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Research Article

ULTRASOUND-ASSISTED SYNTHESIS AND DOCKING STUDY OF NOVEL CHROMONE-THIADIAZOLE INTEGRATED PHOSPHONATE DERIVATIVES TARGETING TOPOISOMERASE II AS ANTICANCER AGENTS

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ABSTRACT

Objective: The present studies discuss the anticancer activity of different topoisomerase II inhibitors with docking study.

Methods: The work reports the synthesis of novel diethyl (4-oxo-4H-chromen-3-yl)(5-substituted phenyl-1,3,4-thiadiazol-2-ylamino)methyl phosphonate derivatives 6(a-j). They were synthesized through one-pot three-component Kabachnik-Fields reaction by using ultrasonicator processor, at room temperature in presence of Zirconium oxychloride ($ZrOCl_2$).

Results: The structures of the synthesized compounds were confirmed by spectral analysis. The synthesized derivatives 6(a-j) were evaluated for their *in vitro* anticancer activity against human cancer cell lines such as DU 145 (Human Prostate Cancer) and MCF-7 (Breast cancer) and also on non-tumor cell lines such as MCF-10 (normal breast epithelial cell) by MTT assay. From the anticancer screening results data, compound 6e was found to be the most potent anticancer compound among the synthesized compounds against MCF-7 and DU 145 cancer cell lines with IC_{50} value of 2.97 µM and 3.11 µM, respectively. The compound 6h has shown cell cycle arrest at G2/M phase, along with the decrease of cells at G0/G1 phage. The compound 6h was able to induce apoptosis in DU 145 cell lines. The synthesized compounds 6(a-j) were further evaluated for their topoisomerase II inhibitory activity.

Conclusion: The anticancer screening result and MTT assay, along with the docking study shows that the synthesised derivative are active anticancer agent against MCF-10, DU-145 and MCF-7 cell line.

Keywords: Kabachnik-Fields reaction, Ultrasound, Thiadiazol, Chromone, α-amino phosphonate, *In vitro* anticancer activity, Topoisomerase II, Docking

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INTRODUCTION

Canceris a disease which involves abnormal cell growth. The cancer cells have a potential to invade or spread to other parts of the body [1]. Topoisomerases (topos) are the nuclear enzymes that resolve topological problems which are associated with the DNA during various genetic processes. The essential role of topoisomerases in imperative processes of the cell, their increased level in solid tumors and death of the cell due to their inhibition makes topoisomerases inhibitors as an important class of anticancer agents [2].

Topoisomerases are enzymes that solve the topological problems associated with the DNA. They help by converting the supercoiled DNA to the relaxed form, which allows important cellular processes to lead further [2]. The topos are of two types depending upon their ability a) topo I that cleaves the single strand of DNA at a time; and b) topo II that simultaneously breaks the double strand of DNA [3, 4]. Topo II plays major role during the process of cell proliferation. Because of the importance of topo II function in cell proliferation process, most of the scientist are doing research in the development of effective topo II inhibitors. The synthesis phase (S phase) is considered to be the most vital phase in the cell cycle because at this phase DNA gets duplicated. S phase lies in between the G1 and G2 phases, followed by mitotic phase (M phase). At M phase the mother cell divides into two daughter cells [5]. If the synthesized compound is able to inhibit topo II function, it results in topological problems associated with the DNA and interruption at important stages of cell cycle, which ultimately leads to cell death [6].

Chromone is considered a vital moiety in the field of medicinal chemistry. The chromone core can be a versatile foundation to discover novel anticancer drug with topoisomerase inhibition ability. A synthetic flavonoid MHY336 have the ability to arrests the cell cycle in G2/M or S phase via Topo-II dependent mechanism [7]. Dietary flavonoid fisetin, has been found to act as a dual inhibitor of Topo-I and Topo-II in cells [8]. Psorospermin, a natural antitumor antibiotic [9], it has been shown that it intercalates with DNA and its alkylating potential is significantly increased in the presence of Topo-II [10, 11].

α-amino phosphonates are among the most studied bioactive organophosphorus. The α-amino phosphonates derivatives and have been used as enzyme inhibitors [12], inhibitors of serine hydrolase [13], peptide mimics [14], antiviral [15], antibacterial [16], antifungal [17], anticancer [18], anti-HIV [19], antibiotics [20], herbicidal [21] etc.

In recent years, many promising multicomponent reactions have come up as novel synthetic platform in the field of organic chemistry. Among many multicomponent reactions reported so far, we were particularly interested in exploring the Kabachnik-Fields reaction because it can afford α-amino phosphonates in good yield [22]. The Kabachnik-Fields reaction is one of the well-known multicomponent reactions. The Kabachnik-Fields reaction corresponds to the classical method for the synthesis of organophosphorus compounds. It was discovered in 1952 independently by Kabachnik and Medved [23] and Fields [24]. The reaction occurs in a three-component system consisting of a hydrophosphoryl compound, a carbonyl compound (aldehyde or ketone), and an amine and results in α-aminoalkylphosphonates commonly named as α-amino phosphonates [25, 26].

The designing protocol for the target compound is as shown in fig. 1. Ilmofosine and Edelfosine contains the dialkoxy phosphonate group and are good anticancer agents. An isoflavonoid Genistein, a Flavonoid MHY336, Dietary flavonoid Fisetin and Psorospermin are good Topoisomease inhibitors containing chromone nucleus. Thiadiazole derivatives are reported to be good anticancer agents by many scientists [27-30]. Therefore, we thought it worthwhile to synthesize new hybrid derivatives based on a molecular hybridization strategy to produce new hybrid compounds with improved efficacy. The target compounds contain thiadizole moiety coupled with 3-formyl chromone moiety and α-aminophosphonate linkage with the hope to get novel hybrid derivatives with better anticancer activity and minimized toxicity.

Ten new diethyl (4-oxo-4H-chromen-3-yl)(5-substituted phenyl-1,3,4-thiadiazol-2-ylamino) methyl phosphonate derivative 6(a-j) were synthesized in facile, eco-friendly condition by one pot Kabachnik–Fields reaction using the ultrasonic processor at room temperature. All the synthesized compounds 6(a-j) were screened for their *in vitro* anticancer activity against two human cancer cell lines such as DU 145 (Human Prostate Cancer Cell Line) and MCF-7 (Human Breast Cancer Cell Line) and also on non-tumor cell lines such as MCF-10 (normal breast epithelial cell) by MTT assay method [31].

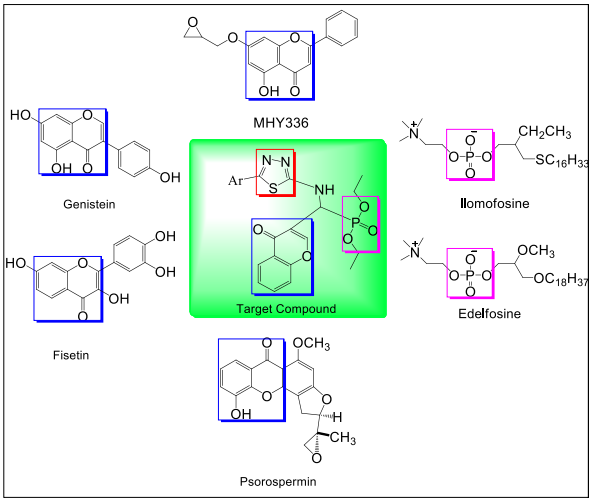


Fig. 1: The designing protocol for the target compound 6(a-j)

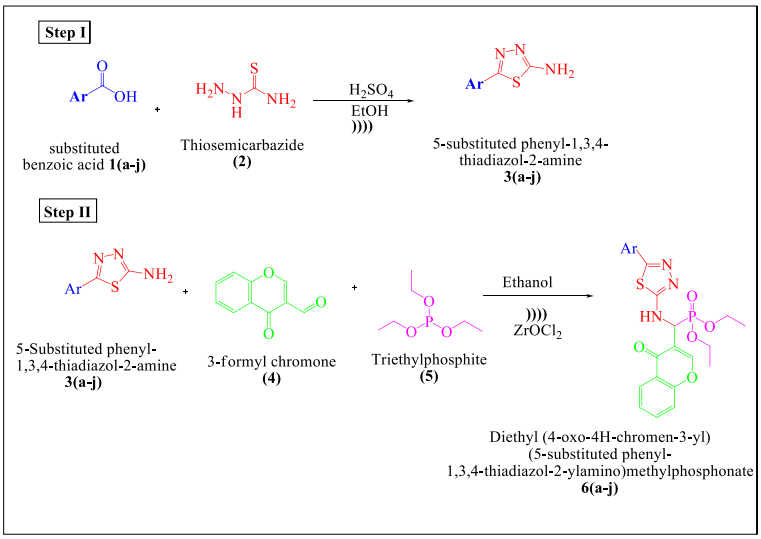
MATERIALS AND METHODS

General

All the chemicals used for the synthesis were of Merck, Sigma, Research lab, Qualigens make and *Hi* media.

