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SYNTHESIS, ANTICANCER ACTIVITIES AND *IN SILICO* SCREENING OF 3-ACETYLCOUMARIN-HYDRAZONE SCAFFOLDS

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ABSTRACT

3-acetylcoumarin hydrazone scaffolds, synthesized from 3-acetylcoumarin and substituted benzoic acid hydrazides are reported with structural characterization using IR, HRMS, ^{1}H and ^{13}C-NMR. The *In vitro* anticancer activities against three human cancer cell lines viz. MCF-7 (human breast cancer cell line), HeLa (human cervical cancer cell line) and SCC-40 (human oral squamous cell carcinoma) are carried out while the tumour selectivity of compounds are tested on the normal human peripheral blood mononuclear cells (PBMCs). The compounds 3ACOH, 3ACDH and 3ACMH exhibited higher sensitivity towards HeLa with GI50 values between 20.4 - 44.1µg/ml and this range of GI50 concentration of hydrazones showed no remarkable toxicity against normal PBMCs. Molecular docking studies revealed commendable binding interactions with cyclooxygenase enzyme (PDB ID 6COX). ADMET analysis shows the hydrazones are showing drug-likeness properties. The reported observations of 3-acetylcoumarin hydrazones suggest their possible role as promising new anticancer drug candidates.

Keywords: 3-acetylcoumarin, Hydrazone, Molecular Docking, MCF-7, HeLa, SCC-40, PBMCs.

1. INTRODUCTION

Anti-cancer drugs are non-specifically cytotoxic and collateral damage to normal tissues is the major side effects associated with radiotherapy. The chemo-protective properties of anticancer drugs such as coumarins against such side effects have been widely studied [1]. Diseases such as Osteoarthritis, Crohn's disease and different types of cancers are associated with the progression of chronic inflammation with activation of pro inflammatory mediators such as interleukins and intracellular enzymes COX and LOX [2]. Under normal conditions, COX is expressed at low levels, however, gets over-expressed during the inflammatory processes, pathogenic stimuli and cancer progression [3].

At the same time, synthesis of hybrid structures from different class of compounds is one of the popular strategies for the development of drug candidates with increased activity and improved specificity [4-6]. Similarly substituted hydrazones also have been reported to show broad range of biological activities such as anticancer, anti-microbial, anti-inflammatory, analgesic, anti-fungal, anti-tubercular, anti-viral, anti-convulsant, cardio protective and these activities are attributed to the distinctive structural features of hydrazones and azomethine group [7-8]. On the other hand, coumarin or 2-oxo-2H-chromene derivatives are also known as therapeutic agents with broad and diverse biological activities [9] such as anti-inflammatory [10], antimicrobial [11], antiviral [12], antioxidant [13], antinociceptive [14], antitumor [15], antiasthmatic [16], antidepressant [17], anti-HIV [18] and antituberculosis [19] being only some of them.

Computer aided drug design (CADD) methods have been used to develop cyclooxygenase targeting anti-inflammatory and anti-cancer drugs [20]. Such processes are useful to identify and develop a potential lead. Likewise, ADME studies are utilized in the drug development process to examine numerous factors which influence on drug activity and viability. These investigations are important to grow a researcher's knowledge to understand the behavior of a candidate drug, including how it influences the establishment,

progression, regression and animosity of specific diseases along with the drawbacks of a drug such as toxicity [21].

Keeping in view the hybrid concept and the anticancer potential of coumarin scaffolds, it was planned to synthesize hybrid compounds those comprise the coumarin nucleus with different benzoic acid hydrazides and their evaluation of anticancer activities. The synthesized hydrazones were characterized and screened for antiproliferative potential against three human cancer cell lines viz. MCF-7, HeLa and SCC-40 and GI50 thus obtained have been compared with cytotoxicity against normal human peripheral blood mononuclear cells (PBMCs). Additional data from *in silico* molecular docking and ADMET studies is incorporated to subtly highlight the binding potential of these ligands with protein structure.

