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Research article

IN-SILICO DESIGN, SYNTHESIS AND EVALUATION OF *IN-VITRO* ANTI-CANCER ACTIVITY OF NOVEL HYDROXYXANTHONE DERIVATIVES

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Abstract

Background*: Xanthones or 9H-xanthen-9-ones (dibenzo-γ-pirone) comprise an important class of oxygenated heterocyclic whose role is well-known in medicinal chemistry. The biological activities of this class of compounds are associated with their tricyclic scaffold but vary depending on the nature and position of the different substituents. The aim at an array of pharmacological effects is presented for synthetic xanthone derivatives, with an emphasis on some significant studies against the anti-cancer activity.* ***Objective:*** *A novel series of 3-(3'-substituted propoxy)-1-hydroxyxanthone and 3-(5'-substituted pentyloxy)-1-hydroxyxanthone derivatives were synthesized.* ***Methods:*** *The synthesis of 3-(3'-substitutedpropoxy)-1-hydroxyxanthone and 3-(5'-*

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substitutedpentyloxy)-1-hydroxy xanthone derivatives. Physico-chemical characterization of the synthesized compounds. Computational and combinatorial chemistry methodology has helped to create a highly selective xanthone derivative which can serve as a novel lead compound for further developments in the field of Topoisomerase-II inhibitors. The structures of the designed ligands were established through the spectroscopic analysis. The bioassay test was conducted for the synthesized compounds by A549 cancer cell lines to find out the inhibitory concentration that kills the cells by 50 %. ***Results and Discussion****: The 3-(3'-substituted propoxy)-1-hydroxyxanthone and 3-(5'-substituted pentyloxy)-1-hydroxyxanthone derivatives act as Topoisomerase-II inhibitors on the basis of molecular docking but which contribute to the possible mechanism of anti-cancer activity of these analogues.* ***Conclusion:*** *The series of 3-(3'-substitutedpropoxy)-1-hydroxyxanthone and 3-(5'-substitutedpentyloxy)-1-hydroxy xanthone derivatives are the potential key approach to design novel anti-cancer agents.*

Keywords: Cancer; Topoisomerase-II inhibitors; Xanthone derivative; 3-(3'-Substituted Propoxy)-1-Hydroxy Xanthone and 3-(5'-Substituted Pentyloxy)-1-Hydroxyxanthone.

Introduction

Cancer is a generic term that involves in disruption of normal cell division and apoptosis and is characterized by the growth (Ferlay *et al.*, 2007; John *et al.*, 2001) of abnormal cells beyond their usual boundaries and that can intrude on the adjoining body parts and spread to other organs (Shudhakar *et al.*, 2017; Ames *et al.*, 1971). Xanthones are secondary metabolites commonly occurring in higher plant families, fungi, and lichens (Singh *et al.*, 2017). Their pharmacological properties (Gobbi *et al.*, 2006) have raised

great interest xanthones are found as the parent polyhydroxylated compounds but most are mono or polymethyl ethers or are found as Glycosides (Bennett *et al.,* 1989; Hostettmann *et al.,* 1997). DNA topoisomerases- II α and topoisomerase- II Beta catalyze the ATP-dependent transport of one intact DNA double helix through another (Nainwal *et al.,* 2014; Larsen *et al.,* 2003; Chang *et al.,* 2010). Structural and biochemical studies showed topo- II selectively negatively supercoil or decadent DNA (Fortune *et al.,* 2000; Cho *et al.,* 2009; Heck *et al.,* 1986; Jensen *et al.,* 1997). Topo- II α plays a key role in DNA replication with main functions are chromosome segregation, chromosome condensation (Holder *et al.,* 2001; Wilstermann *et al.,* 2003), arrest in meiosis I and recombination suppression (Block *et al.,* 1992; Chaffer *et al.,* 2011). Our group has carried out work with series of 3-(5-substituted pentyloxy)-1-hydroxyxanthone derivatives that exhibited antibacterial activity then 3, 6-bis (3-substituted propoxy) xanthone derivatives were also studied for antibacterial activity (Lin *et al.,* 1996; Sun *et al.,* 2002). Another 3, 6-bis (3'-substitutedpropoxy) xanthone and 3, 6-bis (5'-substitutedpentyloxy) xanthone derivatives were also explored for having antibacterial activity followed by another series namely 3, 6-bis (3'substituted propoxy) xanthones and 3, 6-bis (5'-substituted pentyloxy) xanthone derivatives were studied targeting prostaglandin endoperoxide synthase-II (Grover *et al.,* 1995; Negi *et al.,* 2013). Also series 3-(5'-substitutes pentyloxy)-1-hydroxy xanthone and 6-(5'-substitutedpentyloxy)-1-hydroxy xanthone derivatives were also being studied targeting prostaglandin endoperoxide synthase-II and all the activities of the above series were reported (Liu *et al.,* 2016; Yu-Chain *et al.,* 2007; Chu *et al.,* 2002).

Materials and Methods

The total experiment is done completing four different stages namely design and *in-silico* study, synthesis of designed compound, physiochemical characterization of synthesized compound, anti-cancer activity evaluation of the synthesized compounds respectively (Liu *et al.,* 2016; Yu-Chain *et al*., 2007; Chu *et al.,* 2002).

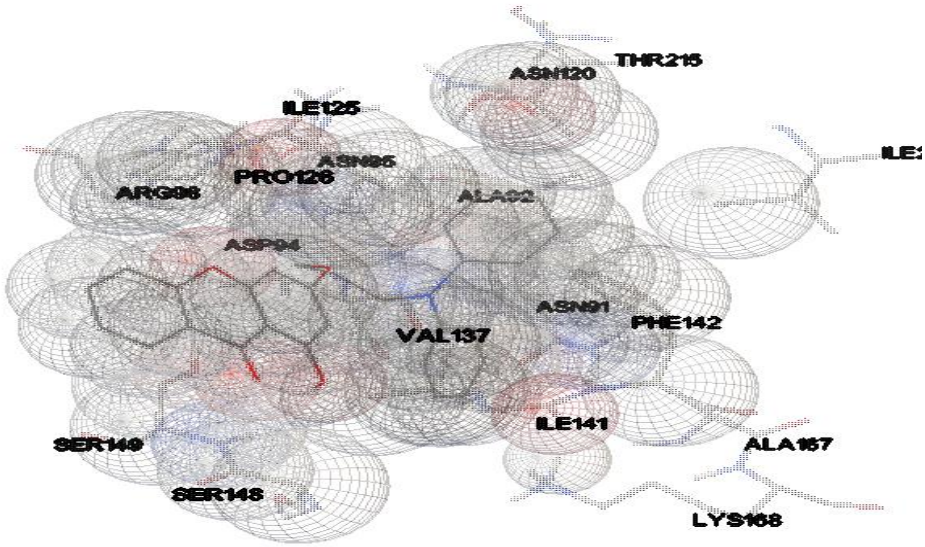
Design and *in-silico* study:

