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Synthesis, Characterization, Docking Studies and *in vitro* Response of New Camptothecin Derivatives towards Oral Squamous Cell Carcinoma

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Evidence suggests heterocyclic rings as the essential component of various available chemotherapeutics. Present study was aimed to carry out the molecular docking, synthesis, characterization and response of new camptothecin derivatives (NCDs) towards OSCC cell lines. To achieve the aim of the study, new camptothecin derivatives were designed to perform molecular docking against the target protein Human DNA Topoisomerase-1 (1T8I). Molecules **2** and **3** with high docking scores were subjected to synthesis. In current investigation, NCDs were synthesized by cyclization of imino analogue (**2**) into a new azetidinone derivative (**3**) on treatment with triethylamine and chloroacetyl chloride. Synthesized NCDs were characterized using IR, NMR and mass analysis. The NCDs were further subjected to antiproliferation study using CAL-27 (OSCC), followed by *in vitro* DNA relaxation assay and cell cycle analysis. The results of the docking of CPT-11 against Human DNA Topoisomerase-1 Duplex (PDB ID: 1T8I) in present study revealed compound **2** and **3** exhibited high docking score among all camptothecin analogues. Present study successfully synthesized and elucidated the structures of NCD **2** and **3**. The antiproliferation study results revealed that NCD **2** and NCD **3** offered an IC_{50} of 34.73 µg/mL and 62.5 µg/mL, respectively. The DNA relaxation assay exhibited the inhibition action of synthesized NCDs (IC_{50} concentration) against topoisomerase enzyme. Moreover, the cell cycle analysis revealed that both NCDs arrested cancer cells in 'S' phase. Though the present study highlights the potential of NCDs against oral squamous cell carcinoma, however, the present study also recommends that the synthesized NCDs must be further evaluated for preclinical and clinical significance.

Keywords: Azetidinone, Characterization, Irinotecan, Imine, Synthesis.

INTRODUCTION

Evidence suggest commercially available 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy analogue of camptothecin (CPT-11), the semisynthetic derivative of camptothecin (CPT) is commonly used to treat various types of cancers. Compound CPT-11 generally undergoes hydrolyzation into 7-ethyl-10-hydroxycamptothecin (SN-38) the active metabolite in human body [1]. Today across the globe, oral squamous cell carcinoma (OSCC) considered to be as the most prevalent cause for morbidity and mortality [2]. Evidence data of Global Cancer Observatory (GLOBOCAN) in year 2020 witnessed 0.38 million new cases and 0.18 million mortality cases related to oral cancers [3] and globally, OSCC stands at 16th position among the most common cancers [4]. Conventional management of oral cancer included surgery followed by either radiotherapy or chemo-

therapy [5,6]. The action of chemotherapeutic agents on the actively multiplying "cycling" tumour cells initiate the activation of the apoptosis and also make these cells susceptible to chemotherapeutic action [7]. However, multidrug resistance by tumor cells found to be significant limitation for these chemotherapeutic agents. Irinotecan is commonly used for treatment of several forms of cancer [8].

Since OSCCs have been associated with high Top-1 activity, the antitumour activity of irinotecan (CPT-11) among other Top-1 inhibitor are investigated and demonstrated modest activity in cancer of head & neck [9,10]. Till date, only two CPT analogues (irinotecan and topotecon) have been approved by US Federation Drug Administration (FDA) for cancer treatment [11], however associated side effect is a concern. Evidence suggests incorporation of pharmacophoric moieties such as imino and azetidinone ring in various organic moieties enhances the antitumor activity [12]. Latest studies reported synthetic routes to turn hydrazides into imino and azetidinones moieties using aromatic aldehydes and chloroacetylchloride (CAC), respectively [13]. Molecular docking plays an important role in drug design and development. It predicts the binding mode of already known ligands and identifies the novel and potent ligands by determining the binding affinity of a molecule [14]. Hence based on the ability of imine and azetidinone nucleus to enhance the anticancer potential of chemical moieties, the current investigation study was designed to perform synthesis and evaluation of safety and anticancer potential of NCDS towards oral squamous cell carcinoma.