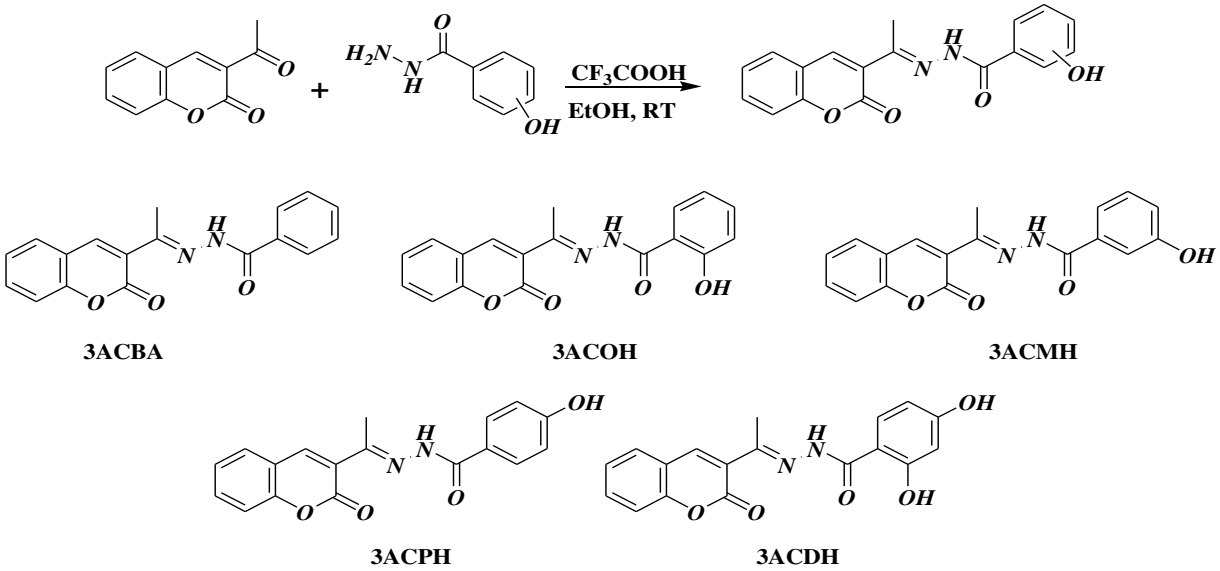
2. EXPERIMENTAL

2.1. Material

The solvents used were purchased from commercial sources and were further dried by standard protocols. The starting materials such as 3-acetylcoumarin, Trifluoroacetic acid and different benzoic acid hydrazides were obtained from Alfa Aesar, Mumbai. DMEM and FBS were purchased from Himedia, Mumbai. The MTT (3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide) reagent was obtained from G-Biosciences, USA. TLC was monitored using commercially available Aluminium TLC plates coated with silica gel GF254 and the developed plates were visualized by UV light and iodine vapors. Melting points of synthesized compounds were determined with open capillary tube on a VEEGO melting point apparatus. The FTIR and NMR spectroscopic data were obtained from CIF, Savitribai Phule Pune University, Pune and HRMS from NCL, Pune. The *in vitro* anticancer activities were carried out at Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Navi Mumbai and ASR Lab, Abeda Inamdar Senior College, Pune.

2.2. Syntheses of 3-acetylcoumarin hydrazones

A mixture of 3-acetylcoumarin and different benzoic acid hydrazides were dissolved in 1:1 molar ratio in absolute ethanol. Few drops of trifluoro acetic acid were added and the mixture was stirred at room temperature for 8-10 min. The reaction was monitored by TLC (Scheme 1). After completion of the reaction, the solid obtained was filtered and washed with cold ethanol and recrystallized from ethanol.



Scheme 1

2.3. *In vitro* anticancer activity (SRB Assay)

The compounds were tested for *in vitro* anticancer activities according to SRB assay protocols available in literature [22]. Cell lines were cultured, inoculated and counted in 96 well plates at four dose levels of test compound concentrations 10, 20, 40, 80μg/ml. Each experiment was repeated three times. After incubation with different concentrations of test compounds, the

cell cultures were stained with SRB dye. The unbound dye was removed by washing with 1% acetic acid and the protein bounded dye was extracted using Tris-HCl buffer base (100 µl, 0.01 M, pH 10.4). The optical density was determined at 540 nm on 96-well plate ELISA reader. The cell viability was expressed as a percentage of the control values.

2.4. *In vitro* cytotoxicity assay against non-cancerous cells

To study the tumour selectivity, the synthesized analogues were tested against non-cancerous normal human peripheral blood mononuclear cells (PBMCs). Isolation of peripheral blood mononuclear cell was done using Ficoll-Hypaque according to the standard method [23] and MTT cytotoxic assay was used to evaluate the cytotoxicity of all the synthesized hydrazones. 1×10^6 cells per well were seeded in 96-well plate, then exposed to different concentrations of hydrazones (10, 20, 40 and 80 µg/ml) for 24 hours along with a control well. After 24 hours, the culture medium containing hydrazones was removed with one PBS wash. Then 10 µl of MTT solution (5mg/ml) was added and incubated for 4 hours followed by addition of 100µl of DMSO. After 10-20 minutes, absorbance was recorded at 570 nm using Readwell Touch Automatic Elisa Plate Reader (Robonik India Private Limited).

2.5. *In silico* Molecular Docking

In order to understand the probable binding affinities of the synthesized derivatives, molecular docking studies were carried out at active site of human cyclooxygenase enzyme (COX-2) PDB ID: 6COX using Auto Dock 4.2.614 software. The PDB file of human cyclooxygenase enzyme was downloaded from Royal Society Protein Data Bank (https://www.rcsb.org). The PDB was processed in Discovery Studio for removal of ligand, water molecules and to make the binding sites free for interaction with the synthesized compounds. The processed PBD file was converted to PDBQT file by adding Kollmann charges and further used for docking studies. The images were created with the help of Pymol Molecular Viewer [25].

2.6. ADMET and pharmacokinetic studies

ADMET and pharmacokinetic properties were checked using pkCSM (A Cambridge online source, link: http://biosig.unimelb.edu.au/pkcsm/prediction). The structures of all the synthesized hydrazones and their physicochemical properties were drawn and calculated using Chem Draw 12.0 software. Simultaneously, the SMILE file format of all compounds was obtained from Chem Draw 12.0 to obtain the drug likeness data. pkCSM predictor provides information regarding absorption parameters like Human Intestinal Absorption (HIA), Oral bioavailability, Caco-2 permeability, distribution parameters like Plasma Protein Binding (PPB), Blood Brain Barrier (BBB), metabolism parameters like Cytochrome P450 2D6 (CYP2D6) inhibitor and Cytochrome P450 3A4 (CYP3A4) inhibitor, excretion parameters like renal clearance and toxicity parameters like organ toxicity and genomic toxicities.