ChemDraw Ultra 8.0 was used for drawing and converting 2D chemical structure of the compound to 3D structure (mol. format) before submission for docking. The energies of ligand structures were previously minimized using the Molecular modelling 2 (MM2) force-field method with ChemDraw Ultra 3D software (Lipinski *et al.,* 2001; Cheng *et al.,* 2011). Then the protein was to be prepared and accordingly the 3D fabric (D5, D9, D14, D18 and D22) of the protein was computerized from Research Collaboratory for Structural Bioinformatics (RCSB), Protein Data Bank (PDB). Molecular docking was used to detect the protein-ligand orientation and interaction against protein 1ZXM which is shown in figure 1. AutoDock Tools package version 1.5.6 was utilized to create the docking input files. AutoDock Tools was used for creating Protein Data Bank, partial charge (Q) and atom type (T), (PDBQT) files from Protein Data Bank (PDB) files (Khodade *et al.,* 2007). Ligands were also saved as PDBQT file format after adding torsional energy for docking simulation. Binding site analysis for the identification and visualization of possible binding sites and the distribution of surrounding residues in the active sites were shown in Table 1 and the binding interactions were depicted in figure 1 (*i.e.*, binding energy in kcal/mol) of ligand and protein which is confirmed by binding energy were calculated after docking (Baruah *et al.,* 2013; Jun *et al.,* 2011). The search process begins from a random ligand location and orientation inside and outside the

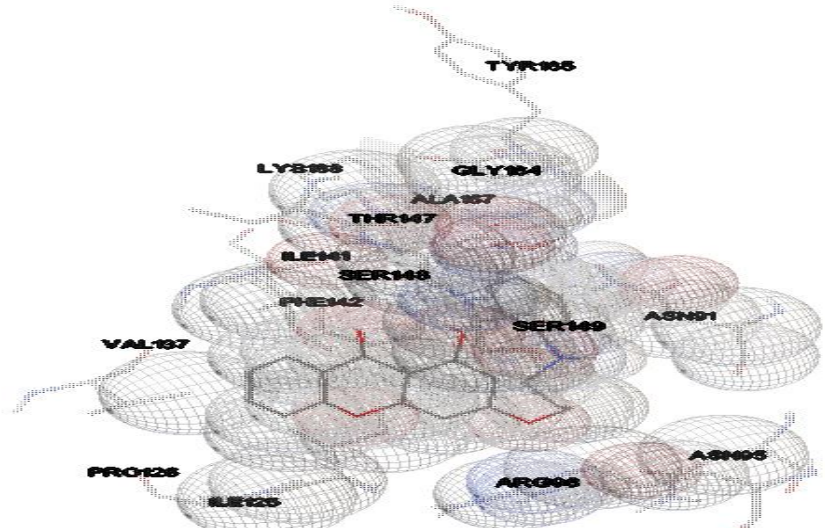
binding pocket. By exploring the value of ligand translations, rotations and internal degree of freedom, it eventually reached the bound conformation.

Table1: Synthesized compounds with different residues present on the binding sites of the protein (PDB ID: 1ZXM) during docking simulations.

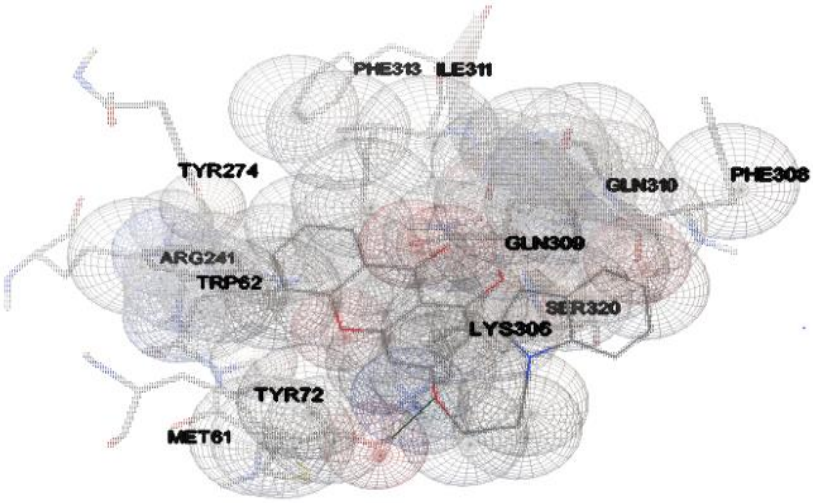
Molecular Code	**Residues involved in docking interactions**	**Binding energy (Kcal/mol)**
D5	Asn120, Thr215, Ile125, Phe142, Ile217, Ala167, Val137 Pro126, Ser148, Ser149, Lys168.	-6.42
D9	Tyr165, Gly164, Phe142, Ser149, Val137, Ile141, Pro126, Ile125, Thr147, Lys168, Ala167, Ser148.	-6.43
D14	Gln309, Phe313, Ile311, Phe308, Gln310, Ser320, Tyr274, Arg241, Lys306.	-6.20
D18	Asn258, Lys233, Ile109, Val236, Pro111, Glu112, Ala237, Val240, Asp110, Asn113.	-5.14
D22	Gln309, Lys306, Arg241, Asp245, Phe313, Ile311, Ser320, Gln310, Ser312, Thr310.	-5.94



