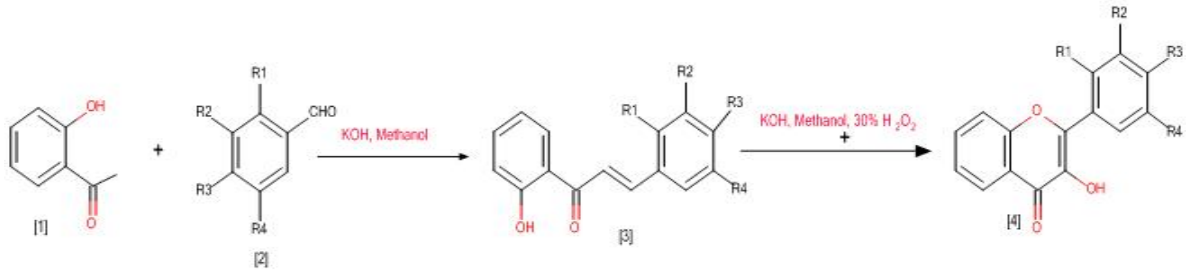
Chalcones, 1,3-diphenylpropenones constitute one of the major classes of flavanoids with widespread distribution in vegetables, fruits, tea and soy[3,11]. Prehistoric therapeutic applications of chalcones can be associated with the thousand-year old use of plants and herbs for the treatment of different medical disorders [12]. Contemporary studies report a generous variation of significant pharmacological activities of chalcones including antiproliferative, antioxidant, anti inflammatory and anti cancer effects[11, 13-15]. The chromone ring system, 1-benzopyran-4-one, is the core fragment in several flavanoids such as flavones, flanols and isoflavones [16].

2. Materials and Methods

The all chemicals used for the synthesis were of laboratory grade and analytical grade. The prognosis and the completion of reaction was determined by TLC plate and spots were visualized under U.V chamber. The melting point of newly synthesized flavanoid compounds were determined by open capillary method. The IR spectra of synthesized compounds were recorded by FT-IR spectrometer . The H^1-NMR spectra were obtained from Bruker Avance II 400 MHz spectrometer using TMS as an internal standard in $CDCl_3$. All the Mass spectra (MS, HR-MS) of synthesized compounds were recorded on a LTQ-orbitrap linear ion trap high resolution mass spectrometer.

General procedure for the synthesis of target compounds [17]: 2-hydroxy acetophenone [(1), 1.36gm, 0.01mol] and benzaldehyde [(2), 1.06gm, 0.01mol) were added to a solution of potassium hydroxide (1.12gm, 0.02 mol) in methanol (50 ml) at 0-5^oC. The reaction mixture was stirred over night at room temperature and then poured over crushed ice and acidified to P^H 6 with 2M Hcl. The resulting yellow solid was filtered and the filter cake washed with water to give the crude product that either be crystallized from ethanol to afford pure 2-hydroxy chalcones 3A or used directly in the next reaction without further purification. 30% hydrogen peroxide (10 ml) was added to a well stirred solution of 3A (1.57gm, 0.007 mol) and 20% (w/w) aqueous potassium hydroxide (10 ml) in methanol (20 ml) at 0-5^oC in a drop wise manner over 1 hour. The resulting reaction mixture was stirred for 10 hours and then poured on crushed ice and neutralized with 2M Hcl. Ethyl aceto acetate (50 ml) was added and the organic layer was washed successfully with water, a saturated solution of sodium bicarbonate, water and brine and then dried over anhydrous magnesium sulphate. The solvent was removed in vacuum and the residue was purified by column chromatography on silica gel (ACOEt/ n-hexane = 1/3 to 1/1) to give the title compound **4A** as a white solid.