3. RESULTS AND DISCUSSION

The compounds show stretching frequencies related to -C=N-, -NH, -OH and >C=O (lactone) besides several others. The peak at 1717 to 1737 cm^{-1} is due to lactone group and that at 1581 to 1606 cm^{-1} is due to -C=N- group. The IR spectrum of all the hydrazones showed one peak in between 3085 to 3186 cm^{-1} for -NH and hydrazones 3ACOH, 3ACMH, 3ACPH and 3ACDH showed a peak in between 3313 to 3439 cm^{-1} for -OH. In addition, a peak a in between 1625 to 1687 cm^{-1} is also observed due to carbonyl of acid hydrazide. The ^{1}HNMR of these hydrazones showed singlet at about δ10.5 for -NH and -OH proton appeared at δ = 9.5. The Olefin proton on C4 of coumarin ring appeared as a sharp singlet at δ = 8.2. All other aromatic protons are observed clearly in the range of δ 7.2 to 7.8. However, ^{13}CNMR of these hydrazones showed characteristic peaks of amide, lactone and imine carbons at about 162-164, 159 and 153 ppm respectively. The following HRMS data of all hydrazones also support the confirmed structures.

3.1. Spectral data of synthesized compounds

3.1.1. (12E)-N'-(1-(2-oxo-2H-chromen-3-yl) ethylidene)benzohydrazide (3ACBA)

Off white powder, yield (95%), MP 164-166°C; IR (KBr, cm^{-1}): 3186.50 (-NH), 3021 (Ar-H), 1717.87 (Lactone, >C=O), 1658.11 (>C=O), 1604.07 (-C=N-), 1532.80 (Aromatic, C-C); ^{1}H-NMR (400 MHz, DMSO) δ 2.32 (s, 3H), 10.83 (s, 1H, -NH), 8.24 (s, 1H, olefinic), 7.88 (d, J = 7.04Hz, 3H),), 7.37 to 7.66 (multiplet, 6H); ^{13}C-NMR (125 MHz, DMSO) δ 164.77 (Amide), 159.70 (Lactone), 153.88 (imine), 142.15, 134.47, 132.91, 132.12, 128.79, 128.58, 127.34, 125.31, 125.08, 124.55, 119.34, 116.56, 16.81(CH3); HRMS (EI) : $C_{18}H_{14}N_2O_3$: 307.10.

3.1.2. (12E)-2-hydroxy-N'-(1-(2-oxo-2H-chromen-3-yl)ethylidene)benzohydrazide (3ACOH)

Orange powder, yield (92%), MP 214-216°C; IR (KBr, cm^{-1}): 3311.53 (-OH), 3085.44 (-NH), 3052.63 (Ar-H), 1718.45 (Lactone, >C=O), 1687.37 (>C=O), 1588.39 (-C=N-), 1552.75 (Aromatic, C-C); ^{1}H-NMR (400 MHz, DMSO) δ 2.3 (s, 3H), 12.8 (s, 1H, -NH), 11.4 (s, 1H, -OH), 8.3 (s, 1H, olefinic), 7.99 (d, J = 6.8Hz, 1H), 7.90 (d, J = 6.8 Hz, 1H), 7.65 (t, J = 7.2 and 7.6 Hz, 1H), 7.4 (multiplet, J = 7.2, 7.2,and 7.6 Hz, 3H), 7.10 (multiplet, J = 8.42, 6.8 and 7.2 Hz, 2H), 7.0 (multiplet, J = 8.42, 7.2 and 6.8 Hz, 1H), 6.97 (doublet, J = 8.42 and 6.8Hz, 1H); ^{13}C-NMR (125 MHz, DMSO) δ 162.48(Amide), 159.78 (Lactone), 156.89 (C-OH), 153.98(imine), 142.15, 134.01, 133.57, 131.04, 129.71, 127.01, 125.37, 120.26, 119.61, 118.84, 118.17, 117.27, 116.69, 16.15(CH3); HRMS (EI): $C_{18}H_{14}N_2O_4$: 323.11.

3.1.3. 3(12E)-3-hydroxy-N'-(1-(2-oxo-2H-chromen-3-yl)ethylidene)benzohydrazide (3ACMH)

Brown powder, yield (85%), MP 210-212°C; IR (KBr, cm^{-1}): 3313.39 (-OH), 3151.45 (-NH), 3053.09 (Ar-H), 1720.55 (Lactone, >C=O), 1625.39 (>C=O), 1581.98 (-C=N-), 1520.93 (Aromatic, C-C); ^{1}H-NMR (400 MHz, DMSO) δ 2.32 (s, 3H), 10.75 (s, 1H, -NH), 9.74 (s, 1H, -OH), 8.13 (s, 1H, olefinic), 7.87 (d, J = 6Hz, 1H), 7.65 (t, J = 7.2, 7.6Hz, 1H), 7.45 (d, J = 8Hz, 1H), 7.31 (multiplet, J= 8, 7.6 and 7.2 Hz, 3H), 7.20 (d, J = 8Hz, 1H), 6.97 (s, 1H); ^{13}C-NMR (125 MHz, DMSO) δ 164.59(Amide), 159.71 (Lactone), 158.11 (C-OH), 153.77(imine), 142.45, 135.80, 132.88, 129.90, 129.51, 127.32, 125.34, 125.06, 124.75, 119.34, 119.05, 118.55, 116.54, 16.81(CH3); HRMS (EI): $C_{18}H_{14}N_2O_4$: 323.10.