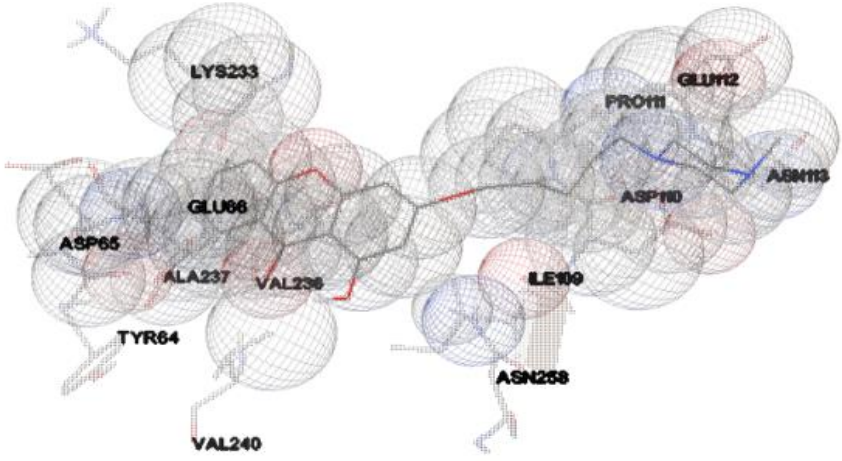
D5: 3-(3-(diphenylamino)propoxy)-1-hydroxy-9H-xanthen-9-one



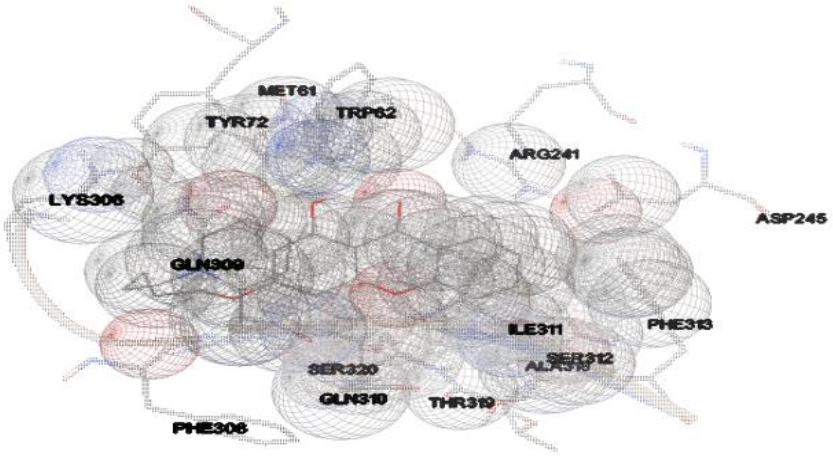
D9: 3-(3-(4-nitrophenylamino)propoxy)-1-hydroxy-9H-xanthen-9-one



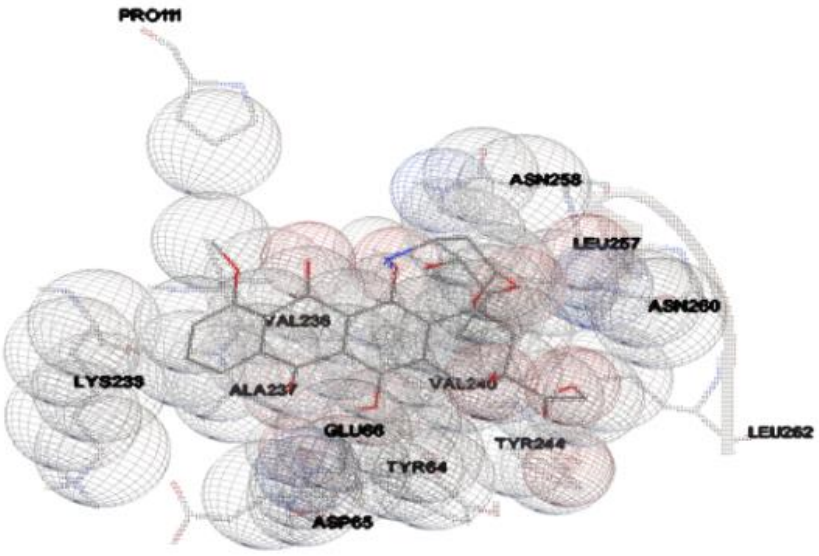
D14: 3-(3-(2-methyl-1H-indol-1-yl)propoxy)-1-hydroxy-9H-xanthen-9-one



D18: 3-(5-(4-methylpiperazin-1-yl)pentyloxy)-1-hydroxy-9H-xanthen-9-one



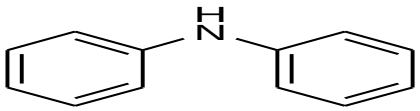
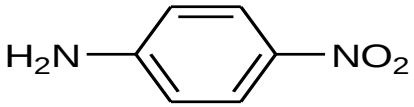
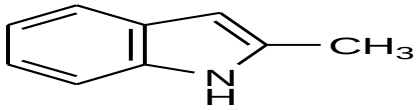
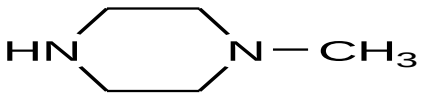
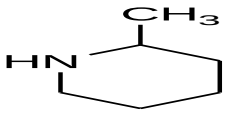
D22: 3-(5-(2-methylpiperidin-1-yl)pentyloxy)-1-hydroxy-9H-xanthen-9-one



Doxorubicin

Fig 1: Interaction of the ligands against the protein 1ZXM

Table 2: List of the proposed compounds to be synthesized

Sl. No.	Molecular code	Substituent (X)	Binding energy (Kcal/mol)
1	D5		-6.42
2	D9		-6.43
3	D14		-6.20
4	D18		-5.14
5	D22		-5.94