Chemistry

Diethyl (4-oxo-4H-chromen-3-yl)(5-substituted phenyl-1,3,4-thiadiazol-2-ylamino)methyl phosphonate derivatives 6(a-j) were synthesized as outlined in Scheme 1. 5-substituted phenyl-1,3,4,-thiadiazol-2-amine 3(a-j) were synthesized by reacting substituted benzoic acid 1(a-j) with thiosemicarbazide (2) in the presence of few drops of sulphuric acid (H_2SO_4) catalyst by the conventional method in ethanol and were also synthesized by ultrasonicaton method with few drops of H_2SO_4. α-Amino phosphonate derivatives 6(a-j) were synthesized by the Kabachnic-Fields synthesis method by reacting5-substituted phenyl-1,3,4,-thiadiazol-2-amine 3(a-j), 3-formyl chromone (4) and triethyl phosphite (5) via one-pot synthetic step in the presence of Zirconium oxychloride ($ZrOCl_2$) as a catalyst.



Scheme 1: Scheme of synthesis of Diethyl (4-oxo-4H-chromen-3-yl)(5-substituted phenyl-1,3,4-thiadiazol-2-ylamino)methyl phosphonate derivatives6(a-j)

Synthesis

Synthesis of 5-phenyl-1, 3, 4-thiadiazol-2-amine 3(a-j)

A*)* Conventional method

An ethanolic solution of substituted benzoic acid (0.05 mol) 1(a-j) was added to thiosemicarbazide (0.05 mol) (2) with constant sterring; few drop of Sulphuric acid was added and refluxed for 140-160 min. at 80-90 °C, after completion of the reaction (TLC), the reaction mixture was cooled and poured to ice-cold water, filtered, dried and recrystallized from ethanol.

B) Ultrasonication method

To the equimolar quantity of substituted benzoic acid (0.05 mol) 1(a-j), thiosemicarbazide (2) (0.05 mol) in 5-7 ml of ethanol, a catalytic amount of sulphuric acid was added and a beaker containing reaction mixture was kept inside an acoustic chamber and solid probe of ultrasonicator inserted in a beaker and reaction was carried out at 25 °C and 60 % amplitude with 9 sec pulse on and 12 sec pulse off time for a specific time period such as 30-40 min. The completion of the reaction was checked by TLC. After completion of the reaction, the reaction mixture was poured into the ice-cold water, filtered, dried and recrystallized from ethanol [40].

General procedure for synthesis of diethyl ((4-oxo-4H-chromen-3-yl)((5-sustituted phenyl-1,3,4-thiadiazol-2-yl)amino)methyl) phosphonate derivatives 6(a-j)

A) Conventional method

A mixture of substituted thiadiazole derivatives 3(a–j) (0.05 mol), 3-formyl chromone (4) (0.05 mol), triethylphosphite(5) (0.05 mol) and the catalytic amount of zirconium oxychloride in absolute ethanol was added and refluxed for a specified time as shown in table 2. After completion of the reaction, the reaction mixture was cooled and poured to ice-cold water, filtered, dried and recrystallized from ethanol.

B) Ultrasonication method

A mixture of substituted thiadiazole derivatives 3(a–j) (0.05 mol), 3-formyl chromone (4) (0.05 mol), triethyl phosphite(5) (0.05 mol) and the catalytic amount of zirconium oxychloride in absolute ethanol was taken. Then the reaction mixture was kept inside an Ultrasonicator acoustic chamber at 25 °C at 60 %amplitude for a specified time as shown in table 2. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured into the ice-cold water and filtered under suction; the precipitate thus obtained was washed with water and recrystallized from ethanol.

Our work represents the one pot Kabachnik-Fields synthesis of diethyl ((4-oxo-4H-chromen-3-yl)((5-sustituted phenyl-1,3,4-thiadiazol-2-yl)amino)methyl) phosphonate derivatives from 5-substituted phenyl-1,3,4,-thiadiazol-2-amine3(a-j), 3-formyl chromone (4) and triethyl phosphate (5) by using ultrasonic processor and $ZrOCl_2$as a catalyst at room temperature with yield from 84-95 %.[40]

Biological evaluation

***In vitro* anticancer screening**

All the synthesized compounds 6(a-j) were evaluated for their *in vitro* anticancer activity against two human cancer cell lines DU 145 and MCF-7 by MTT assay. The human breast cancer cell line (MCF-7) and Human prostate cancer cell line (DU-145) were grown in DMEM medium containing 10 % foetal bovine serum and 0.7 % antibiotics. Cells were seeded into 96 well microtiter plates in 100 µl of media at a plating density of 5000 cells/well. Seeded cells were incubated at 37 ^{0}C, 5 % CO_2, 95 % air and 100 % humidity for 24 h. At 24 h, old media was changed with fresh media followed by treatment with each compound at 10 µM, 1 µM, and 0.1 µM. After 24 h treatment, cell viability was assessed by 3-(4,5-dimethylthiazol)-2,5-diphenyltetrazolium bromide (MTT), cell were incubated with 20 µl of MTT (5 mg/ml) in PBS for 4 h at 37 ºC. The medium was removed and formazan crystal was dissolved in DMSO. MTT reduction was quantified by measurement of absorbance at 570 nm using a multimode reader, Synergy Mx of BioTek [41].

Flow cytometric analysis of the cell cycle

Cell cycle progression was evaluated using a flow cytometer, BD LSR Fortessa with software FACS Diva Version 6.2. In brief, DU 145, human prostate cancer cells were incubated for 24 h with a given compound 6h, each at two concentrations closer to IC_{50} values, obtained during the cell viability assay. After 24 h of treatment, cells were harvested, washed with PBS and fixed in ice-cold 70 % ethanol for overnight in 4 ºC. Next day, all samples were centrifuged at 3000 RPM for 4 min and stained with propidium iodide (PI) (5 mg/ml) followed by the addition of RNase and analyzed [41, 42].

Apoptosis induction

Apoptosis was determined by staining the cells with Annexin V fluorescein isothiocyanate (FITC) and counterstaining with PI using the Annexin V-FITC/PI apoptosis detection kit (BD Biosciences, San Diego, CA) according to the manufacturer's instructions. Briefly, $4x10^6$ cell/T 75 flasks were exposed to compound 6h at its IC_{50} concentration for 24 and 48 h. The cells then were collected by trypsinization and $0.5x10^6$ cells were washed twice with phosphate-buffered saline (PBS) and stained with 5 µl Annexin V-FITC and 5 µl PI in 1x binding buffer for 15 min at room temperature in the dark. Analyses were performed using FACS Calibur flow cytometer (BD Biosciences, San Jose, CA) [43, 44].

Measurement of topoisomerase II inhibitory activity

The most active anticancer agents from the synthesized series were further evaluated for prediction of their mode of action using Topo II drug screening kit (TopoGEN, Inc., Columbus) according to procedure reported by Patra *et al.* Adriamycin was used as standard in this evaluation.

The reaction was started upon incubation of a mixture consisted of, human Topo II (2 µl), substrate super coiled pHot1 DNA (0.25 µg), 50 µg/ml test compound (2 µl), and assay buffer (4 µl) in 37 °C for 30 min. In order to terminate the reaction, 10% sodium dodecylsulphate (2 µl) and proteinase K (50 µg/ml) were added at 37 °C for 15 min. followed by incubation for 15 min at 37 °C. Then, the DNA was run on 1% agarose gel in BioRad gel electrophoresis system for 1-2 h followed by staining with GelRedTM stain for 2 h and destained for 15 min with TAE buffer. The gel was imaged via BioRad's Gel DocTMEZ system. Both supercoiled and linear strands DNA were incorporated in the gel as markers for DNA-Topo II intercalators. The results were reported as IC_{50} (50% inhibition concentration) values [45, 46].