Synthetic scheme:



[1] = 2-Hydroxy acetophenone
[2]=Various aromatic aldehydes
[A]=KOH, CH_3OH
[B]= KOH, CH_3OH, 30% H_2O_2

Spectral data:

Compound 4A: 2-(4-aminophenyl) 3-hydroxy-4H-Chromen-4-one. M.F: $C_{15}H_{11}NO_3$, M.W : 253.252, M.P-180^{O}c, R_f-0.55, Yield-68.9%, FT-IR (KBr) : 3212 cm^{-1} (Ar-OH), 1622 cm^{-1} (C=O , Pyrone ring), ^{1}H-NMR (400MHz, $CDCl_3$) : δ (ppm) : 3.91 (s, H, -NH_2), 7.01 (s, 1H, OH), 7.00-8.25 (m, 7H, Ar-H), MS(ESI^+)m/z : 253.2 $(M+H)^+$, HR-MS(ESI^+)m/z: 253.07 $(M+H^+)$.

Compound 4B: 2-(4-dimethylaminophenyl) 3-hydroxy-4H-Chromen-4-one. M.F: $C_{17}H_{15}NO_3$, M.W : 281.105, M.P-175^{O}c, R_f-0.45, Yield-65.9%, FT-IR (KBr) : 3213 cm^{-1} (Ar-OH), 1625 cm^{-1} (C=O , Pyrone ring), ^{1}H-NMR (400MHz, $CDCl_3$) : δ (ppm) : 3.06 [s, 6H, -$N(CH_3)_2$], 6.87 (s, 1H, OH), 6.84-8.27 (m, 7H, Ar-H), MS(ESI^+)m/z : 281.3 $(M+H)^+$, HR-MS(ESI^+)m/z: 281.105 $(M+H^+)$.

Compound 4C: 2-[4-dimethylaminophenol) 2-hydroxy phenyl] 3-hydroxy-4H-Chromen-4-one. M.F: $C_{17}H_{15}NO_4$, M.W : 297.1000, M.P-181^{O}c, R_f-0.47, Yield-63.9%, FT-IR (KBr) : 3218 cm^{-1} (Ar-OH), 1618 cm^{-1} (C=O , Pyrone ring), ^{1}H-NMR (400MHz, $CDCl_3$) : δ (ppm) : 3.07 [s, 6H, -$N(CH_3)_2$], 6.88 (s, 1H, OH), 6.56 (s, 1H, OH), 6.87-8.27 (m, 7H, Ar-H), MS(ESI^+)m/z : 297.3 $(M+H)^+$, HR-MS(ESI^+)m/z: 297.1000 $(M+H^+)$.

Compound 4D: 2-(4-aminopheno-2-hydroxyphenyl) 3-hydroxy-4H-Chromen-4-one. M.F: $C_{15}H_{11}NO_4$, M.W : 269.068, M.P-172^{O}c, R_f-0.39, Yield-68.5%, FT-IR (KBr) : 3224 cm^{-1} (Ar-OH), 1617 cm^{-1} (C=O , Pyrone ring), ^{1}H-NMR (400MHz, $CDCl_3$) : δ (ppm) : 3.93 [s, 2H, -NH_2], 6.88 (s, 1H, OH), 6.59 (s, 1H, OH), 6.83-8.22 (m, 7H, Ar-H), MS(ESI^+)m/z : 269.2 $(M+H)^+$, HR-MS(ESI^+)m/z: 269.06 $(M+H^+)$.

Compound 4E: 3-hydroxy-2-(4-hydroxy-3-methyl-phenyl)-4H-Chromen-4-one. M.F: $C_{16}H_{12}O_4$, M.W : 268.073, M.P-181^{O}c, R_f-0.45, Yield-66.2%, FT-IR (KBr) : 3213 cm^{-1} (Ar-OH), 1620 cm^{-1} (C=O , Pyrone ring), ^{1}H-NMR (400MHz, $CDCl_3$) : δ (ppm) : 3.92 (s, 3H, -OCH_3), 7.02 (s, 1H, OH), 6.44 (s, 1H, OH), 6.81-8.21 (m, 7H, Ar-H), MS(ESI^+)m/z : 268.07 $(M+H)^+$, HR-MS(ESI^+)m/z: 268.26 $(M+H^+)$.