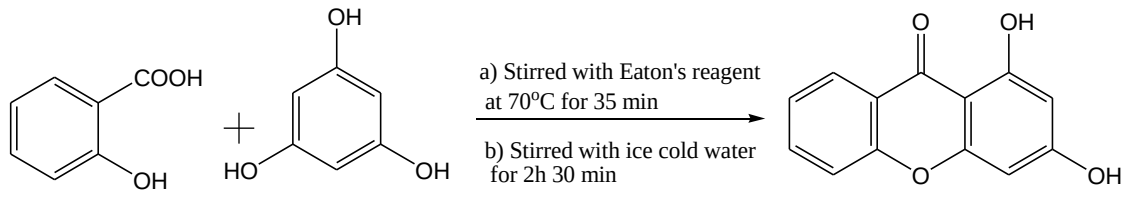
Synthesis:

Synthesis was carried out in two sections namely synthesis of 3-(3'-substitutedpropoxy)-1-hydroxyxanthone and 3-(5'-substitutedpentyloxy)-1-hydroxyxanthone derivatives and physico-chemical characterization of the synthesized compounds Table 2. Synthesis of all the designed compounds (D5, D9, D14, D18 and D22) involves three steps reactions figure 2 (Liu *et al.*, 2016; Yu-Chain *et al.*, 2007; Chu *et al.*, 2002). All the targeted compounds were synthesized using laboratory grade chemicals and solvents. Basically, two series were designed namely 3-(3'-substitutedpropoxy)-1-hydroxyxanthone derivatives and 3-(5'-substitutedpentyloxy-hydroxyxanthone respectively.

1. Synthesis of 3-(3'-substitutedpropoxy)-1-hydroxyxanthone derivatives

Synthetic scheme:

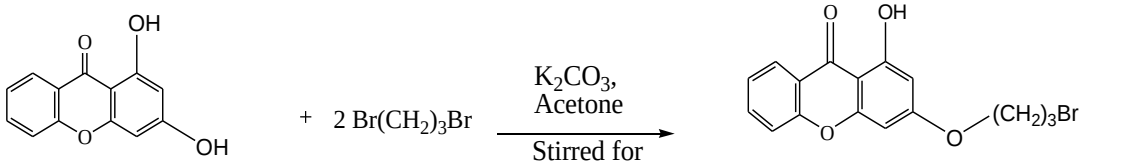
a.



Salicylic Acid Phloroglucinol 1,3-dihydroxyxanthone

Int-1

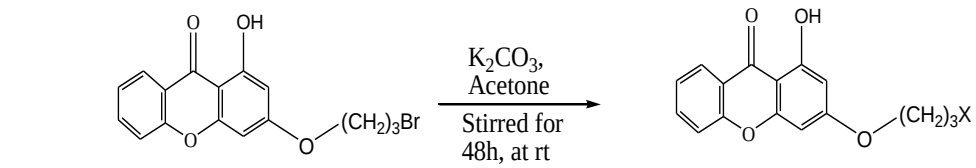
b.



1,3-dihydroxyxanthone 1,3-Dibromopropane 3-(3'-bromopropoxy)-1-hydroxy xanthone

Int.-2

c.



3-(3'-bromopropoxy)-1-hydroxy xanthone

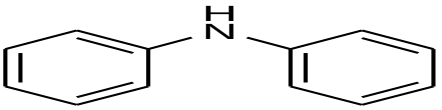
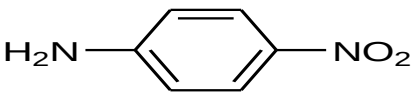
X= Primary or secondary amine

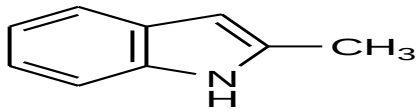
3-(3'-substituted propoxy)-1-hydroxy xanthone

Fig 2: Synthesis of 3-(3'-substitutedpropoxy)-1-hydroxyxanthone derivatives

The substituted amines 'X' as given is figure 2

Table 3: List of substitute amines 'X' with binding energy

Sl. No.	Molecular code	Substituent (X)	Binding energy (Kcal/mol)
1	D5		-6.42
2	D9		-6.43

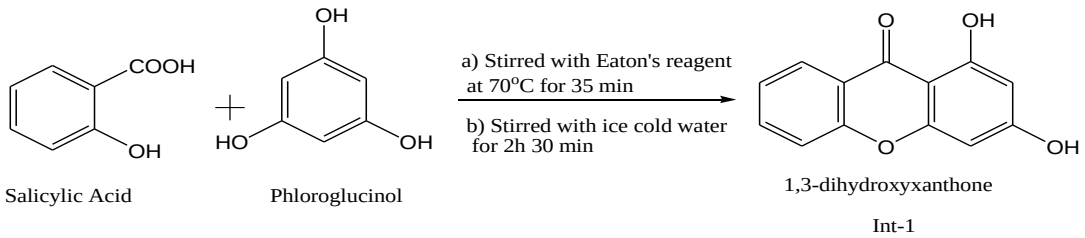
3	D14		-6.20

Procedure: Eaton's reagent was prepared by dissolving phosphorus pentoxide in methanesulphonic acid in 1:10 ratio. 100 ml of Eaton's reagent was added slowly to a mixture of phloroglucinol (60 mmol) and salicylic acid (60 mmol). The mixture was warmed up to 70 °C for 35 min under stirring. Then cooled to room temperature and poured the reaction mixture into ice and stirred for 2h 30 min. The resulting solid collected by filtration, washed with water until pH 6 and dried at 60 °C. Potassium carbonate (0.4 mmol) was added to a solution of 1, 3-dihydroxyxanthone (0.2 mmol) [Intermediate 1, *i.e.*, Int. 1] and 1, 3-dibrompropane (0.4 mmol) in acetone. The mixture was refluxed for 4 hours at 50-55 °C (Diderot *et al.*, 2006; Eaton *et al.*, 1973). The resulting solid was collected by filtration and extracted with ethyl acetate to obtain a yellow pale solid. Now, 3-(3'-bromopropoxy)-1-hydroxyxanthone (10 mmol) and selected amine (20 mmol) were dissolved in 20 ml of acetone containing (20 mmol) potassium carbonate and the reaction mixture was refluxed for 6 hours at 65-70 °C figure 2. The end of the reaction was confirmed by Thin Layer Chromatography (TLC) with the formation of only one spot using acetone: hexane (2:1). The mixture was filtered and collected as a yellow solid. Finally, the amines were substituted at position X Table 3.