3.1.4. (12E)-4-hydroxy-N'-(1-(2-oxo-2H-chromen-3-yl)ethylidene)benzohydrazide (3ACPH)

Yellow powder, yield (90%), MP 206-208°C; IR (KBr, cm^{-1}): 3333.12 (-OH), 3122.52 (-NH), 3049 (Ar-H), 1723.34 (Lactone, -CO), 1647.11 (>C=O), 1602.29 (-C=N-), 1534.07 (Aromatic, C-C); ^{1}H-NMR (400 MHz, DMSO) δ 2.31 (s, 3H), 10.56 (s, -NH, 1H), 10.09 (s, -OH, 1H), 8.22 (s, olefinic, 1H), 7.87 (d, J = 8.4 Hz, 2H), 7.78 (d, J = 7.52 Hz, 1H), 7.68 (dd, J = 7.52 and 7.92 Hz, 1H), 7.45 (d, J = 8.28 Hz, 1H), 7.38 (dd, J = 7.52 and 7.92 Hz, 1H), 6.85 (d, J = 8.4 Hz, 2H); ^{13}C-NMR (125 MHz, DMSO) δ 161.14 (Amide), 159.74(Lactone), 158.19(C-OH), 153.89 (imine), 141.94, 134.93, 132.83, 131.24, 129.5, 127.42, 125.24, 124.63, 119.27, 116.59, 115.34, 16.62(CH3); HRMS (EI): $C_{18}H_{14}N_2O_4$: 323.10.

3.1.5. **(12E)-2,4-dihydroxy-N'-(1-(2-oxo-2H-chromen-3-yl)ethylidene)benzohydrazide (3ACDH)**

Off white powder, yield (95%), MP 244-246°C; IR (KBr, cm^{-1}): 3439.27 (-OH), 3378.13 (-OH), 3112.88 (-NH), 3073.34 (Ar-H), 1737.62 (Lactone, >C=O), 1678.68 (>C=O), 1606.43 (-C=N-), 1570.40 (Aromatic, C-C); ^{1}H-NMR (400 MHz, DMSO) δ 2.30 (s, 3H), 10.66 (s, -NH, 1H), 9.70 (s, -OH, 2H), 8.22 (s, olefinic, 1H), 7.95 (d, J = 8.12 Hz, 1H), 7.85 (dd, J = 7.24 and 8.32 Hz, 1H), 7.66 (dd, J = 7.44 and 7.20 Hz, 1H), 7.40 (d, J = 8.32 Hz, 1H), 6.90 (s, 2H), 6.70 (s, 1H); ^{13}C-NMR (125 MHz, DMSO) δ 164.89 (Amide), 159.72(Lactone), 158.66 (C-OH), 153.78 (imine), 142.39, 136.40, 132.86, 129.50, 127.33, 125.38, 125.03, 124.67, 119.34, 116.08, 106.02, 16.71(CH3); HRMS (EI): $C_{18}H_{14}N_2O_5$: 339.09.

3.2. Anticancer studies

The *in vitro* anticancer activities against three human cancer cell lines viz. human breast cancer cell line (MCF-7), human cervical cancer cell line (HeLa) and human oral squamous cell carcinoma (SCC-40) were carried out while the tumour selectivity of compounds were tested on the normal human peripheral blood mononuclear cells (PBMCs). The results for each hydrazone are expressed as the growth percent of treated cells compared to untreated control cells and the growth curves of the cell lines are shown in Fig. 1 to 3. The GI50 which is 50% growth inhibition of cells was calculated using the graph obtained by plotting drug concentration and % control growth. The calculated values of GI50 for test compounds and standard drug Adriamycin (ADR) are shown in Table 1.

In terms of GI_{50} values, all the derivatives showed prominent activity against Human cervical cancer cell line (HeLa). The GI_{50} value for 3ACOH and 3ACDH against HeLa is 20.4μg/ml and 25.7μg/ml respectively. While the GI_{50} values of all these hydrazones are more than 80μg/ml for MCF-7 and SSC-40 which is far more than the highest concentration of the drug. The standard ADR showed GI_{50} value < 10 μg/ml. Compared with the standard drug Adriamycin, the synthesized hydrazones are moderately active against the three

cancer cell lines. Among the synthesized hydrazones, 3ACPH is effective against MCF-7 with percent growth of 74.9 at 10 µg/ml concentration. 3ACPH showed percent growth of 39.1 against SSC-40 at 10 µg/ml concentration. The results of anti-cancer screening data reveal that the hydrazones show considerable good activity against human cervical cancer cell line (HeLa) and moderately active against MCF-7 and SCC-40.