Docking study

The molecular docking study was initiated with the sketching of 2D form of the structure of all synthesized compounds using Maestro build panel and optimized to lower energy conformers using Lig prep v3.5.9 (Schrodinger, LLC). 2D form of the compounds was then subjected form ligand library preparation module by keeping the preparation protocol as Surface for searching where it generates the single lowest strain energy tautomer/stereoisomer and all necessary structural properties were added and final 3D prepared conformation of each compounds are stored in Mol2 file format. To perform molecular docking, three dimensional X-ray crystal structure of topoisomerase II α (PDB ID: 3QX3) complex with Genistein was used. Molecular docking study of synthesized series of compounds 6(a-j) was subjected for an understanding of binding interactions with Human topoisomerase II α enzyme (PDB ID: 3qx3) was performed Maestro 9.1 using Glide v6.8 (Schrodinger) package following standard procedure. Many topoisomerase II α inhibitors have been produced and tested in the clinic by now. The crystal structures of topoisomerase II α were picked up from the Protein Data Bank (PDB) (http://www.rcsb.org/pdb/explore/explore.dostructureId=3QX3) (PDB code: 3qx3). All the synthesized compounds 6(a-j) showed very good binding interactions in the active site of the selected receptor. The most active compounds in the study which have shown very high docking scores value against selected receptors and had a good binding affinity predicated by non-covalent interactions such as Hydrogen bond interaction, VDW interaction, Carbon, Hydrogen bond interaction, Pi-Anion interaction, Pi-Pi shaped interaction, Alkyl interaction, Pi-Sigma and Pi-Alkyl interactions. To represent the details of the docking score following terms is used as a total score: as total docking score is shown in table 5.

***In silico* bioavailability predictions**

By using a molinspirationthe bioavailability properties were predicted and it was observed that the synthesized compounds 6(a-j) posses good % absorption (41.65 % to 73.27 %) as shown in table 6. Absorption (% ABS) was calculated by: % ABS = 109-(0.345 X TPSA) [47]. In the present study, the calculation of molecular volume (MV), molecular weight (MW), logarithm of partition coefficient (miLog P), number of hydrogen bond acceptors (n-ON), number of hydrogen bonds donors (n-OHNH), topological polar surface area (TPSA), number of rotatable bonds (n-ROTB), number of rigid bonds (Rig Bond), Rings, and Lipinski's rule of five [48]. All the synthesized compounds 6(a-j) were found to be nontoxic, as predicted by using mol-inspiration.

RESULTS AND DISCUSSION

All the synthesized compounds were characterized and confirmed by spectral analysis like; FTIR, ^{1}H NMR, ^{13}C NMR, [31]P NMR, MS and Elemental analysis. Open capillary tubes method was used to determine the melting points of the synthesized compounds and is uncorrected. Details of the synthesis of 5-substituted phenyl-1,3,4-thiadiazol-2-amine derivatives 3(a-j) by conventional and ultrasound method is as shown in table 1. Physical constants data and time required for completion of reaction for Diethyl (4-oxo-4H-chromen-3-yl)(5-substituted phenyl-1,3,4-thiadiazol-2-ylamino) methyl phosphonate derivatives 6(a-j) using conventional method and ultrasound methodis as shown in table 2.

Table 1: Details of synthesis of 5-substituted phenyl-1,3,4-thiadiazol-2-amine derivatives 3(a-j) by conventional method and ultrasound method

Entry	Ar	Time required for conventional method (min)	% Yield	Time required for ultrasound method (min)	% Yield
3a	Phenyl	150	86	30	90
3b	4-chloro phenyl	140	87	35	91
3c	4-hydroxy phenyl	140	90	35	93
3d	4-methyl phenyl	160	93	40	95
3e	2-hydroxy phenyl	160	91	30	95
3f	4-nitrophenyl	140	89	30	95
3g	4-bromo phenyl	145	86	35	90
3h	5-styryl	150	91	30	95
3i	2-phenyl acetate	150	89	30	95
3j	3,4-dinitro phenyl	140	87	30	90

Table 2: Physical constants data for diethyl (4-oxo-4H-chromen-3-yl)(5-substituted phenyl-1,3,4-thiadiazol-2-ylamino)methyl phosphonate derivatives 6(a-j)

Code	Ar	Molecular formula	Mol weight (gm)	Time required conventional method (min)	% Yield	Time required ultrasound method (min)	% Yield	Melting point (°C)
6a	Phenyl	$C_{22}H_{22}N_3O_5PS$	471.47	130	60	80	90	195-196
6b	4-chloro phenyl	$C_{22}H_{21}ClN_3O_5PS$	505.91	135	66	70	92	150-152
6c	4-hydroxy phenyl	$C_{22}H_{22}N_3O_6PS$	487.47	135	68	75	95	176-178
6d	4-methyl phenyl	$C_{23}H_{24}N_3O_5PS$	485.49	140	60	85	90	180-182
6e	2-hydroxy phenyl	$C_{22}H_{22}N_3O_6PS$	487.47	150	62	90	90	188-190
6f	4-nitrophenyl	$C_{22}H_{21}N_4O_7PS$	516.46	130	58	80	88	140-142
6g	4-bromo phenyl	$C_{22}H_{21}BrN_3O_5$	550.36	140	64	75	94	112-114
6h	5-styryl	$C_{24}H_{24}N_3O_5PS$	497.50	130	62	80	92	160-162
6i	2-phenyl acetate	$C_{24}H_{24}N_3O_7PS$	529.50	135	56	80	87	178-180
6j	3,4-dinitro phenyl	$C_{22}H_{20}N_5O_9PS$	561.46	130	50	80	84	176-178

Biological evaluation

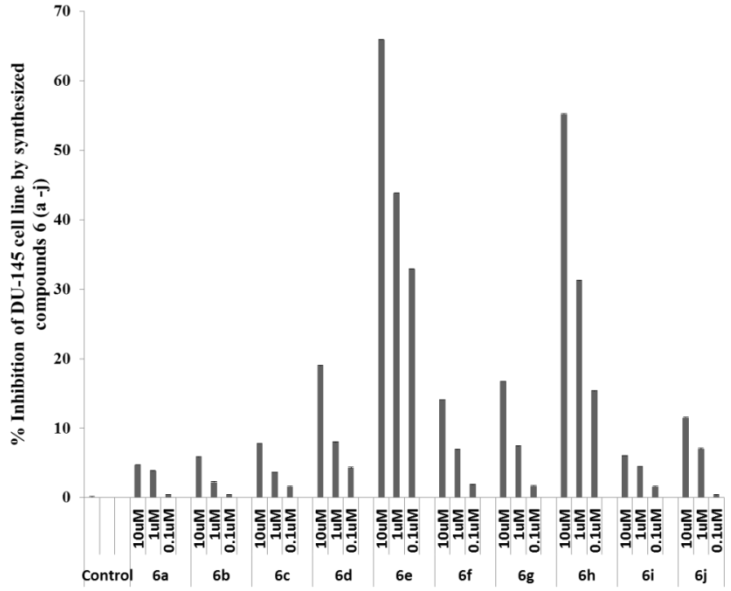
Cell viability assay

The *in vitro* anticancer activity for the novel series of diethyl (4-oxo-4H-chromen-3-yl)(5-substituted phenyl-1,3,4-thiadiazol-2-ylamino)methyl phosphonate derivatives 6(a-j) were evaluated by MTT assay against two human cancer cell lines such as DU-145 and MCF-7. They were also evaluated against non-tumor cell lines such as MCF-10 (normal breast epithelial cell). The results obtained are as shown in table 3.

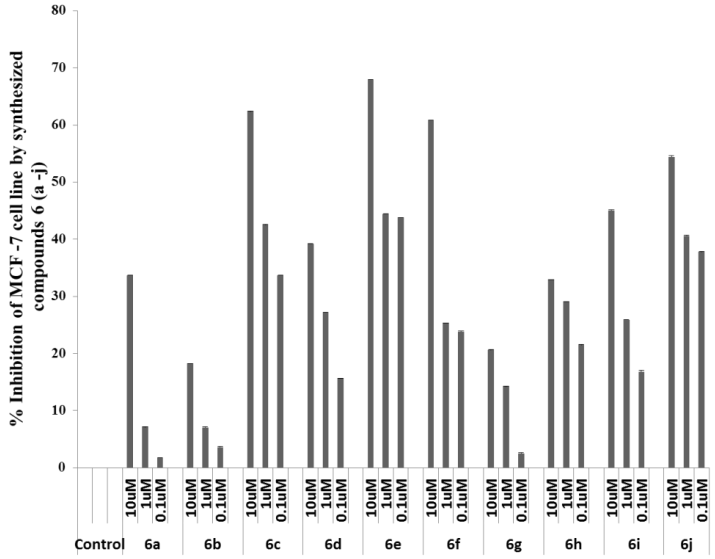
Table 3: *In vitro* anticancer activity of the synthesized compounds 6(a-j)