2. Synthesis of 3-(5'-substitutedpentyloxy)-hydroxyxanthone derivatives

Synthetic scheme:

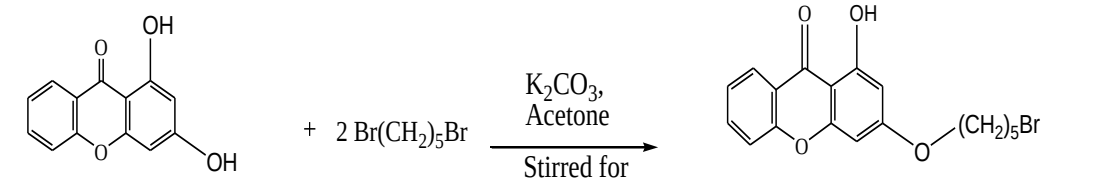
a.



Salicylic Acid Phloroglucinol 1,3-dihydroxyxanthone

Int-1

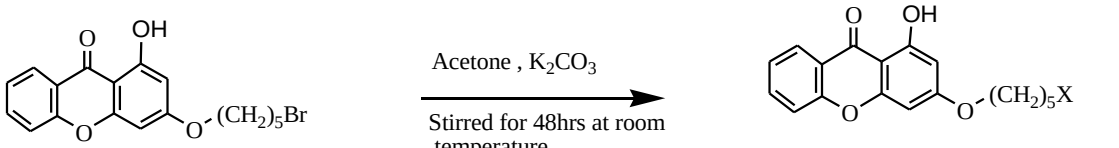
b.



1,3-dihydroxyxanthone 1,5-Dibromopentane 3-(5'-bromopentyloxy)-1-hydroxy xanthone

Int.-2

c.



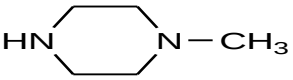
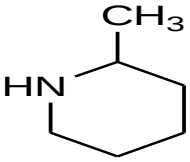
3-(5'-bromopentyloxy)-1-hydroxy xanthone

3-(5'-substitutedpentyloxy)-1-hydroxy xanthone

Fig 3: Synthesis of 3-(5'-substitutedpentyloxy)-hydroxyxanthone derivatives

The substituent amines 'X' is given as figure 3:

Table 4: List of substitute amines 'X' with binding energy

Sl. No.	Molecular code	Substituent (X)	Binding energy (Kcal/mol)
1	D18		-5.14
2	D22		-5.94

Procedure: Eaton's reagent was prepared by dissolving phosphorus pentoxide in methanesulphonic acid in 1:10 ratio *i.e.,* 10 gm phosphorus pent oxide was dissolved in 100 ml methanesulphonic acid. 100 ml of Eaton's reagent was added slowly to a mixture of phloroglucinol (60 mmol) and salicylic acid (60 mmol). The mixture was warmed up to 70 °C for 35 min under stirring. Then cooled to room temperature and poured the reaction mixture into ice and stirred for 2 h 30 min. The resulting solid collected by filtration, washed with water until pH 6 and dried at 60 °C. Potassium carbonate (0.4 mmol) was added to a solution of 1, 3-dihydroxyxanthone (0.2 mmol) [Intermediate 1, *i.e.,* Int. 1] and 1, 3-dibrompentane (0.4 mmol) in acetone. The mixture was refluxed for 4 hours at 50-55 °C (Diderot *et al.,*

2006; Eaton *et al.,* 1973). The resulting solid was collected by filtration and extracted with ethyl acetate to obtain a yellow pale solid 3-(5'-bromopentyloxy)-1-hydroxyxanthone (10 mmol) and selected amine (20 mmol) were dissolved in 20 ml of acetone containing (20 mmol) potassium carbonate and refluxed at 65-70 $^{\circ}$C for 6 h. The end of the reaction was confirmed by TLC with the formation of only one spot using acetone: hexane (2:1) figure 3. The mixture was filtered and collected as a yellow solid. Finally, the position X was substituted with the respective amines Table 4.

Physicochemical characterization of compounds

Thin layer chromatography (TLC) was done for identification and characterization (R_f value) as well as to test the completion of reaction (Sthal *et al.,* 1969). The TLC plate was prepared by using Silica gel G. The different mobile phases were selected according to the assumed polarity of the products. The spots were visualized by exposure to iodine vapor and UV light**.** Melting point range of the synthesized compounds was determined by melting point apparatus (Veego, Model No. MP-I). The compounds were placed in one end sealed capillary and then the capillary was placed in caves of the instrument. The temperature ranges at which the compound melted was measured by a thermometer as a melting point range. For carrying out solubility test, various solvents of different polarity were taken for dissolving the intermediates and final products Table 5. The 0.5 mg of each compound was weighed and added to 5 ml of solvent and determined the solubility of the intermediates and final products.

The wavelength of maximum absorption (λ_{max}) of the compounds was recorded on Shimadzu, UV-1700, and Ultra Violet Visible (UV-VIS) spectrophotometer instrument. Now, the Fourier Transform-Infrared Spectroscopy (FT-IR) spectra of the synthesized compounds were recorded on OPUS, Bruker-Alpha and FT-IR spectrometer using KBr pellet technique.

Then, the H^1-nuclear magnetic resonance (H^1-NMR) spectra of the synthesized compounds were recorded in MeOD at 400.40 MHz by Bruker Avance-II 400 NMR spectrometer and ^{13}C-nuclear magnetic resonance (^{13}C-NMR) also recorded in MeOD at 100 MHz by Bruker Avance-II 100 NMR spectrometer. Chemical shifts are expressed as δ values (ppm), downfield from Tetramethylsilane (TMS) used as internal standard. Significant H^1-NMR data was written in order: multiplicity (s, singlet; d, doublet; t, triplet; m, multiple), number of protons, coupling constants in hertz, assignment. Finally, the mass spectra of the synthesized compounds were recorded in methanol on Water ZQ-4000 equipped with an Electrospray ionization technique as an ionization method. The mass/charge (m/z) values of the intense peaks with abundance were mentioned. The data's obtained from physicochemical studies have been given below.