To evaluate tumour selectivity of hydrazones, cell cultures of PBMCs were exposed to synthesized hydrazones for about 24 hours. The results are shown in Fig. 4. These results of MTT assay showed that the hydrazones with a concentration of 10µg/ml did not cause significant cytotoxicity. However, higher concentrations of these hydrazones led to slightly increased cytotoxicity. The graphs show that on treatment with 80µg/ml concentration of active hydrazones, the percentage growth of cancer cells falls to nearly 20% in all the cell lines and more than 70% of normal cells on treatment with same concentration. This concentration is far higher than GI50 value of the compound against cancer cell lines as mentioned above. These observations indicate that all the synthesized hydrazones are selective against cancer cells.

Table 1: Calculated GI50 values of synthesized compounds (µg/ml)

Cell Lines	MCF-7	HeLa	SSC-40
Codes	GI50*	GI50*	GI50*
3ACBA	>80	72.2	>80
3ACOH	>80	20.4	>80
3ACMH	>80	44.1	>80
3ACPH	>80	>80	>80
3ACDH	>80	25.7	>80
ADR	<10	<10	<10

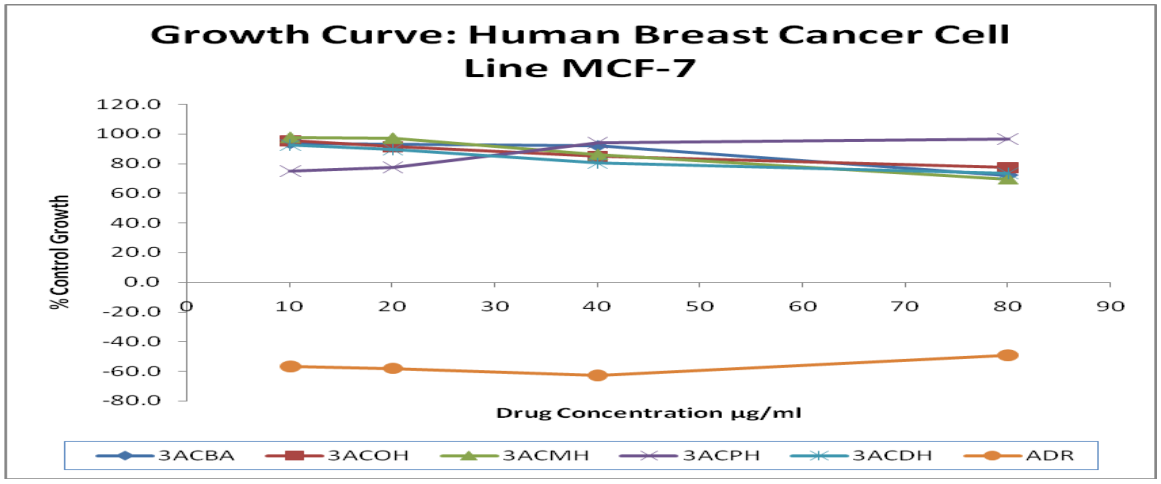


Fig. 1: Growth Curve: Human Breast Cancer Cell Line (MCF-7)

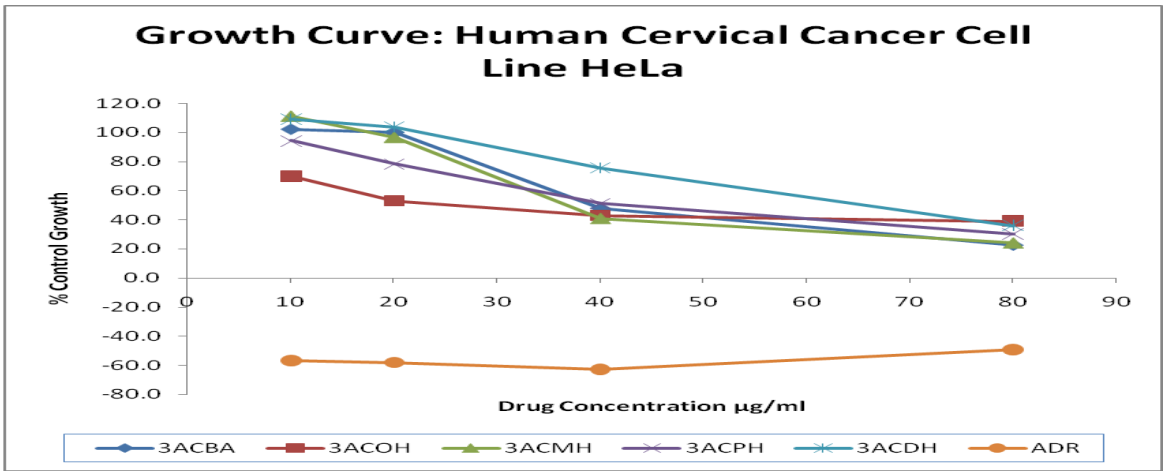


Fig. 2: Growth Curve: Human Cervical Cancer Cell Line (HeLa)