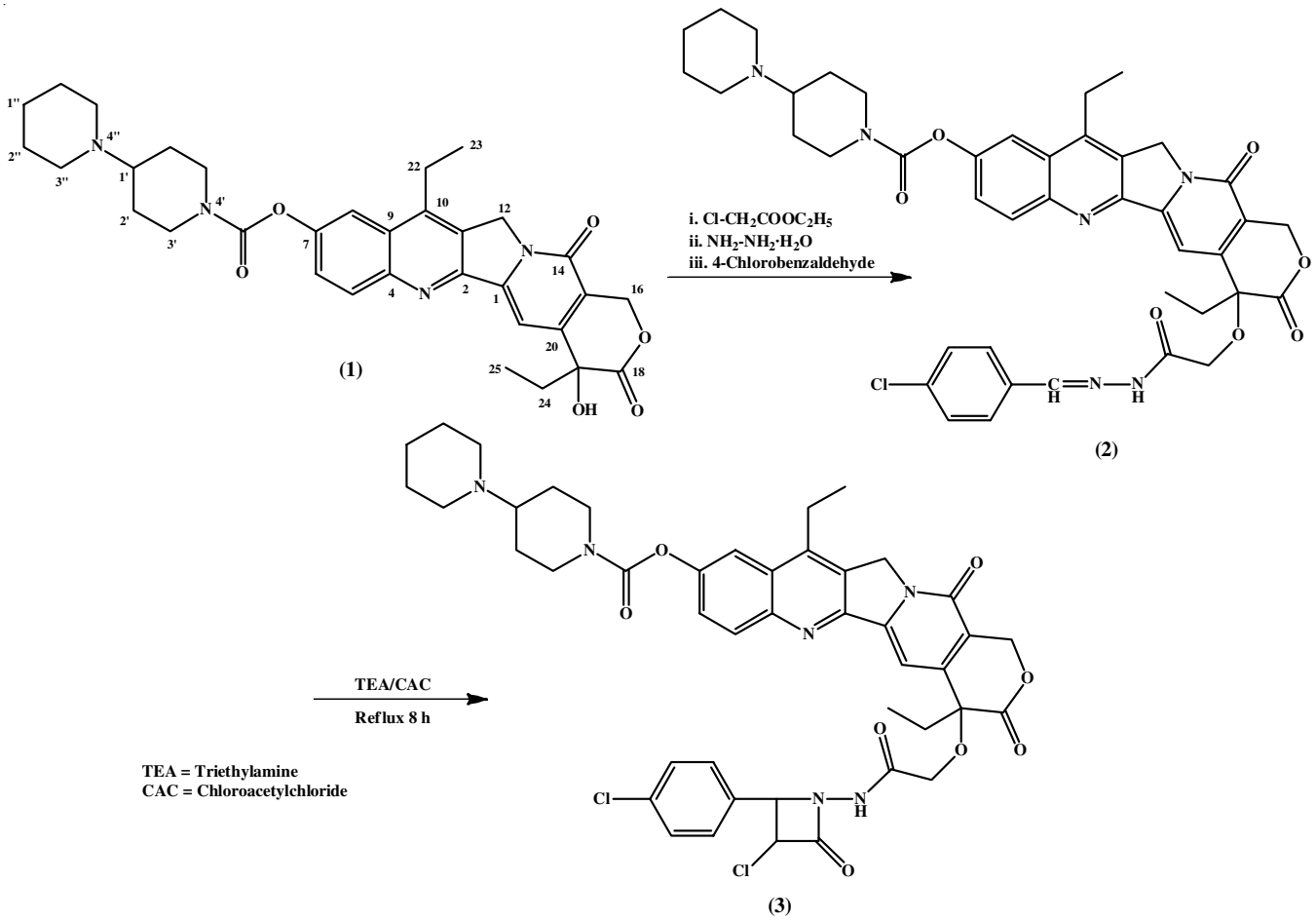
EXPERIMENTAL

In present study, *in silico* docking experiment was done using AutoDock Vina. The chemicals and biologicals were procured from Friendemann Schmidt, Sigma-Aldrich, Merck KGaA, Qrec Chemicals and HmbG® Chemicals. The progress of reaction during synthesis experiment was monitored by thin layer chromatography and melting point measurements using Spectroline UV CM-26 and Stuart Analogue Melting Point SMP11, respectively. The chemical structures of the synthesized compounds were confirmed based on attenuated total reflectance - infrared, ^{1}H & ^{13}C nuclear magnetic resonance and direct infusion mass spectrometry spectral data.

***in silico* Molecular docking of novel camptothecin analogues against Topoisomerase-1:** In present study, the docking of camptothecin analogues was carried out to assess their interaction and binding modes with the target protein Human DNA Topoisomerase-1 (70 Kda) complexed with A 22 Base Pair DNA Duplex (1T8I) using an Intel i7 with RAM of 16 GB. To prepare the protein, structure drawing and conversion to the working format the Open-source software including Discovery Studio, ChemDraw [15] and OpenBabel [16] were used. All the designed compounds chemical structures were modelled using Chemsketch software. The 2D structures of CPT-11 analogues were generated and conversion into respective 3D structures was done using Ligplot. The designed structures of CPT-11 analogues were optimized, followed by energy minimization using AutoDock software and the process of molecular docking [17]. Vina structure-based drug design was performed using AutoDock Vina and visualization was done by Discovery Studio Visualizer [18]. The 3D structures of Human DNA Topoisomerase-1 (Top-1, 70 Kda) complexed with A 22 base pair DNA Duplex (PDB ID: 1T8I) was downloaded from RCSB Protein Data Bank (PDB). The Discovery Studio Visualizer was used to prepare the downloaded Top-1 protein by removing the heteroatoms and water molecules. Whereas for addition of hydrogen and assignment of the charges Molecular Graphics Laboratory (MGL) tools were used. AutoDock Vina was used only for defining grid parameters and docking. The docking results were further analyzed using Discovery Studio Visualizer [18].

Synthesis of 10,19-diethyl-14,18-dioxo-19-{[N′-(phenylmethylidene)hydrazinecarbonyl]methoxy}-17-oxa-3,13-diazapentacyclo[11.8.0.0^{2,11}.0^{4,9}.0^{15,20}]hemicos-1(21),2,4,6,8,10,15(20)-heptaen-7-yl[1,4′-bipiperidine]-1′-carboxylate (NCD 2): NCD 2 was obtained as per the method stated in standard literature with minor modification [19,20]. Briefly, hydrazinated derivative of irinotecan **1** {(that was obtained after esterification and hydrazination of irinotecan (CPT-11)} was refluxed with chlorobenzaldehyde in equimolar concentration for 8 h. For the reaction, just quantity sufficient (Q.S.) of absolute ethanol was added. After completion of reaction the excess of ethanol was distilled off. Next the reaction mixture was cooled and the crude product was filtered off. The obtained crude product was purified using ethanol solvent to offer NCD **2**. During the reaction anhydrous conditions were maintained and to catalyze the reaction one drop of glacial acetic acid was added (**Scheme-I**). The progress and monitoring of reaction was done by TLC. Brown solid (yield: 86%, m.p.: 242 ºC); ATR-IR (cm^{-1}): 3341 (N-H), 3058 (=C-H *str.*), 2961 (-C-H *str.*), 1709 & 1653 (C=O *str.*), 1586 (C=N); ^{1}H NMR (DMSO, ppm) δ: 0.8 (3H, t, *J* = 7.6, H-25), 1.3 (6H, m, H-1″, 2″& 6″), 1.4 (4H, m, H-2′, 6′), 1.8 (2H, q, *J* = 7.6 & 7.6, H-24), 2.2 (4H, t, *J* = 7.6, H-3″ & 4″), 2.5 (2H, t, *J* = 7.6, H-22), 3.3 (4H, t, *J* = 7.6, H3′ & 5′), 4.2 (2H, s, H-12), 4.3 (2H, s, H-26), 4.8 (2H, s, H-16), 6.5 (1H, s, H-21), 6.8-7.9 (7H, m, Ar-H), 8.60 (1H, s, NH-C=O), 8.8 (1H, s, CH=N); ^{13}C NMR (DMSO, ppm) δ: 8 (C25), 22 (C23), 24 (C22), 26 (C1″), 27 (C24), 30 (C2″ & C6″), 39 (C2′ & C6′), 44 (C3′ & C5′), 45 (C3″ & C5″), 46 (C12), 52 (C1″), 57 (C26), 59 (Ar-O-C), 61 (C26), 65 (C16), 72 (C19), 101, 106, 114, 115, 119, 126, 127, 128, 131, 135, 145, 146, 150, 152 (Ar-C), 153 (C14), 157 (C7′), 159 (C=N), 169 (C27), 172 (C18); MS: *m/z*: 780.