Table 5: Physicochemical properties of the synthesized compounds

Sl. No.	**Compound code**	**Sate**	**Colour**	**Solubility**	**Melting point**	**Rf value**
1	D5	Solid	Yellowish	DMSO, Acetone, Acetonitrile, Ethanol.	185-190 °C	0.54
2	D9	Solid	Yellowish-brown	DMSO, Acetone, Acetonitrile, Ethanol.	190-195 °C	0.56
3	D14	Solid	Brownish-yellow	DMSO, Acetone,	225-230 °C	0.65

				Acetonitrile, Ethanol.		
4	D18	Solid	Yellowish	DMSO, Acetone, Acetonitrile, Ethanol.	241-245 °C	0.76
5	D22	Solid	Yellowish	DMSO, Acetone, Acetonitrile, Ethanol.	230-234 °C	0.67

*Solvent system for TLC- Acetone: Ethyl acetate: Hexane: 3:1:1

Spectral analysis

All the synthesized compounds were characterized (Table 5) with UV-VIS spectrophotometry, FTIR, ^{1}H NMR and MS. Respective data are described below and spectra's given in appendices (Pavia *et al.,* 2008; Silverstein *et al.,* 2005; Wiberg *et al.,* 1960).

Compound D5: 3-(3-(diphenylamino)propoxy)-1-hydroxy-9H-xanthen-9-one

UVλmax (in DMSO): 330 nm, **FTIR** (cm^{-1}): 3374.68 (O-H str., phenolic), 1659.43 (N-H bend, sec. amine), 1584.24 (C-C str., aromatic), 1298.66 (C-N str., sec. amine), 3215.44 (N-H str., amine), 1705.41 (C=O str., ketone).

^{1}HNMR (400 MHz) $DMSOd_6$, δ (ppm): 7.249, 6.841 (CH, benzene), 3.650, 3.617 (CH_2, methylene), 4.512, 4.321 (OH, aromatic, C-OH), 3.437 (NH, aromatic, C-NH)

^{13}CNMR (400 MHz) $DMSOd_6$, δ (ppm): 31.09, 39. 35, 40.18 (CH_2, aliphatic), 120.12, 129.24, 143.68(CH, 1 benzene)

MASS (m/z %): 437

Compound D9: 3-(3-(4-nitrophenylamino)propoxy)-1-hydroxy-9H-xanthen-9-one

UVλmax (in DMSO): 350 nm, **FTIR** (cm^{-1}): 3355.21 (O-H str., phenolic), 1659.51(N-H bend, sec. amine), 1580.69 (C-C str., aromatic), 1288.99 (C-N str., sec. amine), 3213.10 (N-H str., amine), 1468.25 (C-NO_2., aromatic)

^{1}HNMR (400 MHz) $DMSOd_6$, δ (ppm): 6.753, 6.592 (CH, benzene), 3.463, 1.056(CH_2, methylene), 4.497, 4.421(OH-aromatic, C-OH)

^{13}CNMR (400 MHz) $DMSOd_6$, δ (ppm): 40.40, 56.37, 66.70(CH_2, aliphatic), 112.43, 117.68, 126.60, 155.80 (CH-1 benzene), 18.88 (CH_3, aliphatic), 180.48 (C, 1- carbonyl)

MASS (m/z %): 405

Compound D18: 3-(5-(4-methylpiperazin-1-yl)pentyloxy)-1-hydroxy-9H-xanthen-9-one

UVλmax (in DMSO): 361 nm, **FTIR** (cm^{-1}): 3366.58 (O-H str., phenolic), 1604.94 (N-H bend, sec. amine), 1315.02 (C-O str., phenolic), 1207.92 (C-N str., sec. amine).

^{1}HNMR (400 MHz) $DMSOd_6$, δ (ppm): 7.355, 7.276 (CH, benzene), 3.412(CH_2, methylene), 2.336 (CH, 4-CH_3-piperazine)

^{13}CNMR (400 MHz) $DMSOd_6$, δ (ppm): 39.29, 40. 38, 56.35 (CH_2-aliphatic), 18.88 (CH_3-aliphatic)

MASS (m/z %): 395

Compound D22: 3-(5-(2-methylpiperidin-1-yl)pentyloxy)-1-hydroxy-9H-xanthen-9-one

UVλmax (in DMSO): 342 nm, **FTIR** (cm^{-1}): 3206.29 (O-H str., phenolic), 1551.19 (N-H bend, sec. amine), 1590.27 (C-C str., aromatic), 1053.72 (C-N str., sec. amine).

^{1}HNMR (400 MHz) $DMSOd_6$, δ (ppm): 7.669, 7.598 (CH, benzene), 3.351, 1.074 (CH_2, methylene), 2.324 (C-N, 2-CH_3-piperidine),

^{13}CNMR (400 MHz) $DMSOd_{6,}$ δ (ppm): 40.36, 56.26(CH_2-aliphatic), 113.97, 120.85, 138.16, 152.18(CH, 1 benzene), 18.58 (CH_3-aliphatic)

MASS (m/z %): 394

Compound D14: 3-(3-(2-methyl-1H-indol-1-yl)propoxy)-1-hydroxy-9H-xanthen-9-one

UVλmax (in DMSO): 335 nm

^{1}HNMR (400 MHz) $DMSOd_{6,}$ δ (ppm): 7.655, 7.124 (CH, benzene), 3.165, 1.577 (CH_2, methylene), 2.330 (CH_2, CH_3-indole)

^{13}CNMR (400 MHz) $DMSOd_{6,}$ δ (ppm): 39.89, 40.32, 56.36 (CH_2-aliphatic), 116.09, 130. 26, 163.19 (CH, 1 benzene), 18.86 (CH_3-aliphatic)