Entry	Cancer cell lines							
	DU-145 cell line				MCF-7 cell line			
	% Cancer cell viability at 10µM	% Cancer cell death at 10µM	Std. dev.	IC_{50} (µM)	% Cancer cell viability at 10µM	% Cancer cell death at 10µM	Std. dev.	IC_{50} (µM)
Control	100	0	±0.03		100	0	±0.16	
6a	95.26	4.75	±0.04		66.32	33.68	±0.06	
6b	94.14	5.86	±0.08		81.81	18.19	±0.03	
6c	92.22	7.78	±0.07		37.66	62.34	±0.08	4.99
6d	81.03	18.97	±0.06		60.77	39.23	±0.02	
6e	34.0	65.92	±0.06	3.11	32.08	67.92	±0.01	2.97
6f	85.88	14.12	±0.02		39.17	60.83	±0.02	5.84
6g	83.27	16.73	±0.05		79.39	20.61	±0.06	
6h	44.83	55.17	±0.08	8.03	67.06	32.94	±0.006	
6i	93.97	6.03	±0.07		54.98	45.02	±0.10	
6j	88.51	11.49	±0.14		45.59	54.41	±0.24	6.99

IC_{50}: Growth inhibition of 50 %; Std dev.: Standard deviation; DU-145: Prostate cancer cell lines; MCF-7: Breast cancer cell lines; Control: DMSO.



2(a)



2(b)

Fig. 2.2(a) represents % inhibition of DU-145 cancer cell lines by the synthesized compounds 6(a-j), 2(b) represents % inhibition of MCF-7 cancer cell lines by the synthesized compounds 6(a-j)

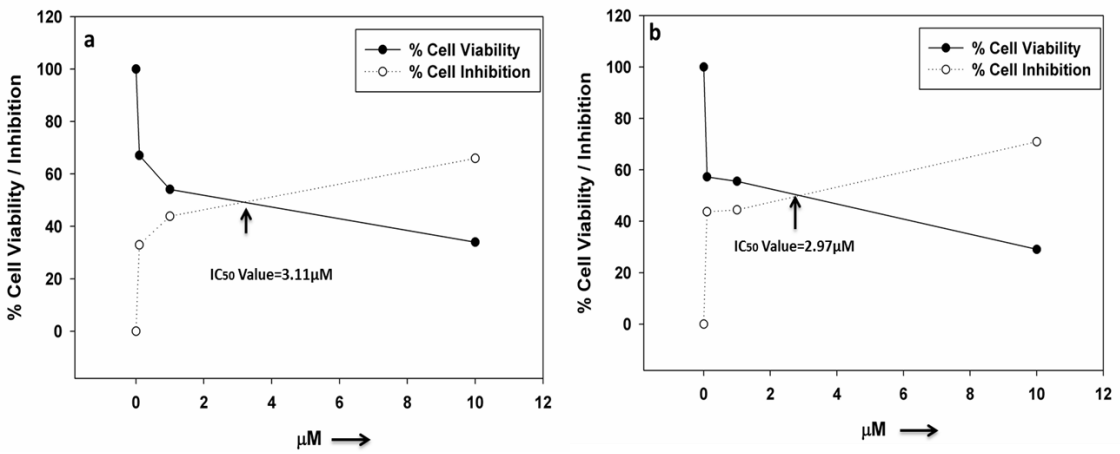
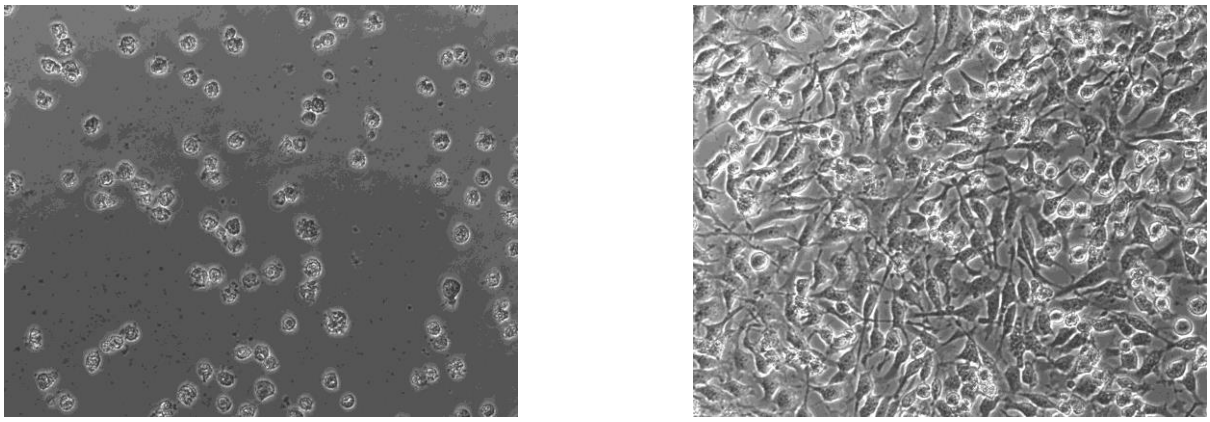


Fig. 3: The IC_{50} value calculation graph of the most potent synthesized compound 6e

Fig. 2 represents % inhibition of cancer cell lines by the synthesized compounds 6(a-j). Fig. 2 represents % inhibition of DU-145 cancer cell lines by the synthesized compounds 6(a-j) and fig. 2(b) represents % inhibition of MCF-7 cancer cell lines by the synthesized compounds 6(a-j). Fig. 3 represents IC_{50} value calculation graph of the most potent synthesized Compound 6e.

From the *in vitro* anticancer screening, the compound 6e (which bears ortho hydroxy group on the phenyl ring) was found to be the most potent anticancer compound among the synthesized compounds against MCF-7 and DU-145 cancer cell lines with IC_{50} value of 2.97 µM and 3.11 µM respectively. The compound 6f was found to be the second most potent anticancer compound among the synthesized compounds against MCF-7 cancer cell lines with IC_{50} value of 5.84 µM with 60.83 % of cancer cells death. The compound 6h (which bears acetate group on the phenyl ring) was found to be the second most potent anticancer compound among the synthesized compounds against DU-145 cancer cell lines with IC_{50} value 8.03 µM with 55.17 % of cancer cells death.

Cell morphology was observed at the IC_{50} concentration of synthesized compound 6e and photographs were taken under the Eclipse Ti-S Inverted Research Microscope-Nikon and the images were processed using NIS-Elements software. The fig. 4 represents the changes shown by the synthesized compound 6e at its IC_{50} concentration and control i.e. DMSO solvent on DU-145 cancer cell line. It can be clinched from fig. 4 that at the IC_{50} concentration of the most active compound 6e there were distinctive changes observed such as cells detachment, cell membrane deformation, cell shrinkage and reduced number of viable cells in DU-145 cancer cell lines in comparison to control cells. Similarly in the fig. 5 represents the changes shown by the synthesized compound 6e at its IC_{50} concentration and control i.e. DMSO solvent on MCF-7 cancer cell line. It can be concluded from fig. 5 that at the IC_{50} concentration of the most active compound 6e there were distinctive changes observed, such as cells detachment, cell membrane deformation, cell shrinkage and reduced number of viable cells in MCF-7 cancer cell lines in comparison to control cells.