Synthesis of 19-({[3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl]carbamoyl}methoxy)-10,19-diethyl-14,18-dioxo-17-oxa-3,13-diazapentacyclo[11.8.0.0^{2,11}.0^{4,9}.0^{15,20}]-hemicosa-1(21),2,4,6,8,10,15(20)-heptaen-7-yl[1,4′-bipiperidine]-1′-carboxylate (NCD 3): The synthesis of the **NCD 3** was done as per the standard protocol with slight modification [21,22], briefly, in a round-bottom flask, equimolar concentration of compound **NCD 2**, triethylamine (TEA) and chloroacetylchloride (CAC) using dioxane was refluxed for 8 h. To the reaction mixture, just quantity sufficient (Q.S.) of absolute ethanol was added. After completion of reaction the excess of ethanol was distilled off. Next the reaction mixture was cooled and the crude product was filtered off. The crude product was purified to obtain **NCD 3** (**Scheme-I**). During the



Scheme-I: Synthesis of NCD **2** and **3**

reaction anhydrous conditions were maintained. Periodically during reflux, the TLC analysis and determination of melting point of the synthesized compound was determined. Yellow solid (yield: 82%, m.p.: 246 ºC) ATR-IR (cm^{-1}): 3298 (N-H), 3063 (=C-H *str.*), 2926 (C-H *str.*), 1688 (C=O *str.*), 1594 (C=N); ^{1}H NMR (DMSO, ppm) δ: 0.8 (3H, t, *J* = 7.6, H-25), 1.3 (6H, m, H-1″, 2″& 6″), 1.4 (4H, m, H-2′,6′), 1.8 (2H, q, *J* = 7.6 & 7.6, H-24), 2.2 (4H, t, *J* = 7.6, H-3″ & 4″), 2.5 (2H, t, *J* = 7.6, H-22), 3.3 (4H, t, *J* = 7.6, H3′ & 5′), 4.2 (2H, s, H-12), 4.3 (2H, s, H-26), 4.8 (2H, s, H-16), 5.0 (1H, d, *J* = 7.2, CH-N of Azet), 5.4 (1H, d, *J* = 7.2, CH-Cl of Azet), 6.5 (1H, s, H-21), 6.8-7.9 (7H, m, Ar-H), 8.6 (1H, s, NH-C=O); ^{13}C NMR (DMSO, ppm) δ: 8 (C25), 22 (C23), 24 (C22), 26 (C1″), 27 (C24), 30 (C2″ & C6″), 39 (C2′ & C6′), 44 (C3′ & C5′), 45 (C3″ & C5″), 46 (C12), 52 (C1″), 57 (C26), 59 (Ar-O-C), 61 (C26), 65 (C16), 63 (C-N, Azet), 64 (C-Cl, Azet), 72 (C19), 101, 106, 114, 115, 119, 126, 127, 128, 131, 135, 145, 146, 150, 152 (Ar-C), 153 (C14), 157 (C7′), 165 (C=O, Azet), 169 (C27), 172 (C18); MS: *m/z*: 856.

Antiproliferation activity: The characterized **NCDs** were further subjected to evaluation of their anticancer activity using MTT assay as per the standard protocol with minor changes [23,24]. Briefly, the CAL27 cells were cultured in 5% inactivated FBS enriched - Dulbecco modified eagle (DME) medium in an incubator maintained at 5% CO_2, 95% relative humidity and 37 ºC. In this study, CAL27 cell were proliferated and incubated overnight on 96-well plate (with 10000 cells density of each well) for cells attachment. The **NCDs** and standard **NCD 1** (irinotecan) were serially diluted with DME, followed by transfer to each well (to make final concentration ranging from 3.9-500 µg/mL), incubation of microplates (as done previously) for 24 h, addition of 10 µL MTT solution into each well of microplate, re-incubation in dark for 4 h (as done previously), pipetting of contents from each well, addition of DMSO (100 µL) into each well and finally measurement of absorbance at 517 nm to calculate the percent cytotoxicity and IC_{50} of **NCDs** using expression (eqn. 1) and the non-linear regression analysis by GraphPad Prism.

$$\text{Cytotoxicity } (\%) = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100 \qquad (1)$$

DNA relaxation assay (topoisomerase-1 inhibition): The inhibition potential of newly synthesized **NCDs** against Topoisomerase enzyme was determined by evaluating the relaxation of supercoiled DNA. Relaxation was performed according to the manufacture's protocol (Topogen) [25]. Reaction assay included pBR322 coiled plasmid, Top-1 reaction buffer, Top-1 enzyme, newly synthesized **NCD 2** & **3** along with **NCD 1** as standard compound and loading dye (bromophenol blue). The assay was performed with 20 µL of reaction volume containing 500 ng of supercoiled DNA pBR322 and 1 unit of human