Anti-cancer activity evaluation

Anti-cancer activity evaluations of the synthesized compounds (D5, D9, D14, D18, and D22) were performed using MTT assay method for the determination of IC_{50} values (Shen *et al.,* 2010; Liu *et al.,* 1995). Cytotoxic Assay was carried out firstly and thus, A549 (Human lung cancer) cell lines (Woessner *et al.,* 1991) was procured from National center for cell sciences (NCCS), Pune, India. Stock cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % inactivated Fetal bovine serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in a humidified atmosphere of 5 % CO_2 at 37 ºC until confluent (Jensen *et al.,* 1990; Micheletti *et al.,* 2011). The cells were dissociated with trypsin solution (0.2 % trypsin, 0.02 % Ethylene Diamine Tetra Acetic Acid (EDTA), 0.05 % glucose in Phosphate buffered saline, PBS). The stock cultures were grown in 25 cm^2 culture flasks, and all experiments were carried out in 96 microtitre plates. The cytotoxic effects of the various synthetic compounds were investigated using the MTT (Sigma, USA) on A549 cells (Skowronski *et al.,* 1993; Sousa *et al.,* 2009). The cells were seeded in 96-well plates at a density of 5 x 10^3 cells per well. After

incubation for 20-24 h, the cells with 70-80 % confluence were treated with the extracts at different concentrations (20, 40, 60, 80 and 100 µg/ml) and incubated for 24 h. Then, 20 µL of MTT (5 mg/ml) solution was added to cells per well, and the plate was moved to a cell incubator for another 4 h. The medium was removed, and 150 ml of Dimethyl sulfoxide (DMSO) was added to the cells. The plate was gently shaken for 15 min to dissolve the formazan crystals generated by proliferating cells, and the measurement was performed using a Spectramax M2 Microplate Reader (Molecular Diagnostic, Inc.) at a wavelength of 550 nm. Relative viability was calculated taking wells with non-treated cells as 100 % control. The results were expressed as mean ± SD, n=3 which were shown in Table 6. The anti-cancer activities of the synthesized compounds were evaluated based on the cell viability percentage of the tested cancer cell at varying concentrations of the compounds by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. The inhibitory concentration that kills cell by 50 %, $IC_{50,}$ for each compound was then obtained from the graph of cell viability versus concentration of drug figure 4 (Pedro *et al.*, 2002; Quan *et al.*, 2011).

Table 6: The percentage cell toxicity of A549 cell lines against synthetic compound

Conc. (µg/ml)	**% cytotoxicity**					
	D1	**D2**	**D3**	**D4**	**D5**	**Doxoru-bicin**
20	12.17±3.6	08.08±4.1	34.99±71	23.70±2.7	25.73±3.4	77.15±2.3

40	18.78±3.4	12.05±3.1	44.98±3.4	37.84±3.5	34.03±3.3	80.99±3.8
60	20.93±1.3	13.72±4.1	56.55±1.3	42.89±1.1	37.04±3.5	88.08±4.1
80	27.29±4.6	20.15±1.5	59.55±0.3	47.78±0.0	41.40±2.6	89.76±5.6
100	30.37±2.6	24.10±1.7	60.89±1.8	48.32±1.4	43.16±0.9	93.36±3.4
IC_{50}	191	161	48.05	171	171	43.06

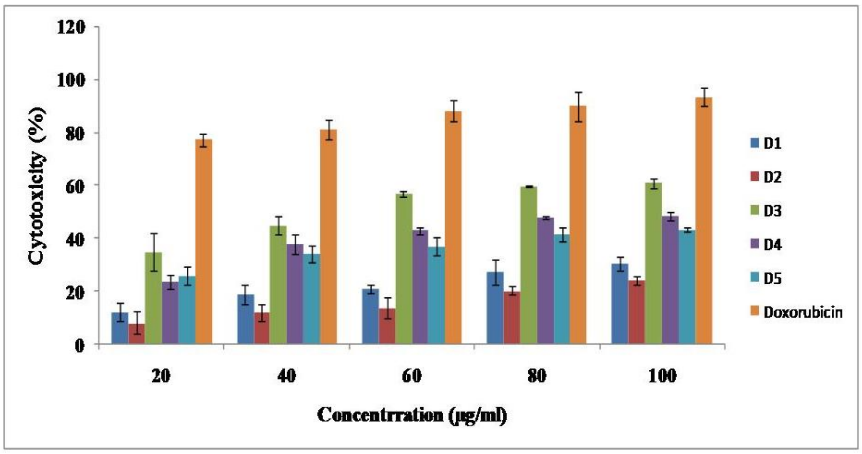


Fig 4: Anti-cancer activity of the synthesized xanthone compounds; Here the serial numbers D1, D2, D3, D4, D5 represents the synthesized compounds D5, D9, D18, D22, D14 respectively**;** D5: 3-(3-(diphenylamino)propoxy)-1-hydroxy-9H-xanthen-9-one; D9: 3-(3-(4-nitrophenylamino)propoxy)-1-hydroxy-9H-xanthen-9-one; D14: 3-(3-(2-methyl-1H-indol-1-yl)propoxy)-1-hydroxy-9H-xanthen-9-one; D18: 3-(5-(4-methylpiperazin-1-yl)pentyloxy)-1-hydroxy-9H-xanthen-9-one, D22: 3-(5-(2-methylpiperidin-1-yl)pentyloxy)-1-hydroxy-9H-xanthen-9-one.

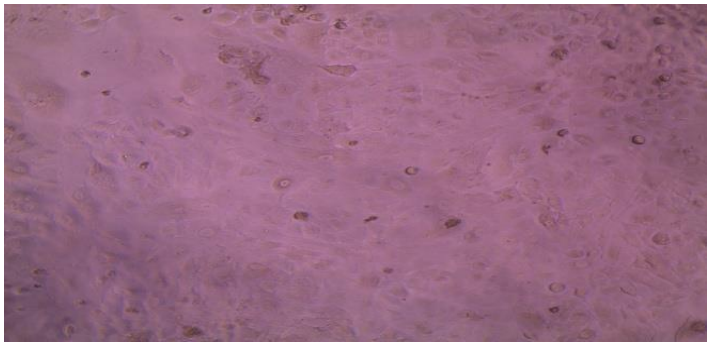


Fig 5: Control (untreated)