6e **Control**

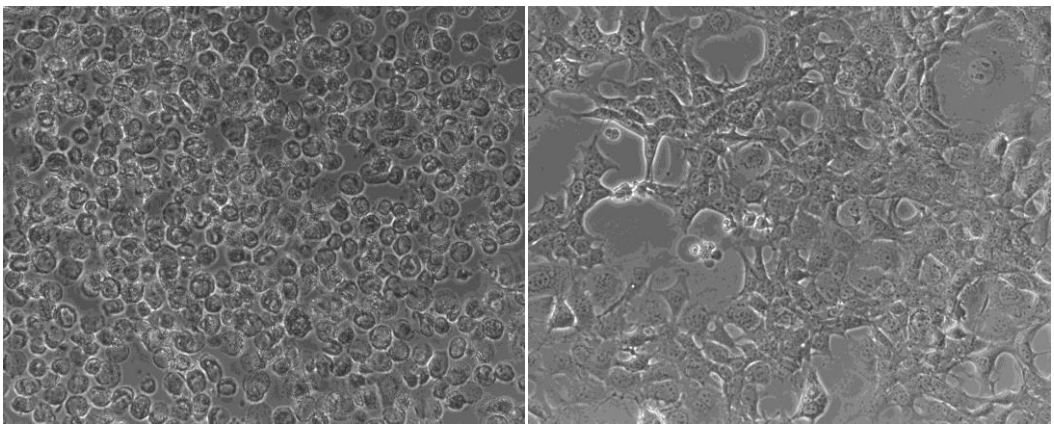
Fig. 4: Images for *in vitro* anticancer activity of compound 6e and control against DU 145 cancer cell line at 100x magnification

Flow cytometric analysis of the cell cycle

In order to understand the mode of action of most active compound, we had examined its effects on cell cycle progression by flow cytometry analysis on, Human prostate cancer cells (DU-145). In this study, DU-145 cells were treated with the active synthesized compound 6h at two concentrations closer to IC_{50} values, obtained during the cell viability assay. The data obtained clearly signposts that the active synthesized compound 6h have an ability to alter the cell cycle progression enormously. The percentage distribution of cells in various phases of the cell cycle is

exemplified in the fig. 6. The fig. 6 revealed that the exposure of DU-145 cells to compound 6h at the IC_{50} concentration for 24 h induced a significant disruption in the cell cycle profile, including a time-dependent decrease in cell population at G1 phase with a concomitant increase at sub G0/G1phase and G2/M phase. This might indicate an ability of the compound 6h to reduce cellular proliferation and to induce cell cycle arrest at G2/M phase and induce DNA fragmentation, the hallmark of cell death, preventing the cells from proceeding towards replication and proliferation.

If there is an increase in the cell percentage at the sub G0/G1 during analysis it means that apoptosis took place. As revealed in fig. 6, the compound 6h has shown dose-dependent arrest of the cell cycle at G2/M phase, along with the decrease of cells at G0/G1 phage. It can be perceived; from fig. 6 that there was an increase in sub G0/G1 population compared to the control. This indicates apoptotic death in the DU 145 cancer cells when treated with the synthesized compound 6h. The increase of cells in sub G0/G1 fraction indicates that DNA fragmentation and apoptosis occurs in cancer cells due to the synthesized compound 6h [32, 33].



6e **Control**

Fig. 5: Images for *in vitro* anticancer activity of compound 6e and control against MCF-7 cancer cell line at 100x magnification

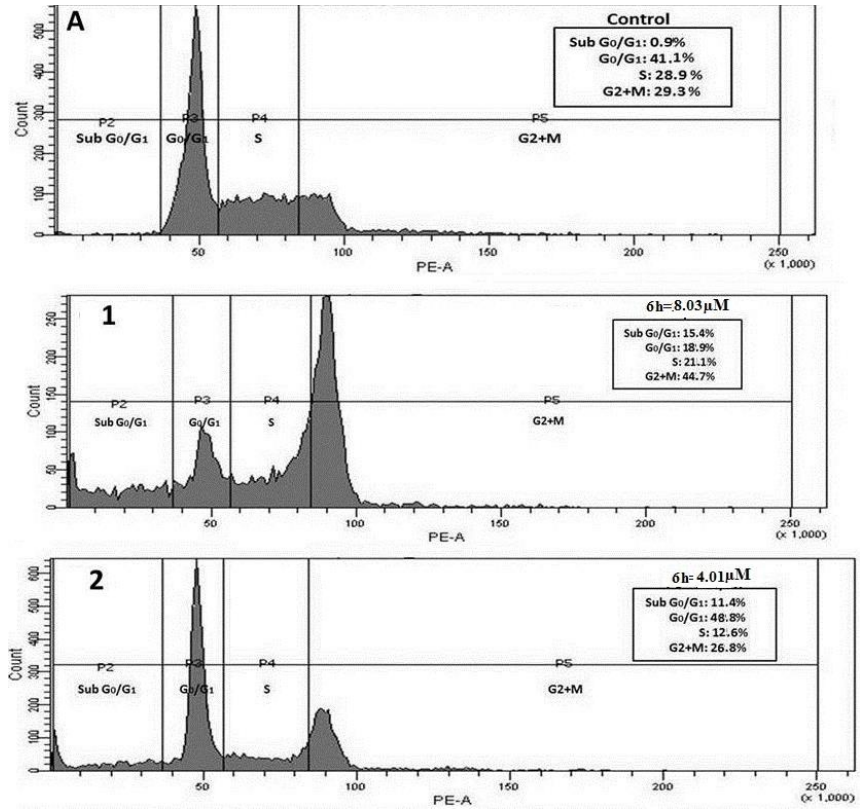


Fig. 6: Flow cytometry cell cycle analysis of control and of compounds 6h treated on DU-145 cancer cells

Apoptosis

Apoptosis, autophagy and necrosis are the major types of cell death. Apoptosis, or programmed cell death, is a normal physiologic process for the removal of unwanted cells. One of the earlier events of apoptosis includes the translocation of membrane phosphatidylserine (PS) from the inner

side of the plasma membrane to the surface. Annexin V, a Ca^{2+} dependent phospholipid-binding protein, has a high affinity for PS. This assay detects PS expressed on the surface of the apoptotic cells and fluoresce green after interacting with the labeled Annexin V. During early apoptosis, membrane asymmetry is lost, and PS translocates from the cytoplasmic side of the membrane to the external leaflet. Propidium iodide (PI), the counter stain used in this assay, has the ability to cross only compromised membranes to intercalate into the DNA. Therefore, PI is used to detect the late stages of apoptosis and necrosis by the presence of red fluorescence.

The ability of the synthesized compound 6h to induce apoptosis was determined using an Annexin V (conjugated to FITC) apoptosis detection kit. From fig. 7 it is clear that the compound 6h have induced early apoptosis and late apoptosis of 16.7% and 4.1%, respectively in comparison with control with early apoptosis of 3.2% and late apoptosis of 1.1%.

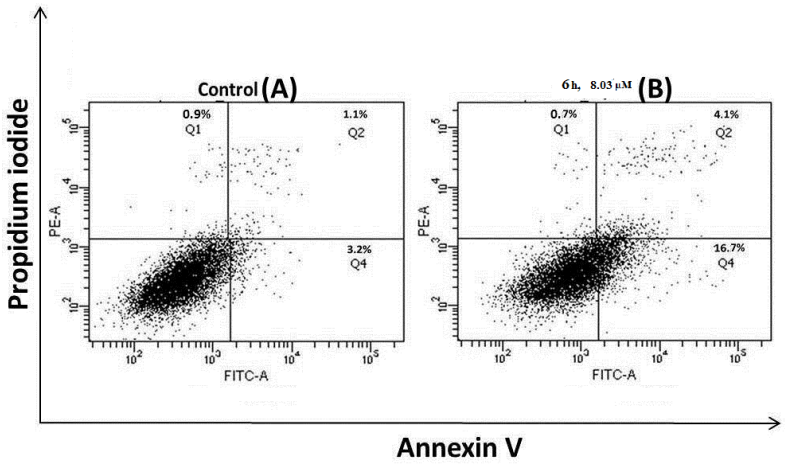


Fig. 7: Apoptosis study by Annexin V/FITC assay of compound 6h

Effect of compound 6h on DU 145 cells treated for 24 h. (A)-Control (with 0.1% DMSO), (B)-treated with compound 6h (8.03 µM). Lower left represents live cells (annexin V-/PI-), lower right represents early apoptotic cells (annexin V+/PI-), upper right represents late apoptotic cells (annexin V+/PI+) and the upper left represents necrotic cells (annexin V-/PI+).

Measurement of topoisomerase II activity

The most active anticancer compounds, according to the *in vitro* anticancer evaluation data are compounds 6c, 6e, 6f, 6h and 6j. These compounds were further evaluated to predict their ability to inhibit the topoisomerase II enzyme. The catalytic activity of Topo II was evaluated according to the procedure reported by the scientist Patra *et al.* [34]. Adriamycin was used as a standard in this evaluation. Adriamycin inhibits the progression of topoisomerase II, an enzyme which relaxes supercoils in DNA for transcription. The data of the measurement of topoisomerase II activity is as presented in table 4.

The synthesized compounds 6c, 6e, 6f, 6h and 6j have an ability to inhibit the topoisomerase II enzyme. The compound 6e was found to be the most potent topoisomerase II enzyme inhibitor. The compound 6c was found to be the second most potent topoisomerase II enzyme inhibitor.

Table 4: The IC_{50} values of the novel synthesized compounds against topo II enzyme

Compound	IC_{50} µM[a]
6a	ND
6b	ND
6c	8.18±0.21
6d	ND
6e	5.98±0.14
6f	15.46±0.05
6g	ND
6h	16.21±0.09
6i	ND
6j	14.08±0.15
Adriamycin	0.94±0.05

[a]Three independent experiments were performed for each concentration; ND: Not determined.