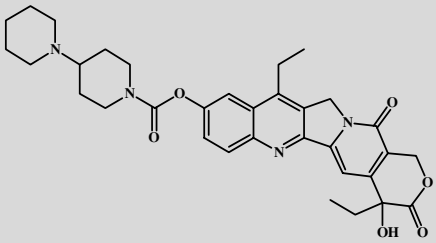
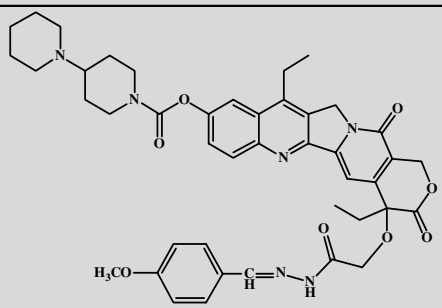
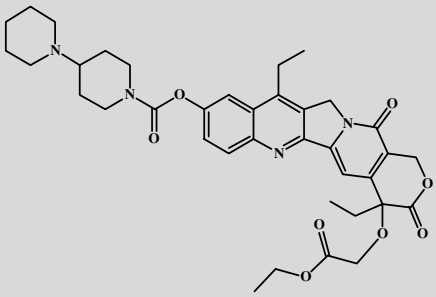
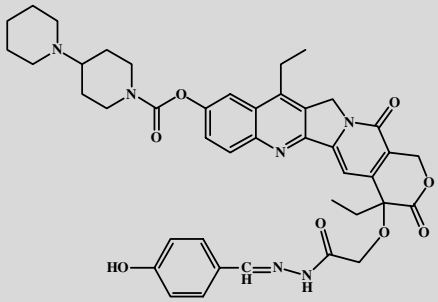
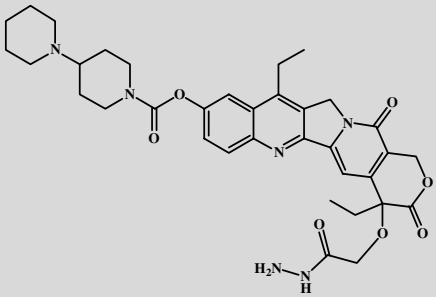
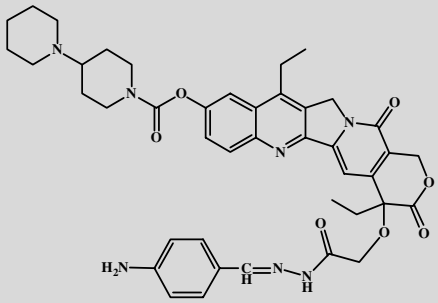
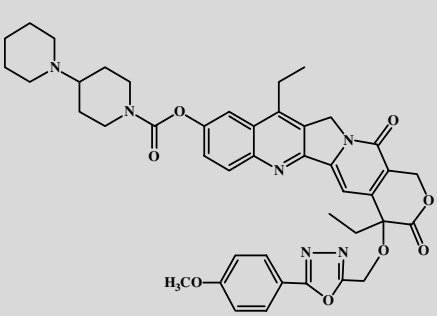
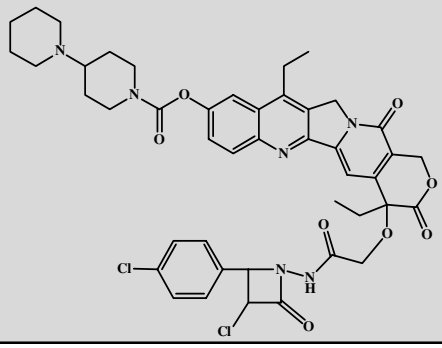
Top-1 along with and without synthesized compounds in the different reaction buffer, respectively. The prepared reaction mixture was incubated at 37 ºC for 10-15 min and terminated by adding 4 µL of DNA loading buffer. Following 1% agarose gel preparation, electrophoresis was performed and the samples were run on a 1% agarose gel with staining of the DNA by 2.5 µg/mL ethidium bromide at 100 V for 10 min. Later, the gel was destained in water and photographed under UV trans-illuminator for DNA bands using Gel Image Viewer.

Cell cycle arrest: The **NCDs** were further subjected to cell cycle analysis by standard protocol of flow cytometry [26]. Briefly, seeding of CAL27 cells was done over 6-well plates for 24 h (with 200000 cells in each well), followed by treatment of cells for another 24 h with predetermined IC_{50} of NCDs, harvesting, fixing and permeation of cells (for 0.5 h with 70% cold ethanol), two time washing with PBS, further incubation for 30 min (with propidium iodide in solution containing Triton X-100 and RNase at 25 ºC) and finally determination of **NCDs** effects on cell cycle were using flow cytometer.

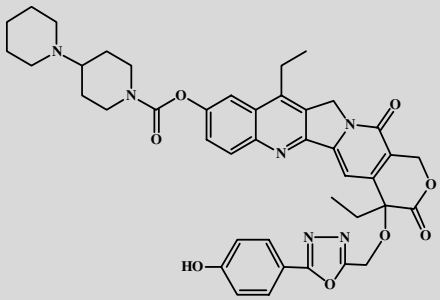
RESULTS AND DISCUSSION

The molecular docking study was performed to establish the binding ability of the camptothecin derivatives to the binding site of Human DNA Topoisomerase-1 (Top-1, 70 Kda) complexed with A 22 Base Pair DNA Duplex (PDB ID: 1T8I) [27]. The docking scores of designed molecules are presented in Table-1.