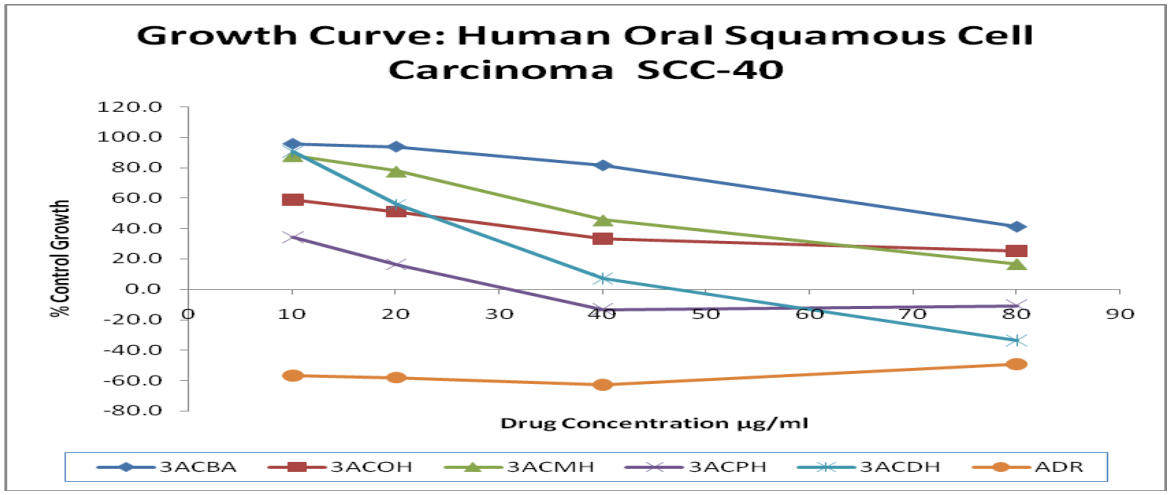


Fig. 3: Growth Curve: Human Oral Squamous Cancer Cell Line (SSC-40)

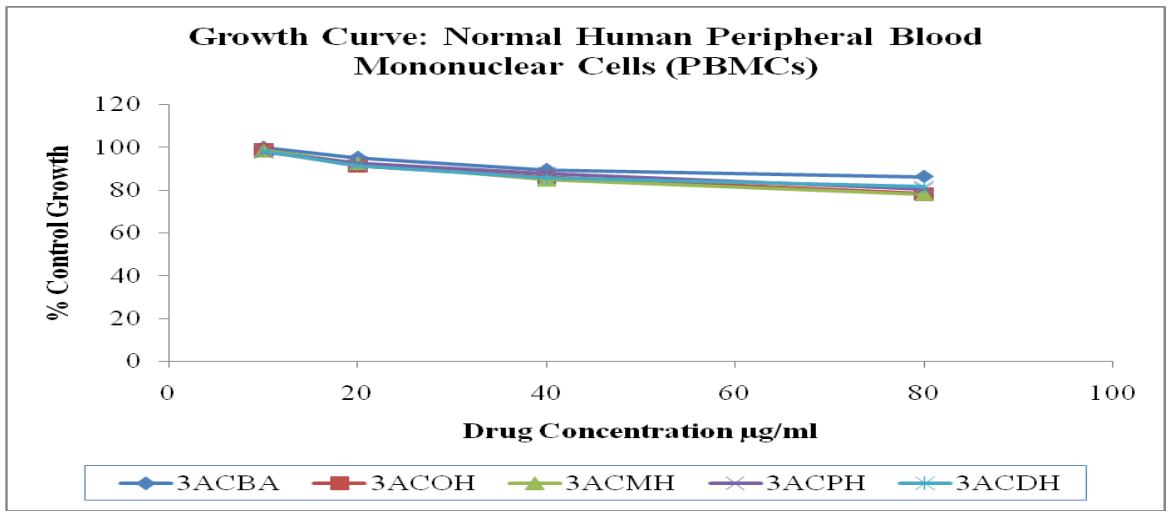


Fig. 4: Growth Curve of Normal Human Peripheral Blood Mononuclear Cells (PBMCs)

3.3. Molecular docking study of synthesized compounds

Molecular Docking study was carried out to check the binding affinities of 3-acetylcoumarin hydrazones with the Cyclooxygenase active site residues of COX-2 enzyme (PDB ID: 6COX) using the software Auto Dock 4.2.614. A grid box covering the cyclooxygenase active site residues of the target protein was generated to get the best conformational state of docking. Docking grid box size was set to 46×46×46 Å dimension and centered at 30.77, -1.64, 25 of X, Y and Z coordinates. The estimated binding energy values and the interacting amino acid residues are given in Table 2. The calculated values of binding energies reveal that all 3-acetyl-coumarin hydrazones fit favorably into the cyclooxy-genase active site of 6COX displaying hydrogen bonding with different amino acid residues of the target protein. The docking ribbon structures of 6COX protein with respective compounds are given in Fig. 6. The binding interactions for these hydrazones involve -NH and -OH functionalities in the structures. The best binding energy was exhibited by 3ACOH followed by 3ACMH, 3ACDH, 3ACBA and 3ACPH. Thus, the compound 3ACOH has stronger binding interactions with 6COX than other derivatives. The compounds show hydrogen bonding interactions with amino acid residues and the hydrogen bond distances of 2.7, 2.1 and 2.2 Å lengths indicate strong interactions. These interactions lead to stabilization of the compounds in the protein cavity. The docking results show that these hydrazones may have *in vitro* significant interactions with human Cyclooxygenase.