Docking study

In order to explore binding affinity, binding mode and molecular interactions of the synthesized compounds 6(a-j), **a** molecular docking study was carried out on topoisomerase II. Human topoisomerase II enzyme is indispensable for numerous processes in the cell. Inhibition of Human topoisomerase II has been shown to be a promising therapeutic strategy. Molecular docking study was carried out using the topoisomerase II α enzyme of Human (PDB ID: 3QX3). Molecular docking was performed using Maestro 9.1 using Glide v6.8 module of Schrodinger package following standard procedure. The crystal structures of Human topoisomerase II α in complex with Genistein were picked up from the Protein Data Bank (PDB) (http://www.rcsb.org/pdb/explore/explore.do?structureId=3QX3) (PDB code: 3QX3) [35, 36].

Depending upon the binding affinity, total score-log(ki), molecular interaction values the novel derivatives such as 6c, 6e, 6i and 6f have shown docking score in between 7.708 and 7.724 are found among the most active one. The compounds 6b, 6d, 6h, 6h and 6j are moderately active with docking score 6.913 to 6.193. The compound 6e is the most active amongst the entire synthesized compounds with a docking score of 7.724 as given in table 5.

Table 5: Molecular docking study data of the synthesized compounds 6(a-j) in the Top II α enzyme of Human (PDB ID: 3QX3)

Compound ID	Total score-log(ki)
6a	7.524
6b	6.193
6c	7.253
6d	6.913
6e	6.015
6f	7.708
6g	6.913
6h	6.542
6i	7.374
6j	6.529

Total score: total docking score.

The most active derivative 6f (7.708), have shown efficient binding mode and penetrating active site cavity in the topoisomerase II α (3QX3) by forming the hydrogen bond interactions with active site residues such as LYS505, GLY504, THR1012, MET782, LEU918, TYR923 and LEU1011 etc as shown in fig. 8. The most active derivative 6f interacts with active site residue ARG503 forming the hydrogen bond with the phenyl ring hydrogen atom. Hydrophobic amino acids LEU1011 and MET782 interact with aryl ring Pi-electrons and alkyl groups to form Pi-alkyl and Pi-sigma interactions.

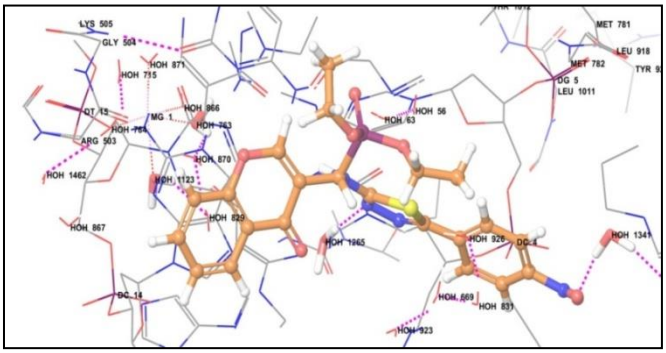


Fig. 8: Binding pose and molecular interactions of 6f into the active site of Top II α

***In silico* ADMET predictions**

Early prediction of the drug like properties of lead compounds is an important task as it decides the time and cost of drug discovery and development. Many of the active agents have shown significant biological activity fail in clinical trials because of an inadequate drug-like properties. The drug like properties has been predicted by analyzing absorption and distribution characteristics. We have calculated and analyzed various physical descriptors and pharmaceutical relevant properties for ADMET prediction by using mol-inspiration [37, 38] the data is summarized in table 6. All the compounds showed significant values for the various parameters analyzed and showed good drug-like characteristics based on Lipinski's rule of five and its variants that characterized that these agents are likely to be orally active [39]. The data obtained for all the synthesized novel derivatives 6(a-j) is within the range of accepted values. None of the synthesized compounds had violated the Lipinski's rule of five. The value of polar surface area (PSA) and Log P value of the synthesized compounds 6(a-j) indicate good oral bioavailability. The parameters, like number of rotatable bonds and the number of rigid bonds are linked with intestinal absorption. All the synthesized compounds 6(a-j) have shown good intestinal absorption. All the synthesized compounds 6(a-j) were found to be nontoxic. *In silico* assessment of all the synthetic compounds has shown that they have very good pharmacokinetic properties which is reflected in their physicochemical values and which is ultimately contributing *for the pharmacological properties of these molecules.

By using mol-inspiration, it was predicted that the compounds exhibited a good % Absorption (ABS) ranging from 41.65 % to 73.27 % as shown in table 6.

Table 6: Pharmacokinetic parameters of the synthesized compounds 6(a-j) for good oral bioavailability

Entry	% ABS	TPSA (A2)	n- ROTB	MV	MW	miLog P	n-ON	n-OHNH	Lipinski violation
Rule 6a	-73.27	-103.56	-9	-396.07	<500 471.47	<5 3.85	<10 8	<5 1	<1 0
6b	73.27	103.56	9	409.61	505.91	4.53	8	1	1
6c	66.29	123.79	9	404.09	487.47	3.38	9	2	0
6d	73.27	103.56	9	412.64	485.49	4.30	8	1	0
6e	66.29	123.79	9	404.09	487.47	3.59	9	2	0
6f	57.46	149.38	10	419.41	516.46	3.81	11	1	2
6g	73.27	103.56	9	413.96	550.36	4.66	8	1	1
6h	73.27	103.56	10	423.49	497.50	4.05	8	1	0

6i	64.19	129.87	11	440.60	529.50	3.36	10	1	1
6j	41.65	195.21	11	442.74	561.46	3.70	14	1	2

% ABS-Percentage of absorption; TPSA-topological polar surface area; n-ROTB-number of rotatable bonds; MV-molecular volume; MW-molecular weight; miLogP-logarithm of partition coefficient; n-ON-number of hydrogen bond acceptors; n-OHNH-number of hydrogen bonds donors.

Spectral analysis

The FTIR spectra were obtained using JASCO FTIR-4000 and peaks were expressed in terms of wave number (cm^{-1}). The ^{1}H NMR and ^{13}C NMR spectra of the synthesized compounds 6(a-j) were recorded on Bruker Avance II 400 NMR Spectrometer at 400 MHz Frequency in DMSO and using TMS as internal standard (chemical shift δ in ppm). The Mass spectra were scanned on Water's Micromass Q-T of the system. The ^{31}P NMR of compounds was recorded at δ 250 to δ 250 in DMSO and using Phosphoric acid (H_3PO_4) as the external standard (chemical shift δ in ppm). Ultrasound synthesizer Vibra Cell VCX-500 (Sonics, Newtown, CT, USA) with solid probe was used for the synthesis of intermediates and final derivatives 6(a-j). The MTT assay for anti-cancer activity evaluation of the synthesized compounds 6(a-j) was performed at the Department of animal biology, University of Hyderabad, Hyderabad 500046, India.

Diethyl (4-oxo-4H-chromen-3-yl)(5-phenyl-1,3,4-thiadiazol-2-ylamino)methylphosphonate 6a

M. P.: 195-197 °C; IR (KBrvmax in cm-1): 3363.25 (N-H stretching), 3016.12 (C-H stretching of aromatic), 2773.14 (C-H stretching of alkyl), 2271.73 (C=N Stretching), 1725.02 (C-O stretching), 1665.10 (C=O stretching), 1527.35 (C-N Stretching), 1041.37 (O-stretching), 663.39(C-S Stretching);^{1}HNMR (400 MHz, DMSO, δ_H ppm): 1.29 (t, 6H, 2×OCH_2CH_3), 4.70 (q, 4H, 2×OCH_2CH_3), 5.05 (d, 1H,-CH), 7.41-8.08 (m, 10H, aromatic), 8.61 (s, 1H,-NH); ^{13}CNMR: (DMSO) δppm: 16.3(CH_3), 16.3(CH_3), 62.3(CH_2), 62.3(CH_2), 61.09CH), 128.7(CH), 123.4(CH), 129.2(CH), 135.2(CH), 125.8(CH), 130.9(CH), 116.9(CH), 123.9(C), 133.5(C), 183.0(C), 150.6(C), 157.2(C), 174.1(C), 164.2(C); [31]PNMR (200 MHz, DMSO) δ: 19.90; Molecular weight: calculated: 471.47. Found: 472.47 (M+1); Molecular formula: $C_{22}H_{22}N_3O_5PS$; Elemental Analysis: (C, H, N): 56.05, 4.70, 8.91, found 56.03, 4.71, 8.90.