Compound 4F: 2-(3,4-dimethoxyphenyl) 3-hydroxy-4H-Chromen-4-one. M.F: $C_{17}H_{14}O_5$, M.W : 298.084, M.P-174^{O}c, R_f-0.48, Yield-62.9%, FT-IR (KBr) : 3225 cm^{-1} (Ar-OH), 1617 cm^{-1} (C=O , Pyrone ring), ^{1}H-NMR (400MHz, $CDCl_3$) : δ (ppm) : 3.94 (s, 3H, -OCH_3), 3.96 (s, 3H, -OCH_3), 7.03 (s, 1H, OH), 7.00-8.24 (m, 7H, Ar-H), MS(ESI^+)m/z : 298, $(M+H)^+$, HR-MS(ESI^+)m/z: 298.2 $(M+H^+)$.

Compound 4G: 3-hydroxy-2-(3,4,5-trimethoxyphenyl)-4H-Chromen-4-one. M.F: $C_{17}H_{14}O_5$, M.W : 328.09, M.P-179^{O}c, R_f-0.47, Yield-69.9%, FT-IR (KBr) : 3225 cm^{-1} (Ar-OH), 1617 cm^{-1} (C=O , Pyrone ring), ^{1}H-NMR (400MHz, $CDCl_3$) : δ (ppm) : 3.94 (s, 3H, -OCH_3), 3.96 (s, 3H, -OCH_3), 3.99 (s, 3H, -OCH_3), 7.03 (s, 1H, OH), 7.00-8.24 (m, 7H, Ar-H), MS(ESI^+)m/z : 328, $(M+H)^+$, HR-MS(ESI^+)m/z: 328.3 $(M+H^+)$.

Compound 4H: 3-hydroxy-2-(4-hydroxy-3,5-dimethoxy phenyl)-4H-Chromen-4-one. M.F: $C_{17}H_{14}O_6$, M.W: 314.079, M.P-177^{O}c, R_f-0.46, Yield-67.9%, FT-IR (KBr) : 3224 cm^{-1} (Ar-OH), 1617 cm^{-1} (C=O , Pyrone ring), ^{1}H-NMR (400MHz, $CDCl_3$) : δ (ppm) : 3.94 (s, 3H, -OCH_3), 3.99 (s, 3H, -OCH_3), 6.03 (s, 1H, OH), 7.02 (s, 1H, OH), 7.00-8.24 (m, 7H, Ar-H), MS(ESI^+)m/z : 314, $(M+H)^+$, HR-MS(ESI^+)m/z: 314.2 $(M+H^+)$.

Compound 4I: 2-(3,5-dichloro-2-hydroxyphenyl)-3-hydroxy-4H-Chromen-4-one. M.F: $C_{16}H_{10}Cl_2O_3$, M.W : 320, M.P-175^{O}c, R_f-0.44, Yield-62.2%, FT-IR (KBr) : 3224 cm^{-1} (Ar-OH), 1617 cm^{-1} (C=O , Pyrone ring), ^{1}H-NMR (400MHz, $CDCl_3$) : δ (ppm) : 6.03 (s, 1H, OH), 7.02 (s, 1H, OH), 7.00-8.24 (m, 7H, Ar-H), MS(ESI^+)m/z : 321.1, $(M+H)^+$, HR-MS(ESI^+)m/z: 320 $(M+H^+)$.

Compound 4J: 2-(5-amino-2-hydroxyphenyl)-3-hydroxy -4H-Chromen-4-one. M.F: $C_{15}H_{11}NO_4$, M.W : 269.06, M.P-174^{O}c, R_f-0.47, Yield-63.6%, FT-IR (KBr) : 3222 cm^{-1} (Ar-OH), 1617 cm^{-1} (C=O , Pyrone ring), ^{1}H-NMR (400MHz, $CDCl_3$) : δ (ppm) : 3.93 [s, 2H, -NH_2], 6.03 (s, 1H, OH), 7.02 (s, 1H, OH), 7.00-8.24 (m, 7H, Ar-H), MS(ESI^+)m/z : 269, $(M+H)^+$, HR-MS(ESI^+)m/z: 269.2 $(M+H^+)$.