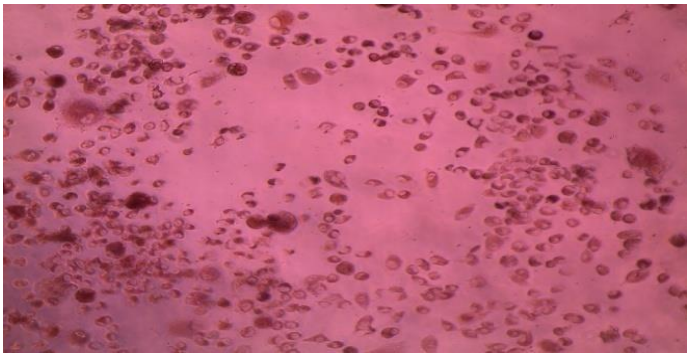


Fig 6: Positive control (doxorubicin 10 µg)

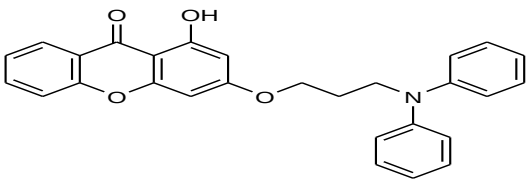
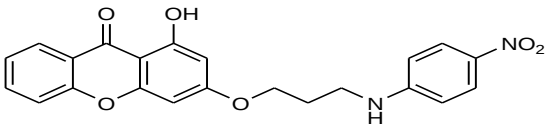
The assay of the compounds was done in various concentrations such as 20, 40, 60, 80, 100 µg/ml. According to the results the synthesized compounds were found less active as compared to standard drug doxorubicin which was observed in cancer cell lines (figure 4 and figure 6). Among the five compounds, compound number have D3 (D18) showed the least IC_{50} value *i.e.,* 48.05 µg/ml, so this compound is more potent against the A549 cell line and is having more cytotoxic effect in comparison to the other compounds.

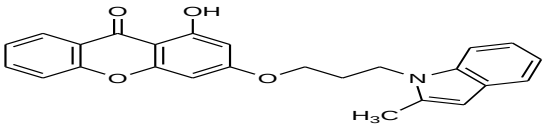
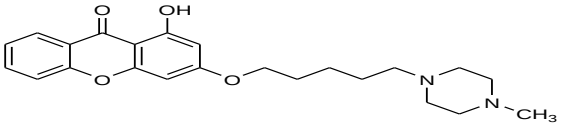
All the compounds were shown dose dependent increase in cytotoxic effect. Thus, as the concentration increases, there is increase in % cytotoxicity. Further, modification in substitution as well as side chains at certain positions of the xanthone moiety were found more potent as compared to standard drug doxorubicin against the cancer cell lines and it has been represented in figure 5 and figure 6 respectively.

Result and discussion

A new series of 3-(3'-substitutedpropoxy)-1-hydroxy xanthone and 3-(5'-substitutedpentyloxy)-1-hydroxy xanthone derivatives have been synthesized from commercially available starting materials Table 7. In this series, the hydroxyl groups at third position of 1, 3-dihydroxyxanthone was substituted with propane and pentane side chain along with different nucleophiles to obtain the targeted compounds. The synthesized compounds are given below in Table 7.

Table 7: List of the synthesized compound

Compound code	Compound structure and name	% Yield
D5	 3-(3-(diphenylamino)propoxy)-1-hydroxy-9H-xanthen-9-one	63.23
D9		64.56

	3-(3-(4-nitrophenylamino)propoxy)-1-hydroxy-9H-xanthen-9-one	
D14		61.25
	3-(3-(2-methyl-1H-indol-1-yl)propoxy)-1-hydroxy-9H-xanthen-9-one	
D18		62.40
	3-(5-(4-methylpiperazin-1-yl)pentyloxy)-1-hydroxy-9H-xanthen-9-one	

Modern medicinal chemistry offers a wide range of different strategies for finding selective Topoisomerase-II inhibitors with a novel structure. Computational and combinatorial chemistry methodology has helped to create a highly selective xanthone derivative which can serve as a novel lead compound for further developments in the field of Topoisomerase-II inhibitors. Frequent use of Topo-II like etoposide may cause hypersensitivity reactions, including vasomotor changes in the pulmonary and gastrointestinal systems. Other toxicities include myelo supression, alopecia, mucositis, nausea, vomiting. We have presented effective hydrogen bond interactions,

which was also supplemented through scoring parameters a new series of 3-(3'-substituted propoxy)-1-hydroxyxanthone and 3-(5'-substituted pentyloxy)-1-hydroxyxanthone derivatives. In conclusion, the 3-(3'-substituted propoxy)-1-hydroxyxanthone and 3-(5'-substituted pentyloxy)-1-hydroxyxanthone derivatives act as Topoisomerase-II inhibitors on the basis of molecular docking runs which contribute to the possible mechanism of anti-cancer activity of these analogues. These molecules were energetically proficient enough to make stable contacts with target protein on account of. Molecular docking study shows that most of the designed ligand reveals better binding potential than standard drugs like Doxorubicin and etoposide. The operational simplicity, good yield in significantly short reaction times, can impose this procedure as a useful and attractive alternative to the currently available anti-cancer drugs. With an objective to investigate a compound for toxicity risks evaluated with the help of Osiris property explorer, we have found that most of the compounds exhibited less side effect. In the light of above observation, xanthone derivatives can act as a lead towards the development of potential Topoisomerase-II inhibitors, these compounds show excellent correlation between docking results, synthetic data, *in-vitro* anti-cancer activity analysis by MTT assay method.

From this research effort, it can be concluded that the compound having secondary nitrogen as substituent of the series; 3-(3'-substitutedpropoxy)-1-hydroxyxanthone and 3-(5'-substitutedpentyloxy)-1-hydroxyxanthone derivatives are the potential key approach to design novel anti-cancer agents. The structures of the designed ligands were established through the spectroscopic analysis. The bioassay test was conducted for the synthesized compounds by A549 cancer cell lines to find out the inhibitory concentration that kills the cells by 50 %. D3 (D18) showed the least IC_{50} value *i.e.,* 48.05 µg/ml, so this compound is more potent against the A549 cell line and is

having more cytotoxic effect in comparison to the other compounds. However, our team is still working out with the series to further synthesize more potent drugs.

An important goal of present and future work is to maximize therapeutic efficacy of therapy using Top-II targeting agents while minimizing the risks of secondary malignancy and other toxicities.

Conflict of interest

There is no conflict of interest among the authors.

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