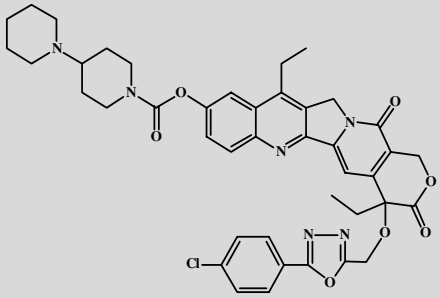
TABLE-1
DOCKING SCORE OF CAMPTOTHECIN DERIVATIVES AGAINST 1T8I

Compd. code	Dock score	Structure of camptothecin derivatives	Compd. code	Dock score	Structure of camptothecin derivatives
1	-12.3		**2a1**	-12.4	
1x	-11.3		**2a2**	-12.2	
1y	-12.6		**2a3**	-12.8	
1a	-13.0		**3**	-13.1	

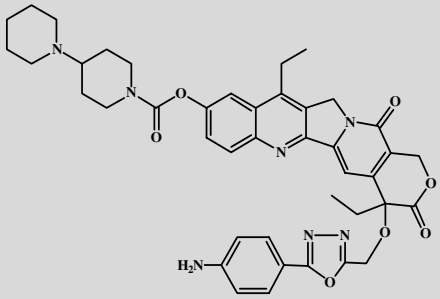
1a1 -12.9



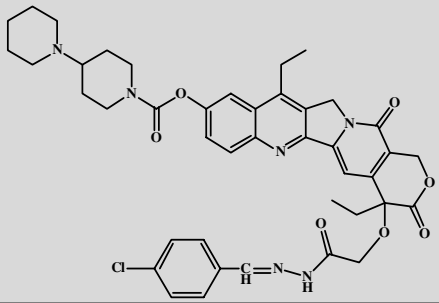
1a2 -12.8



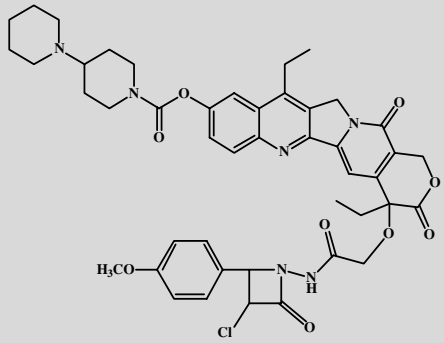
1a3 -11.3



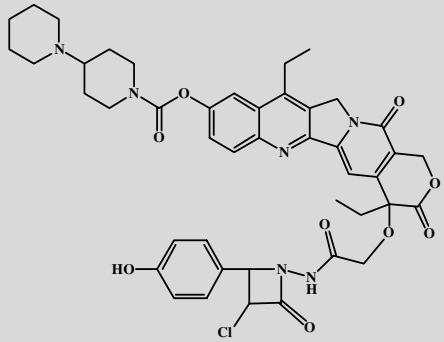
2 -13.2



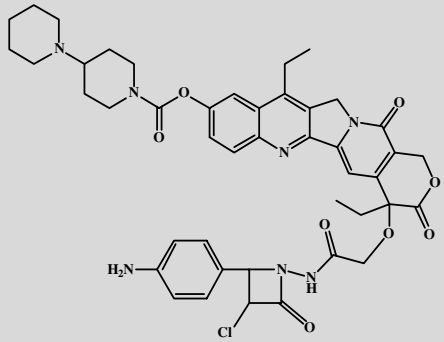
3a1 -12.9



3a2 -12.8



3a3 -11.2



All the compounds were found to completely occupy the active sites of Human DNA Topoisomerase-1 (Top-1, 70 Kda) complexed with A 22 Base Pair DNA Duplex (PDB ID: 1T8I). **NCDs 2** and **3** of the all-titled compounds, found to exhibit the highest D-score. **NCDs 2** and **3** also assumes favourable orientation within the 1T8I binding site. The 2D ligand interaction diagram of compound **2** and **3** given in Figs. 1 and 2, respectively, reveals their significant interactions with specific amino acids of the Topoisomerase-1 (PDB ID: 1T8I). The 2D ligand interaction diagram of **NCD 2** (Fig. 1a) reveals that a hydrogen bond was formed between the oxo group contained in the carboxylate, at 17th and at 18th position of **NCD 2** and the lysine amino acid present in the chain A at the 425, 436 and 751 residues of the Human DNA topoisomerase-1 (1T8I). These interactions are crucial for stabilizing the ligand protein complex and enhancing its binding affinity. The 3D representation of the molecular docking complex confirms the presence of these interactions and provides a view of how **NCD 2** fits within the active site of the Top-1 isomerase (1T8I) presented in Fig. 1b.

The 2D ligand interaction diagram of **NCD 3** (Fig. 2a) reveals that hydrogen bonds were formed between the oxo group at position 18th and in azetidine group the arginine present in the chain A at the 536 and 362 residues; and the oxo group in carbamoyl and azetidine ring of **NCD 3** with glycine in the chain A at the 536 and 365 residues of the Human DNA topoisomerase I (1T8I). These interactions are crucial for stabilizing the ligand protein complex and enhancing its binding affinity.