Screening of *In-Vitro* Antineoplastic Activity by SRB Assay

Principle:

Sulphorodamine B (SRB) is a bright pink aminoxanthine dye with two sulfonic acid group. Under mild acidic conditions SRB dye binds to basic amino acid residues in trichloro acetic acid (TCA) fixed cells to provide a sensitive index of cellular protein content that is linear over a cell density range of visible at least two order of magnitude[18,19]

Cell culture :

Human Prostate cancer cell line DU-145 and Human Leukemia Cancer Cell line MOLT- 4 were provided by National Centre for Cell Science (NCCS), Pune and were grown in Eagles Minimum Essential Medium (EMEM) which contained 10% fetal bovine serum (FBS). All cells were maintained at 37°C, 100% relative humidity, 5% CO2, 95% air and the culture medium was changed twice a week.

Procedure:

The monolayer cell culture was trypsinized and the cell count was adjusted to 0.5-1.0x10^5 cells/ml using medium containing 10% new born sheep serum. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately) 10,000 cells was added. After 24 hrs, when a partial monolayer was formed, the supernatant was flicked off, washed once and 25µl of different test compound concentration were added to the cell in microtitre plate. The plates were incubated at 37^{0}c for 72 hrs in 5% CO_2 incubator, microscopic examination was carried out and observations were recorded every 24 hrs. After 72 hrs , 25µl of 50% TCA was added to wells gently such that it forms a thin layer over the test compounds to form overall concentrations 10%. The plates were incubated at 4^{0}c for 1 hr. The plates were flicked and washed five times with tap water to remove traces of medium sample and serum and were then air dried. The air dried plates were stained with 100 µl SRB and kept for 30 mnts at room temperature. The unbound dye was removed by rapidly washing four times with 1% acetic acid. The plates were then air dried. 100 µl of 10 mM Tris base was

then added to the wells to solubilise the dye [20]. The plates were shaken vigorously for 5 mnts. The absorbance was measured using microplate reader at a 540 nm. The % growth inhibition was calculated by the following formula:

% cell growth inhibition = 100-{(At-Ab/Ac-Ab)}x 100
At = Absorbance value of test compound
Ab = Absorbance value of blank
Ac = Absorbance value of control

3. Results and Discussion

Chemistry:

The synthesis of target compounds (4A-4J) 2- (2, 3, 4, & 5 substituted phenyl) 3-hydroxy-4H-Chromen-4-one were carried out by reacting 2-hydroxy acetophenone and various aromatic aldehydes in the presence of potassium hydroxide, methanol and 30% hydrogen peroxide. The synthesized compounds were characterized by IR, NMR, and Mass spectroscopy. The progress of the reaction was monitored by TLC using solvent systems of different polarities. TLC plates are pre-coated silica gel (HF254-200 mesh) aluminium and spots were visualized under U.V chamber and the proposed structures of the synthesized compounds were ascertained by spectral data.

Biological screening:

Table 1: Various flavanoid derivatives

3	R1	R2	R3	R4	4	R1	R2	R3	R4
3A	H	H	$-NH_2$	H	4A	H	H	H	$-NH_2$
3B	H	H	$N-(CH_3)_2$	H	4B	H	H	$N-(CH_3)_2$	H
3C	OH	H	$N-(CH_3)_2$	H	4C	OH	H	$N-(CH_3)_2$	H
3D	OH	H	$-NH_2$	H	4D	OH	H	$-NH_2$	H
3E	H	$-CH_3$	OH	H	4E	H	CH_3	OH	H
3F	H	$-OCH_3$	$-OCH_3$	H	4F	H	$-OCH_3$	$-OCH_3$	H
3G	H	$-OCH_3$	$-OCH_3$	$-OCH_3$	4G	H	$-OCH_3$	$-OCH_3$	$-OCH_3$
3H	H	$-OCH_3$	OH	$-OCH_3$	4H	H	$-OCH_3$	OH	$-OCH_3$
3I	OH	Cl	H	Cl	4I	H	Cl	Cl	H
3J	OH	H	H	$-NH_2$	4J	OH	H	H	$-NH_2$