Table 2: Binding energies and the interacting surfaces of the compounds

Sr.	Code	B.E. in kcal/mole	Binding amino acid Residue	Bond Length in Angstrom
1	3ACBA	-9.3	CYS-47	1.9
2	3ACOH	-9.9	ASN-34, GLY-135, ALA-156	2.7, 2.1, 2.2
3	3ACMH	-9.7	GLN-42, CYS-47, GLU-465	3.4, 2.3, 3.4
4	3ACPH	-9.3	CYS-47	3.4
5	3ACDH	-9.4	ASN-39, ARG-44, PRO-154, GLN-461	2.4, 2.6, 3.4, 2.5

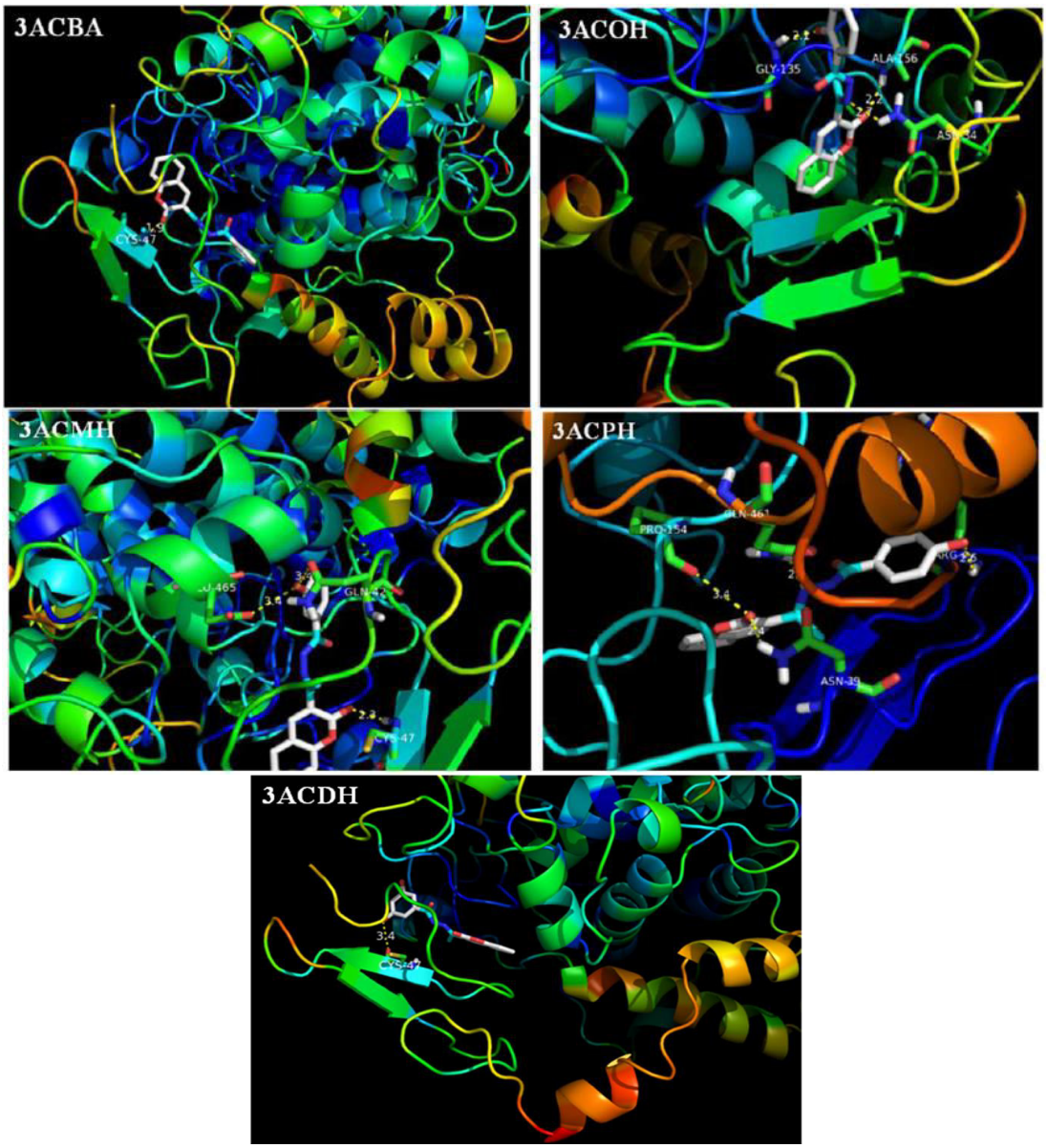


Fig. 5: Binding of 3-acetylcoumarin Hydrazones into active site of 6COX assessed from molecular docking

3.4. ADMET and Pharmacokinetic studies

The predicted pharmacokinetic parameters and other physicochemical properties are important for both *in silico* and *in vitro* evaluation of drug-like properties [25]. To make sure that the synthesized molecules show the potential of a drug, their ADMET and pharmacokinetic properties were checked using pkCSM and the predicted values are shown in Table 3. The molecules have a great solubility potential, both in water as well as $CaCO_2$ permeability. The values of intestinal absorption

(human), skin permeability, CNS permeability are in the permitted range. The molecule's ability to inhibit CYP2D6 and CYP3A4 shows that the four molecules except 3ACPH, will not allow the metabolism of xenobiotics in the body. The values of total clearance, which represents the rate of drug elimination divided by its plasma concentration, is quite favorable for all molecules and this implies that the molecules would not accumulate in the body and hence are nontoxic. The hydrazones didn't show hepatotoxicity except 3ACDH. These results depict that the compounds have got good ADMET values and have drug-likeness properties.