Diethyl(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-ylamino)(4-oxo-4H-chromen-3-yl)methylphosphonate 6b

M. P.:150-152 °C; IR (KBrvmax in cm-1): 3303.25 (N-H stretching), 3096.12 (C-H stretching of aromatic), 2783.14 (C-H stretching of alkyl), 2241.73 (C=N Stretching), 1715.02 (C-O stretching), 1685.10 (C=O stretching), 1527.35 (C-N Stretching), 1041.37 (O-stretching), 660.39(C-S Stretching); ^{1}HNMR (400 MHz, DMSO, δ_H ppm): 1.28 (t, 6H, 2×OCH_2CH_3), 4.71 (q, 4H, 2×OCH_2CH_3), 5.05 (d, 1H,-CH), 7.47-8.08 (m, 9H, aromatic), 8.61 (s, 1H,-NH); ^{13}C NMR: (DMSO) δ ppm: 16.3(CH_3), 16.3(CH_3), 62.3(CH_2), 62.3(CH_2), 61.6(CH), 123.4(CH), 135.2(CH), 125.8(CH), 128.9(CH), 116.1(CH), 129.3(CH), 128.9(CH), 129.3(CH), 116.9(C), 123.9(C), 131.6(C), 183.0(C), 150.6(C), 157.2(C), 134.3(C), 174.1(C), 164.2(C); [31]PNMR (200 MHz, DMSO) δ: 19.84; Molecular weight: calculated: 505.91. Found: 507.91(M+2); Molecular formula: $C_{22}H_{21}ClN_3O_5PS$; Elemental Analysis: (C, H, N,): 52.23, 4.18, 8.31, found 52.21, 4.19, 8.29.

Diethyl (5-(4-hydroxyphenyl)-1,3,4-thiadiazol-2-ylamino)(4-oxo-4H-chromen-3-yl)methylphosphonate 6c

M. P.:176-178 °C; IR (KBrvmax in cm-1): 3303.25 (N-H stretching), 3106.12 (C-H stretching of aromatic), 2773.14 (C-H stretching of alkyl), 2271.73 (C=N Stretching), 1709.02 (C-O stretching), 1675.10 (C=O stretching), 1527.35 (C-N Stretching), 1051.37 (O-stretching), 698.39(C-S Stretching); ^{1}HNMR (400 MHz, DMSO, δ_H ppm): 1.29 (t, 6H, 2×OCH_2CH_3), 4.71 (q, 4H, 2×OCH_2CH_3), 5.09 (d, 1H,-CH), 5.35 (s, 1H, OH), 6.87-8.08 (m, 9H, aromatic), 8.60 (s, 1H,-NH); ^{13}CNMR: (DMSO) δ ppm: 16.3(CH_3), 16.3(CH_3), 62.3(CH_2), 62.3(CH_2), 61.0(CH), 123.4(CH), 135.2(CH), 125.8(CH), 128.9(CH), 116.4(CH), 116.1(CH), 128.9(CH), 116.4(CH), 116.9(CH), 123.9(CH), 126.1(C), 158.5(C), 183.0(C), 150.6(C), 157.2(C), 174.1(C), 164.2(C);[31]PNMR (200 MHz, DMSO) δ: 19.54; Molecular weight: calculated: 487.47. Found: 488.47 (M+1); Molecular formula: $C_{22}H_{22}N_3O_6PS$; Elemental Analysis: (C, H, N): 54.21, 4.55, 8.62, found 54.20, 4.56, 8.60.

Diethyl (4-oxo-4H-chromen-3-yl)(5-p-tosyl-1,3,4-thiadiazol-2-ylamino)methylphosphonate 6d

M. P.: 180-182 °C; IR (KBrvmax in cm-1): 3357.25 (N-H stretching), 3016.82 (C-H stretching of aromatic), 2763.14 (C-H stretching of alkyl), 2271.73 (C=N Stretching), 1705.02 (C-O stretching), 1695.10 (C=O stretching), 1597.35 (C-N Stretching), 1041.37 (O-stretching), 676.39(C-S Stretching); ^{1}HNMR (400 MHz, DMSO, δ_H ppm): 1.29 (t, 6H, 2×OCH_2CH_3), 2.34 (s, 3H, aromatic C-CH_3),4.71 (q, 4H, 2×OCH_2CH_3), 5.09 (d, 1H,-CH), 5.35 (s, 1H, OH), 7.40-8.09 (m, 9H, aromatic), 8.60 (s, 1H,-NH); ^{13}CNMR: (DMSO) δppm: 16.3(CH_3), 16.3(CH_3), 21.3 (CH_3), 62.3(CH_2), 61.0(CH), 123.4(CH), 135.2(CH), 129.5(CH), 125.8(CH), 127.4(CH), 116.1(CH), 129.5(CH), 127.4(CH), 131.7(CH), 123.9(CH), 116.9(C), 123.9(C), 130.5(C), 183.0(C), 150.6(C), 157.2(C), 174.1(C), 164.2(C);[31]PNMR (200 MHz, DMSO) δ: 19.54; Molecular weight: calculated: 485.49. Found: 486.49 (M+1); Molecular formula: $C_{23}H_{24}N_3O_5PS$; Elemental Analysis: (C, H, N): 56.90, 4.98, 8.66, found 56.89, 4.99, 8.64.

Diethyl (5-(2-hydroxyphenyl)-1,3,4-thiadiazol-2-ylamino)(4-oxo-4H-chromen-3-yl)methylphosphonate 6e

M. P.:188-190 °C; IR (KBrvmax in cm-1): 3363.25 (N-H stretching), 3016.12 (C-H stretching of aromatic), 2753.14 (C-H stretching of alkyl), 2281.73 (C=N Stretching), 1710.02 (C-O stretching), 1685.10 (C=O stretching), 1617.35 (C-N Stretching), 1081.37 (O-stretching), 663.39(C-S Stretching); ^{1}HNMR (400 MHz, DMSO, δ_H ppm): 1.29 (t, 6H, 2×OCH_2CH_3), 4.70 (q, 4H, 2×OCH_2CH_3), 5.08 (d, 1H,-CH), 5.35 (s, 1H OH), 7.01-8.08 (m, 9H, aromatic), 8.55 (s, 1H,-NH); ^{13}CNMR: (DMSO) δ ppm: 16.3(CH_3), 16.3(CH_3), 62.3(CH_2), 62.3(CH_2), 61.60(CH), 123.4(CH), 135.2(CH), 125.8(CH), 128.9(CH), 116.4(CH), 116.1(CH), 128.9(CH), 116.4(CH), 116.9(CH), 123.9(CH), 126.1(C), 158.5(C), 183.0(C), 150.6(C), 157.2(C), 174.1(C), 164.2(C); [31]PNMR (200 MHz, DMSO) δ: 19.04; Molecular weight: calculated: 487.47. Found: 488.47 (M+1); Molecular formula: $C_{22}H_{22}N_3O_6PS$; Elemental Analysis: (C, H, N): 54.21, 4.55, 8.62, found 54.20, 4.57, 8.60.

Diethyl(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-ylamino)(4-oxo-4H-chromen-3-yl)methylphosphonate 6f

M. P.: 140-142 °C; IR (KBrvmax in cm-1): 3293.25 (N-H stretching), 3016.12 (C-H stretching of aromatic), 2773.14 (C-H stretching of alkyl), 2271.73 (C=N Stretching), 1725.02 (C-O stretching), 1715.10 (C=O stretching), 1527.35 (C-N Stretching), 1041.37 (O-stretching), 689.39(C-S Stretching); ^{1}HNMR (400 MHz, DMSO, δ_H ppm): 1.28 (t, 6H, 2×OCH_2CH_3), 4.71 (q, 4H, 2×OCH_2CH_3), 5.09 (d, 1H,-CH), 7.47-8.38 (m, 9H, aromatic), 8.61 (s, 1H,-NH); ^{13}CNMR: (DMSO) δppm: 16.3(CH_3), 16.3(CH_3), 62.3(CH_2), 62.3(CH_2), 61.60CH), 123.4(CH), 135.2(CH), 125.8(CH), 128.9(CH), 116.4(CH), 116.1(CH), 128.9(CH), 116.4(CH), 116.9(CH), 123.9(CH), 126.1(C), 158.5(C), 183.0(C), 150.6(C), 157.2(C), 174.1(C), 164.2(C); [31]PNMR (200 MHz, DMSO) δ: 19.64; Molecular weight: calculated: 516.46. Found: 517.46 (M+1); Molecular formula: $C_{22}H_{21}N_4O_7PS$; Elemental Analysis: (C, H, N): 51.16, 4.10, 10.85, found 51.14, 4.12, 10.83.