These synthesized compounds (4A-4J) were screened for their *in vitro* antineoplastic activity by using SRB assay. A preliminary screening against both Human Prostate cancer cell line DU-145 and Human Leukemia Cancer Cell line MOLT- 4 displayed that the compounds 4A-4J (at concentration 25µg/ml) as well as standard drug 5-FU and doxorubicin at concentration 15µg/ml were able to inhibit the proliferation of more than 50% cells (Fig: 1, 2, 3 and 4). It was appeared that compounds 4A-4J displayed antineoplastic activity with IC_{50} values below 100 µg/ml against these both cancer cell lines. In the USNCI screening program a compound is generally considered to have *in vitro* antineoplastic activity, if the IC_{50} value following incubation between 48 hrs and 72 hrs is less than 4 µg/ml or 10 µM [12]. In the present study IC_{50} values below 4 µg/ml were displayed compound 4A (IC_{50} of 2.2 µg/ml), 4B (IC_{50} of 2.3 µg/ml), 4C (IC_{50} of 2.6 µg/ml), 4D (IC_{50} of 2.8 µg/ml), 4E (IC_{50} of 3.1 µg/ml), 4F (IC_{50} of 2.9 µg/ml), 4G (IC_{50} of 2.3 µg/ml), 4H (IC_{50} of 3.4 µg/ml), 4I (IC_{50} of 3.8 µg/ml) and 4J (IC_{50} of 2.0 µg/ml) against DU-145 cell line and 4A (IC_{50} of 2.3 µg/ml), 4B (IC_{50} of 2.4 µg/ml), 4C (IC_{50} of 2.5 µg/ml), 4D (IC_{50} of 2.7 µg/ml), 4E (IC_{50} of 2.9 µg/ml), 4F (IC_{50} of 3.1 µg/ml), 4G (IC_{50} of 3.3 µg/ml), 4H (IC_{50} of 4.3 µg/ml), 4I (IC_{50} of 3.9 µg/ml) and 4J (IC_{50} of 2.2 µg/ml) against MOLT-4 cell line. The IC_{50} values of standard drugs 5-FU and doxorubicin were found to be 1.88 µg/ml and 1.91 µg/ml.

Table 2 : For Percentage Cell Growth Inhibition (%) of Synthesized Compounds against DU-145 (Human Prostate Cancer cell line) by SRB Assay

Name of the synthesized compounds	Concentration of the drugs	Absorbance of the drug samples	Inhibition of Cell Growth (%)
4A	25 µg/ml	0.010	96.656
4B	25 µg/ml	0.018	93.979
4C	25 µg/ml	0.023	92.308
4D	25 µg/ml	0.029	90.30
4E	25 µg/ml	0.036	87.96
4F	25 µg/ml	0.033	88.963
4G	25 µg/ml	0.045	84.95
4H	25 µg/ml	0.052	82.609
4I	25 µg/ml	0.061	79.599
4J	25 µg/ml	0.009	96.989
5-FU	**15 µg/ml**	**0.004**	**98.663**
Control		0.299	0

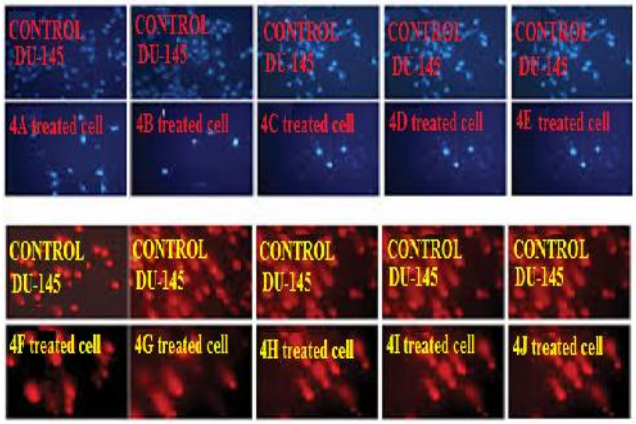


Figure 1: Inhibition of DU-145 cancer cells by 4A- 4J Compounds

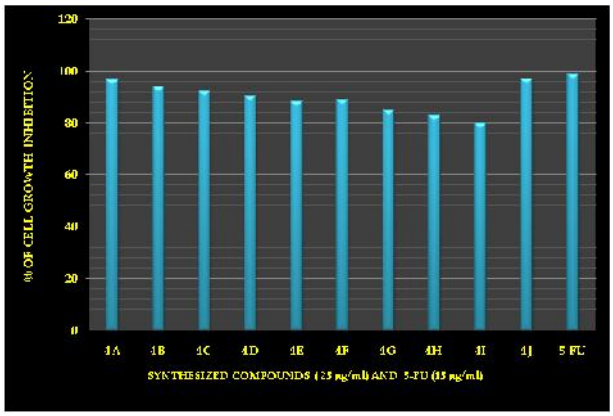


Figure 2: Inhibitory Percentage (%) of the Synthesized Compounds at 25 µg/ml and Standard 5-FU (at 15µg/ml) on DU-145 (Human Prostate) Cancer Cell line