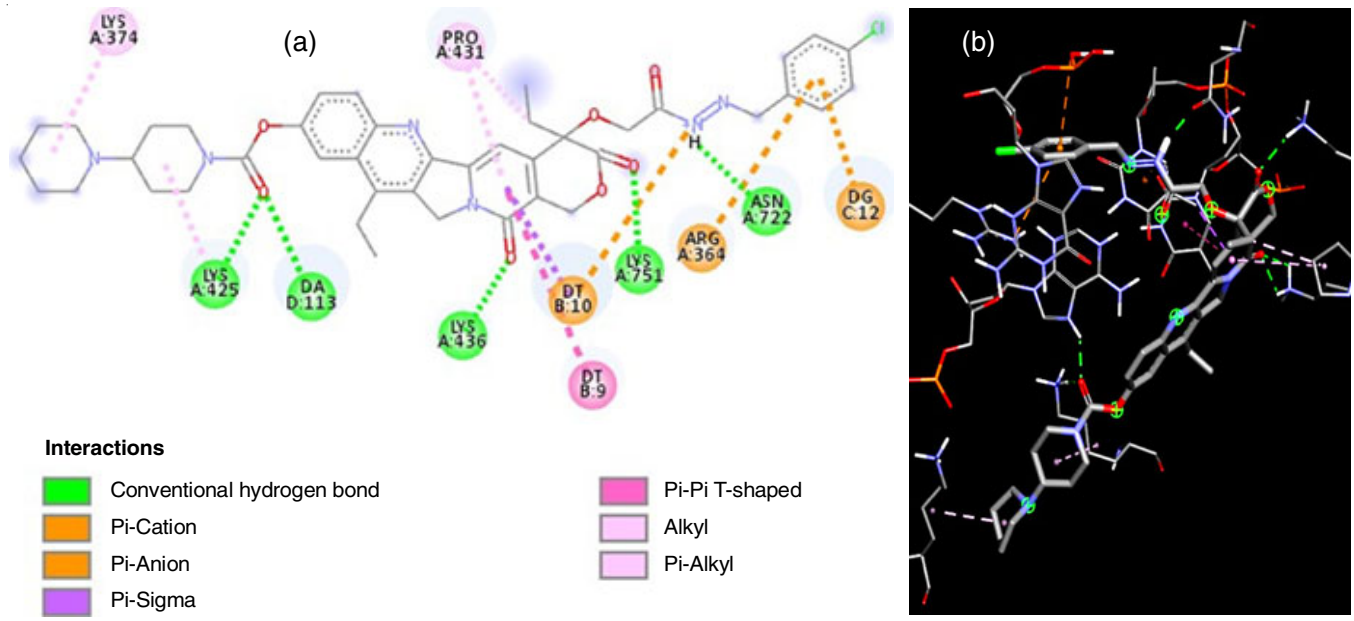


Fig. 1. 2D ligand interaction diagram (a) & 3D docked pose of compound **2** with 1T8I (b)

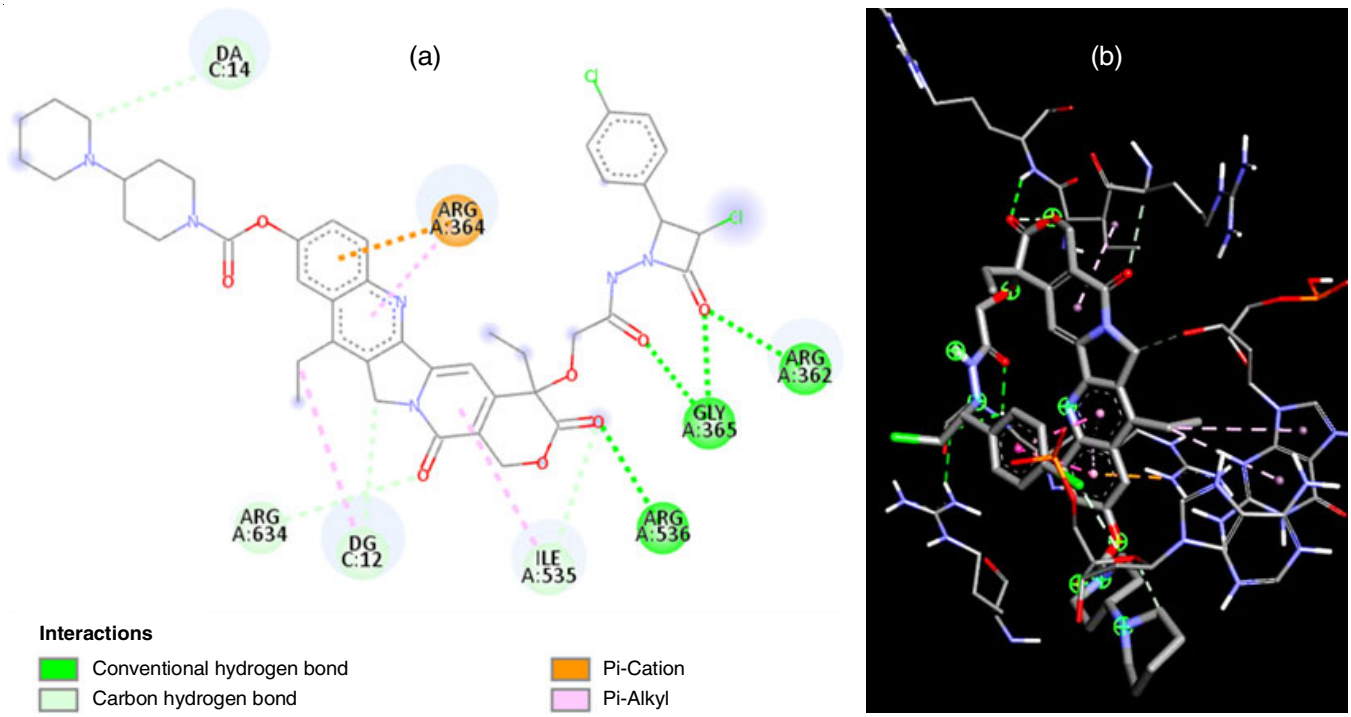


Fig. 2. 2D ligand interaction diagram (a) & 3D docked pose of compound **3** with 1T8I (b)

The 3D representation of the molecular docking complex confirms the presence of these interactions and provides a view of how **NCD 3** fits within the active site of the Top-1 isomerase (1T8I) presented in Fig. 2b.

The **NCDs** were synthesized as per the standard protocol with slight modifications [19-22]. Current study yielded **NCD 3** (azetidinone analogue) by cyclization of **NCD 2** the imino analogue using chloro-acetylchloride. The **NCD 2** was previously synthesized by esterification of CPT-11 (irinotecan) using, followed by hydrazination and Schiff reaction with chlorobenzaldehyde. All the reactions were progressed in anhydrous conditions and purification of **NCDs** was done by recrystallizing with CH_3OH/activated charcoal and purity was determined by melting point and TLC. The synthesis and characterization study results for imine and azetidinone were also correlated with other studies [19-22]. This study successfully synthesized and elucidated structure of imine and azetidinone derivative (**NCDs 2** & **3**), which correlates the synthesis of azetidinones with their physical and chemical structures of the present study [21,22]. Similar procedures and results were also highlighted by other researchers also [23,24]. Appearance of signal of IR at 3341 cm^{-1} for (N-H *str.*), 3058 (=C-H *str.*), 2961 (C-H *str.*),

1709 and 1653 (C=O *str.*), 1586 (C=N); ^{1}H NMR at δ 8.8 corresponding to CH=N protons; ^{13}C NMR at δ 172 (C=N-N); and at *m/z* value of 780 (molecular ion), elucidated **NCD 2** structure. Also, appearance signal of IR at 3298 cm^{-1} (N-H), 3063 (=C-H *str.*), 2926 (C-H *str.*), 1688 (C=O *str.*), 1594 (C=N); ^{1}H NMR at δ 5.4 corresponding to CH-Cl protons; ^{13}C NMR at δ 64 attributed to carbons C-Cl carbon of azetidinone ring; and at *m/z* 856 (molecular ion), confirmed the structure of **NCD 3**. Findings of this characterization study over **NCDs** were also in agreement with other studies findings over imino and azetidinone moieties [19-22].