Diethyl (5-(4-bromophenyl)-1,3,4-thiadiazol-2-ylamino)(4-oxo-4H-chromen-3-yl)methylphosphonate 6g

M. P.: 112-114 °C; IR (KBrvmax in cm-1): 3293.25 (N-H stretching), 3096.12 (C-H stretching of aromatic), 2763.14 (C-H stretching of alkyl), 2271.73 (C=N Stretching), 1715.02 (C-O stretching), 1675.10 (C=O stretching), 1527.35 (C-N Stretching), 1041.37 (O-stretching), 668.39(C-S Stretching);

^{1}HNMR (400 MHz, DMSO, δ_H ppm): 7.10(NH),1.29(CH_3), 1.29(CH_3), 4.07(CH_2), 4.07(CH_2), 3.3(CH), 7.47(CH), 7.56(CH), 8.08(CH), 7.86(CH), 7.55(CH), 7.66(CH), 7.86(CH), 7.66(CH), 4.0(NH); ^{13}CNMR: (DMSO) δppm: 16.3(CH3), 16.3(CH3), 62.3(CH2), 62.3(CH2), 61.0(CH), 123.4(CH), 135.2(CH), 125.8(CH),129.7(CH),116.1(CH), 132.1(CH), 129.7(CH), 132.1(CH), 116.9(C), 123.9(C), 132.5(C), 183.0(C), 150.6(CH), 151.2(C), 123.1(C), 174.1(C), 164.2(C); [31]PNMR (200 MHz, DMSO) δ: 19.94; Molecular weight: calculated: 550.36. Found: 552.36 (M+2); Molecular formula: $C_{22}H_{21}BrN_3O_5PS$; Elemental Analysis: (C, H, N): 48.01, 3.85, 7.63, found 48.00, 3.87, 7.60.

(E)-diethyl (4-oxo-4H-chromen-3-yl)(5-styryl-1,3,4-thiadiazol-2-ylamino)methylphosphonate 6h

M. P.:160-162 °C; IR (KBrvmax in cm-1): 3293.25 (N-H stretching), 3116.12 (C-H stretching of aromatic), 2703.14 (C-H stretching of alkyl), 2298.73 (C=N Stretching), 1710.02 (C-O stretching), 1705.10 (C=O stretching), 1527.35 (C-N Stretching), 1041.37 (O-stretching), 653.39(C-S Stretching); ^{1}HNMR (400 MHz, DMSO, δ_H ppm): 1.28 (t, 6H, 2×OCH_2CH_3), 4.71 (q, 4H, 2×OCH_2CH_3), 5.09 (d, 1H,-CH), 6.59-6.99 (m, 2H,-CH), 7.33-8.10 (m, 9H, aromatic), 8.55 (s, 1H,-NH); ^{13}CNMR: (DMSO) δppm: 16.3(CH_3), 16.3(CH_3), 133.4(CH), 116.9(CH), 62.3(CH_2), 62.3(CH_2), 61.0(CH), 127.9(CH), 128.6(CH), 128.6(CH), 123,4(CH), 135.29(CH), 128.5(CH), 125.8(CH), 116.1(CH), 128.5(CH), 137.5(C), 116.9(C), 123.9(C), 183.0(C), 150.6(C), 157.2(C), 158.9(C), 164.2(C); [31]PNMR (200 MHz, DMSO) δ: 19.65; Molecular weight: calculated: 497.50. Found: 498.50 (M+1); Molecular formula: $C_{24}H_{24}N_3O_5PS$; Elemental Analysis: (C, H, N): 57.94, 4.86, 8.45, found 57.92, 4.88, 8.43.

2-(5-((diethoxyphosphoryl)(4-oxo-4H-chromen-3-yl)methylamino)-1,3,4-thiadiazol-2-yl)phenyl acetate 6i

M. P.:178-180 °C; IR (KBrvmax in cm-1): 3283.25 (N-H stretching), 3056.12 (C-H stretching of aromatic), 2773.14 (C-H stretching of alkyl), 2271.73 (C=N Stretching), 1715.02 (C-O stretching), 1695.10 (C=O stretching), 1598.35 (C-N Stretching), 1089.37 (O-stretching), 679.39(C-S Stretching); ^{1}HNMR (400 MHz, DMSO, δ_H ppm): 1.28 (t, 6H, 2×OCH_2CH_3), 2.28 (s, 3H,-CH_3), 4.71 (q, 4H, 2×OCH_2CH_3), 5.09 (d, 1H,-CH), 7.33-8.10 (m, 9H, aromatic), 8.55 (s, 1H,-NH); ^{13}CNMR: (DMSO) δppm: 16.3(CH_3), 16.3(CH_3), 20.3(CH_3), 62.3(CH_2), 62.3(CH_2), 169.0(C), 61.0(CH), 129.4(CH), 126.0(CH), 129.1(CH), 135.2(CH), 125.8(CH), 127.9(CH), 123.2(CH), 116.1(CH), 116.9(C), 123.9(C), 129.4(C), 151.1(C), 183.0(C), 150.6(CH), 157.2(C), 174.1(C), 164.2(C);[31]PNMR (200 MHz, DMSO) δ: 19.45; Molecular weight: calculated: 529.50. Found: 530.50 (M+1); Molecular formula: $C_{24}H_{24}N_3O_7PS$; Elemental Analysis: (C, H, N): 54.44, 4.57, 7.94, found 54.42, 4.59, 7.90.

Diethyl(5-(3,4-dinitrophenyl)-1,3,4-thiadiazol-2-ylamino)(4-oxo-4H-chromen-3-yl)methylphosphonate6j

M. P.:176-178 °C; IR (KBrvmax in cm-1): 3303.25 (N-H stretching), 3106.12 (C-H stretching of aromatic), 2711.14 (C-H stretching of alkyl), 2225.73 (C=N Stretching), 1710.02 (C-O stretching), 1685.10 (C=O stretching), 1587.35 (C-N Stretching), 1091.37 (O-stretching), 673.39(C-S Stretching); ^{1}HNMR (400 MHz, DMSO, δ_H ppm): 1.29 (t, 6H, 2×OCH_2CH_3), 4.71 (q, 4H, 2×OCH_2CH_3), 5.05 (d, 1H,-CH), 7.10-8.08 (m, 5H, aromatic), 8.49 (s, 1H,-NH), 8.52-8.99 (m, 3H, aromatic); ^{13}CNMR: (DMSO) δppm: 16.3(CH_3), 16.3(CH_3), 62.3(CH_2), 62.3(CH_2), 61.0(CH), 123.4(CH), 135.2(CH), 125.8(CH), 116.1(CH), 134.5(CH), 125.3(CH), 123.7(CH), 116.9(C), 123.9(C), 140.5(C), 144.8(C), 145.3(C), 183.0(C), 150.6(CH), 157.2(C), 174.1(C), 164.2(C); [31]PNMR (200 MHz, DMSO) δ: 19.56; Molecular weight: calculated: 561.46. Found: 562.46 (M+1); Molecular formula: $C_{22}H_{20}N_5O_9PS$; Elemental Analysis: (C, H, N): 47.06, 3.59, 12.47, found 47.04, 3.61, 12.45.

CONCLUSION

The ten novel diethyl (4-oxo-4H-chromen-3-yl)(5-substituted phenyl-1,3,4-thiadiazol-2-ylamino)methyl phosphonate derivatives6(a-j) were synthesized using ultrasonic processor, in presence of $ZrOCl_2$ as a catalyst. The *in vitro* anticancer activity was performed against two human cancer cell lines such as DU 145 and MCF-7 by MTT assay method. From the *in vitro* anticancer screening the, compound 6e was found to be the most potent anticancer compound among the synthesized compounds against MCF-7 cancer cell lines with IC_{50} value of 2.97 µM with 67.92 % of cancer cells death. *In silico* bioavailability studies indicated that compounds have a good *in silico* % absorption (41.65 % to 73.27 %) and all synthesized compounds 6(a-j) were found to be non-toxic. This suggests that the compounds of the present series can serve as lead molecules for the design and development of new Topoisomease II inhibitor as anticancer agents.

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Nil

AUTHORS CONTRIBUTIONS

V. K. C. performed the synthesis; performed the synthesis and performed the enzyme assay; D. K. C. performed molecular docking study, V. K. C. spectral analysis.

CONFLICTS OF INTERESTS

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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