Table 3 : For Percentage Cell Growth Inhibition (%) of Synthesized Compounds against MOLT-4 (Human Leukemia Cancer cell line) by SRB Assay

Name of the synthesized compounds	Concentration of the drugs	Absorbance of the drug samples	Inhibition of Cell Growth (%)
4A	25µg/ml	0.011	96.322
4B	25 µg/ml	0.017	94.315
4C	25 µg/ml	0.020	93.312
4D	25 µg/ml	0.024	91.974
4E	25 µg/ml	0.034	88.629
4F	25 µg/ml	0.040	86.623
4G	25 µg/ml	0.049	83.613
4H	25 µg/ml	0.099	66.89
4I	25 µg/ml	0.077	74.248
4J	25 µg/ml	0.010	96.656
DOXORUBICIN	**15 µg/ml**	**0.005**	**98.327**
Control		0.299	0

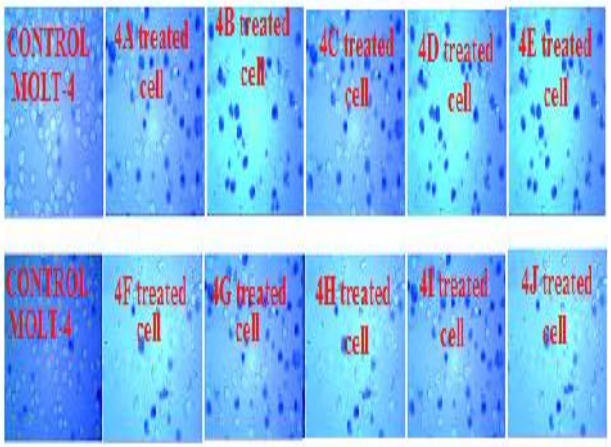


Figure 3: Inhibition of MOLT- 4 cancer cells by 4A-4J Compounds

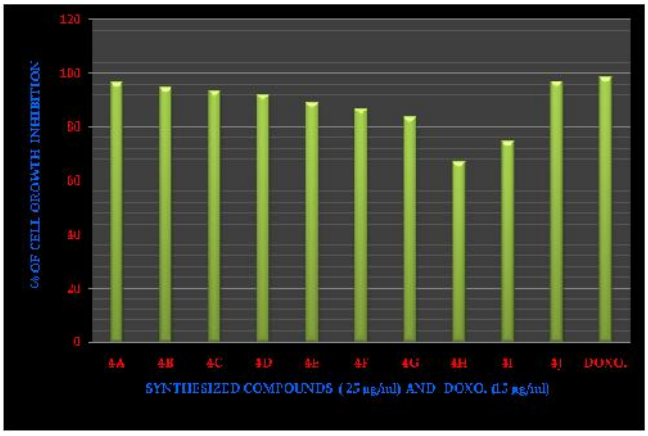


Figure 2: Inhibitory Percentage (%) of the Synthesized Compounds at 25 µg/ml and Standard drug Doxorubicin (at 15µg/ml) on MOLT-4 (Human Leukemia) Cancer Cell line

4. Conclusion

In conclusion, we report here a series of novel 2- (2, 3, 4, and 5 substituted phenyl) 3-hydroxy-4H-Chromen-4-one derivatives (4A-4J) were prepared by reacting 2-hydroxy acetophenone and various aromatic aldehydes in the presence of potassium hydroxide, methanol and 30% hydrogen peroxide and their ability to kill neoplastic cells in vitro. The antineoplastic activity of the compounds 4A-4J against both Human Prostate cancer cell line DU-145 and Human Leukemia Cancer Cell line MOLT- 4 can be considered very good with regards to the USNCI standard.

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