Biological activity: Characterized **NCDs 2** & **3** were tested for *in vitro* antiproliferation activity against CAL27 cells on 96-well plate by MTT assay in triplicate [25]; and percent cell cytotoxicity and IC_{50} were also assessed based on standard procedure [26]. Data of antiproliferation study on **NCDs 2** & **3** mentioned in Table-2, indicates that cytotoxicity of **NCDs 2** & **3** increased with an increase in the concentration of the **NCDs 2** & **3** towards CAL27. Findings of current cytotoxicity investigation confirms that synthesized **NCDs 2** & **3** were efficacious.

TABLE-2
In vitro CYTOTOXICITY ASSAY FOR NCDs COMPOUNDS

Conc. (μg/mL)	CPT-11	NCD2	NCD3
500	89.53 ± 1.36	86.32 ± 0.64****	70.15 ± 0.15****
250	84.83 ± 0.32	74.58 ± 0.20****	66.25 ± 0.29****
125	79.19 ± 1.41	60.78 ± 0.87****	60.98 ± 3.69****
62.5	73.22 ± 0.41	52.00 ± 0.69****	54.72 ± 1.15****
31.25	63.02 ± 1.46	42.72 ± 0.80****	49.13 ± 3.26****
15.62	50.13 ± 2.86	38.82 ± 0.92****	47.20 ± 0.67****
7.81	33.24 ± 4.48	31.19 ± 0.13***	41.36 ± 0.24***
3.9	19.3 ± 5.54	27.18 ± 0.56*	36.98 ± 1.10

The data is presented as mean ± standard deviation, each experiment was performed in triplicate. Statistical analysis was attained through one-way analysis of variance (ANOVA) followed by Dunnett's posthoc test. The comparison was done by mean of standard CPT-11 *vs.* mean of **NCDs 2** & **3** to determine the significant difference among the groups using the GraphPad Prism software version 10. Data were expressed as mean ± standard deviation of compound mean. Statistical significance is indicated by *$p < 0.05$, **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$. The study IC_{50} study revealed that **NCDs 2** & **3** exhibited significant IC_{50} (Table-3).

TABLE-3
ANTICANCER ACTIVITY (IC_{50}) OF NCDs AGAINST CAL27

IC_{50}	CPT-11	NCD2	NCD3
(μg/mL)	13.2	<34.73	<62.5

Topoisomerases are one of the crucial enzymes in the cell, which has vital role in cell division. Therefore, by blocking the action of this enzyme, it is possible to disrupt the process of DNA replication or transcription during the cell division. The inhibition of Top-1 enzyme can be a target to cause decease of cancer cells, thereby exhibiting anticancer activity [26].

As shown in Fig. 3, supercoiled pBR322 plasmid (lane 1) with high intensity staining was used as control. Lane 3 shows the relaxation of the DNA fragments of because of Top-1 enzyme activity on supercoiled pBR322 plasmid. Treatment and incubation with **NCD 1** (IC_{50} = 13.2 μg/mL), supercoiled pBR322 plasmid showed the retention of supercoiled DNA at the loading edge (lane 2). Similarly, pBR322 plasmid treated and incubated **NCD 2** (lane 4) & **NCD 3** (lane 5), at their IC_{50} 34.73 and 62.5 μg/mL, respectively along with Top-1. The intensity exhibited by the relaxed DNA fragments in the gel image viewer showed Top-1 enzyme inhibitory activity against the newly synthesized **NCDs** based on position of supercoiled DNA. Based on the previous studies, it is suggested that newly synthesized analogue compounds exhibit anticancer activity through Top-1 enzyme inhibition [28,29].

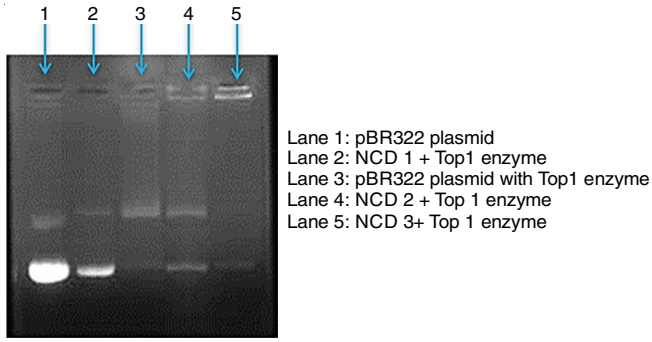


Fig. 3. Topoisomerase-1 inhibition activity

For understanding in which phase **NCDs** arrests cancer cell cycle, **NCDs** were further subjected to cell cycle analysis using flow cytometry [26], that included arrest of cell cycle of CAL27 cells (stained with propidium iodide) by **NCDs 2** & **3** in various phases. Findings of the cell cycle arrest study were represented as graph (histogram) of cell population where peaks of G0/G1 and G2/M phase are separated by the S phase distribution in each phase against DNA content distribution by fluorescence emission.