From the predicted pharmacokinetic parameters (Table 3), it appears that test compounds show good drug likeness properties and could be good drug candidates for further studies. They have Drug Likeness with zero violations as per the standards defined by Lipinski, Ghose, Veber, Egan and Muegge which is evident from the results shown in Table 4. The Medicinal Chemistry parameters for these compounds exhibit their potential with lead like properties with no violations and they fall under the category of PAINS (Pan Assay INterference compoundS), suggesting that these hydrazones can be the starting point in the pursuit of biologically active compounds. However, two alerts are reported on Brenk Model for each compound which is attributable to imine bond and Coumarin fragment.

Table 3: ADMET properties of 3-acetylcoumarin hydrazones calculated from pkCSM online tool

Property	Absorption				Distribution			Metabolism		Excretion	Toxicity			
Model Name	Water solubility (log mol/L)	$CaCO_2$ permeability (log Papp in 10^{-6} cm/s)	Intestinal absorption (human) (% Absorbed)	Skin Permeability (log Kp)	VDss (human) (log L/kg)	BBB permeability (log BB)	CNS permeability (log PS)	CYP2D6 inhibitor (Yes/No)	CYP3A4 inhibitor (Yes/No)	Total Clearance (log ml/min/kg)	AMES toxicity (Yes/No)	Max. tolerated dose (human) (log mg/kg/day)	Oral Rat Acute Toxicity (LD50) (mol/kg)	Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_bw/day)	Hepatotoxicity (Yes/No)
3ACBA	-3.955	0.938	93.534	-2.801	-0.343	-0.198	-1.912	No	No	0.843	No	0.215	2.042	1.214	No
3ACOH	-3.719	0.746	86.846	-2.937	-0.284	-0.298	-2.102	No	No	0.709	No	0.337	1.987	2.052	No
3ACMH	-3.731	0.614	86.842	-2.922	-0.262	-0.293	-2.099	No	No	0.738	No	0.363	1.928	2.039	No
3ACPH	-3.887	0.653	86.844	-2.931	-0.388	-0.326	-2.093	No	Yes	0.719	No	0.394	1.919	1.878	No
3ACDH	-3.526	0.253	76.419	-2.815	-0.432	-1.037	-2.342	No	No	0.648	No	0.598	2.017	2.738	Yes

Table 4: Drug Likeness properties of the synthesized 3-acetylcoumarin hydrazones

Compound	Mol.Wt.	ClogP	No. of H-bond acceptors	No. of H-bond donors	PSA	Lipinski No. of violations	Ghose No. of violations	Veber No. of violations	Egan No. of violations	Muegge No. of violations	Bioavailability Score	PAINS No. of Alerts	Brenk No. of Alerts	Lead likeness No. of Violations
3ACBA	306.32	2.97	4	1	71.67	0	0	0	0	0	0.55	0	2	0
3ACOH	322.31	2.54	5	2	91.9	0	0	0	0	0	0.55	0	2	0
3ACMH	322.31	2.49	5	2	91.9	0	0	0	0	0	0.55	0	2	0
3ACPH	322.31	2.57	5	2	91.9	0	0	0	0	0	0.55	0	2	0
3ACDH	338.31	2.39	6	3	112.13	0	0	0	0	0	0.55	0	2	0

4. CONCLUSION

In the present study, a new series of coumarin hydrazone scaffolds containing an azomethine group were synthesized. All the compounds were tested for their *in vitro* anticancer activity against MCF-7, HeLa and SCC-40 and normal human PBMCs with SRB and MTT assay. The compounds showed moderate anti-cervical cancer activity. Two hydrazones 3ACOH and 3ACDH exhibit distinct activity with GI_{50} value of 20.4µg/ml and 25.7µg/ml against Human Cervical

Cancer Cell Line (HeLa). On the contrary, the derivative 3ACMH exhibits a cytotoxic activity with 69.6% control growth at 80µg/ml against MCF7 cell line. On the basis of such results it may be concluded that the position as well the number of hydroxyl groups both is responsible for the cytotoxicity of cells. The molecular docking studies are also in best agreement with experimental results. The shown activity can be accounted for the presence of polar interactions between the -NH and -OH groups and amino acid residues in the protein. These compounds fit well in the target enzyme's active site pocket and these interactions lead to stabilization of the compounds in the protein cavity. The above results are quite encouraging and these 3-acetylcoumarin hydrazones may be considered as potential antiproliferative agents in future.

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Conflict of interests

Authors have no conflict of interest to declare.

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