Fig. 4 depicts the histograms for CAL27 cells DNA distribution in presence/absence of test compounds (CPT-11, **NCDs 2** and **3**) with IC_{50}, wherein each histogram represents CAL27 cells arrest in specific growth phase. Study indicated that % content accumulation in G0/G1, G2M and S phase was found to be 36.55%, 17.86% and 45.64% respectively in histogram 1 for untreated CAL27 cells (propidium iodide stained); (i) 16.86%, 9.64% and 73.5% for histogram (ii) of CPT-11 (IC_{50} of 13.2 μg) treated CAL27; 33.70%, 13.27% and 53.03% for histogram (iii) of **NCD 2** (IC_{50} of 34.70 μg) treated CAL27; and 11.09%, 27.42% and 61.49% for histogram (iv) of **NCD 3** (IC_{50} of 62.50 μg) treated CAL27. Study indicated that post-treatment of CAL27 cells with **NCDs** lead to increase in S-phase % cell content, offering maximum arrest of cell cycle in S-phase. Therefore, cell cycle arrest study confirms that all **NCDs** arrested maximum cell cycle in S-phase. Antiproliferation and cell cycle arrest studies confirms the anticancer activity and phase on which anticancer agent generally acts [30,31]. In current investigation also, the antiproliferation, topoisomerase-1 inhibition and cell

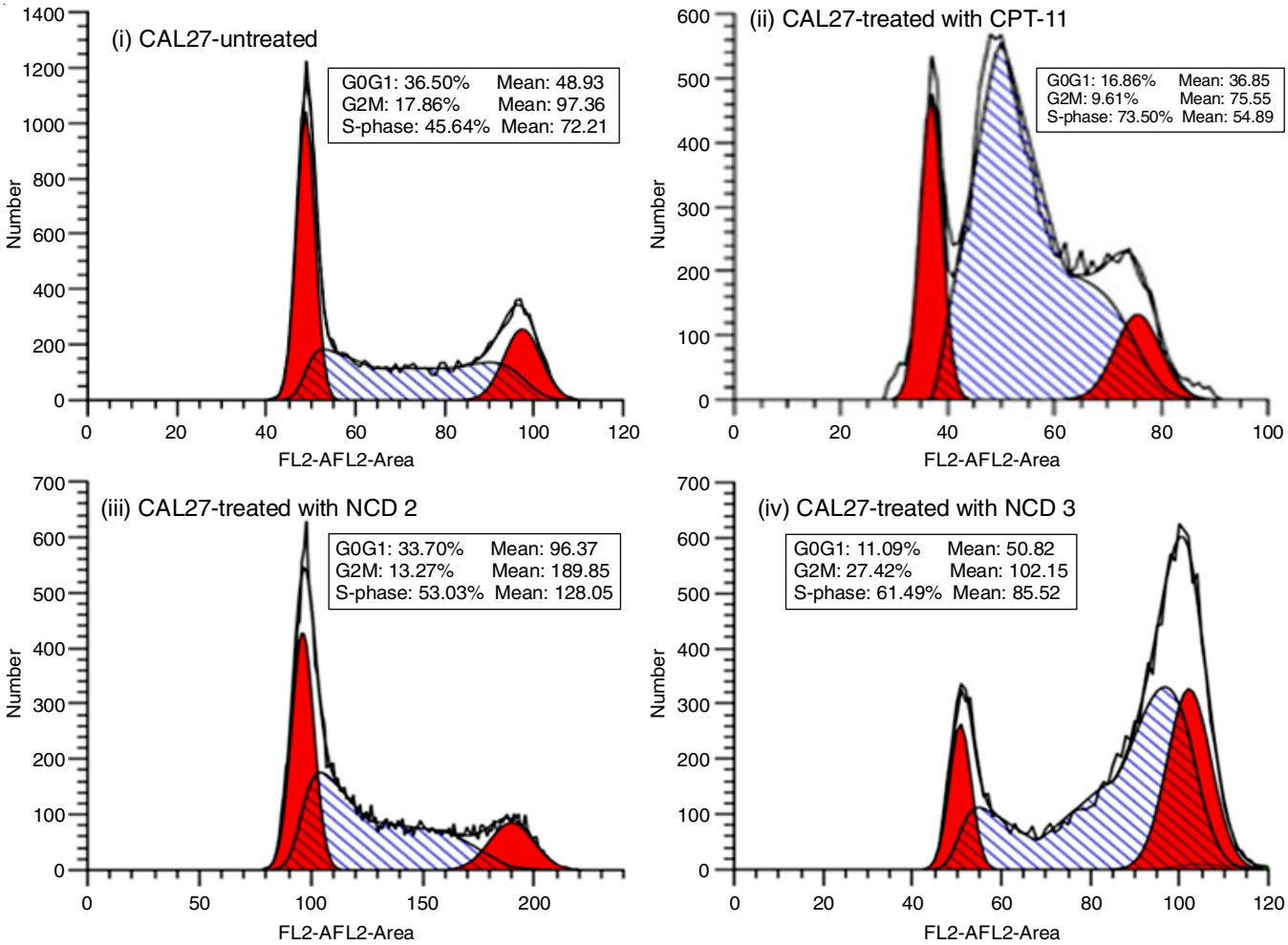


Fig. 4. Histograms for CAL27cell cycle arrest in presence/absence of compounds (CPT-**11**, **2** & **3**)

cycle studies supported the anticancer efficacy of **NCDs 2** & **3** and found that that NCDs offers maximum arrest of cell cycle in S-phase, thereby inhibits the oral squamous cell carcinoma growth. However, **NCDs 2** & **3** should be further investigated for preclinical and clinical significance.

Conclusion

Current investigation successfully synthesized new camptothecin derivatives (NCDs) and structure of synthesized **NCDs** were in agreement with their spectrometric data. Study concludes that NCDs offers significant antiproliferation activity, topoisomerase inhibition and arrest of cell cycle in S-phase against CAL27. Therefore, synthesized **NCDs** are proven as effective anticancer agent for oral squamous cell carcinoma treatment, however, prior preclinical/clinical investigations should be done to establish the safety/efficacy of NCDs in oral squamous cell carcinoma treatment.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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