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1,2,3-triazole tagged 3H-pyrano[2,3-d]pyrimidine-6-carboxylate derivatives: Synthesis, invitro anticancer activity, Molecular Docking and DNA interaction studies

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Abstract: A series of novel ethyl 2,7-dimethyl-4-oxo-3-((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)-4,5-dihydro-3H-pyrano[2,3-d]pyrimidine-6-carboxylate derivatives **7(a-m)** were efficiently synthesized employing click chemistry approach and evaluated for in vitro anti cancer activity against four tumor cell lines: A549 (human lung adenocarcenoma cell line), HepG2 (Human hematoma), MCF-7 (Human breast adenocarcinoma), and SKOV3 (human ovarian carcinoma cell line). Among the compounds tested, the compounds **7a, 7b, 7f, 7l** and **7m** have shown potential and selective activity against human lung adenocarcenoma cell line (A549) with IC_{50} ranging from 0.69 to 6.74 µM. Molecular docking studies revealed that the compounds **7a, 7b, 7f, 7l** and **7m** are potent inhibitors of human DNA topoisomerase-II and also showed compliance with stranded parameters of drug likeness. The calculated binding constants, k_b, from UV-Vis absorptional binding studies of **7a, 7l** with CT-DNA were 10.77 x 10^4, 6.48 x 10^4 respectively. Viscosity measurements revealed that the binding could be surface binding mainly due to groove binding. DNA cleavage study showed that the **7a, 7l** have the potential to cleave pBR322 plasmid DNA without any external agents.

Keywords: Pyrano[2,3-d]pyrimidine, 1,2,3-triazoles, Anti cancer activity, Molecular docking, groove binding

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Introduction

Cancer, the uncontrolled growth of cells, is a major cause of death throughout the world. Every year more than 20% of the population is affected by cancer and the rate of its induction is increasing annually.[1] Although the discovery of anticancer drugs has been an extraordinary challenge, efforts are underway for the acquisition of chemicals that might be useful in the chemotherapy of cancer. A quantum leap in effective cancer chemotherapy requires the discovery and development of new anticancer drugs with unprecedented antitumor activities, specificities, and mechanism of action. Anticancer drugs have well known therapeutic limitations,[2] which have continued to stimulate the search for new agents with enhanced therapeutic efficacy. Henceforth, the augmentation of new anticancer products which are effective and safer from the commercial materials are always on demand. With respect to a recently conducted survey on developing a new pharmacophore model in order to treat any disease or disorder, a complex of two pharmacophores in a single form makes an effective approach in medicinal chemistry. This approach aids in the expedition of highly effective novel compounds. Heterocyclic compounds were found to be crucial in drug design and development[3]. In the past few years, many building blocks have been designed, developed and approved for a range of diseases. Among these, polyfunctionlized pyranopyrimidine derivatives are important synthetic bioactive compounds.

*In the recent years, the synthesis of novel pyranopyrimidine derivatives has gained renewed interest in the area of medicinal chemistry for their wide range of biological activities including anti-tumor,[4] antitubercular,[5] anti-inflammatory,[6] anti-histamine,[7] antimalarial,[8] antigenic,[9] anti-viral,[10] antimicrobial,[11] anti-platelet[12] and hepatoprotective[13] properties. Some of these compounds have also identified as new HA14-1 analogues[14]. Some well-known pyranopyrimidine derivatives (***1, 2****, and* ***3****) were also reported to be antimicrobial agents[15] (***Figure 1****). Similarly, nitrogenous compounds like triazole derivatives have occupied a unique position in heterocyclic chemistry. The 1,2,3-triazoles are of exclusively synthetic origin, remarkably stable and essentially inert to oxidation, reduction and hydrolysis. They have received much attention of researchers because of their high effectiveness, low toxicity and wide applications in medicinal chemistry.[16] 1,2,3-triazole played an important role in agrochemical and pharmaceuticals such as, anti-cancer,[17] anti-inflammatory,[18] antibacterial,[19] antiviral,[20] anti-HIV,[21] fungicidal[22] and insecticidal.[23] For example, the triazole derivative carboxyamidotriazole* **4** *is an angiogenesis inhibitor useful in cancer therapy,[24] Tazobactam* **5** *is a β-lactamase inhibitor used in combination with the β-lactam antibiotic Piperacillin.[25] The 1,4-disubstituted 1,2,3-triazole Rufinamide* **6** *exhibits anticonvulsant activity and has been used to treat childhood mental impairment of the Lennox-syndrome,[26] the cephalosporin analogue, cefatrizine* **7** *is also an antibiotic used in the treatment of bacterial infections of the urinary tract, liver and gallbladder[27] etc (***Figure 1****).*

DNA interaction studies of small molecules are very important in the development of new therapeutic agents.[28] Numerous biological experiments have demonstrated thet DNA is the primary intracellular target of anticancer drugs; interaction between small molecules and DNA can cause damage in cancer cells, blocking the division and resulting in cell death.[29] Many heterocycles exhibited their anticancer activity by inhibiting DNA.[30] Hence, an additional attempt was made to understand the possible molecular action mode of these compounds investigating their interaction with DNA.[31]

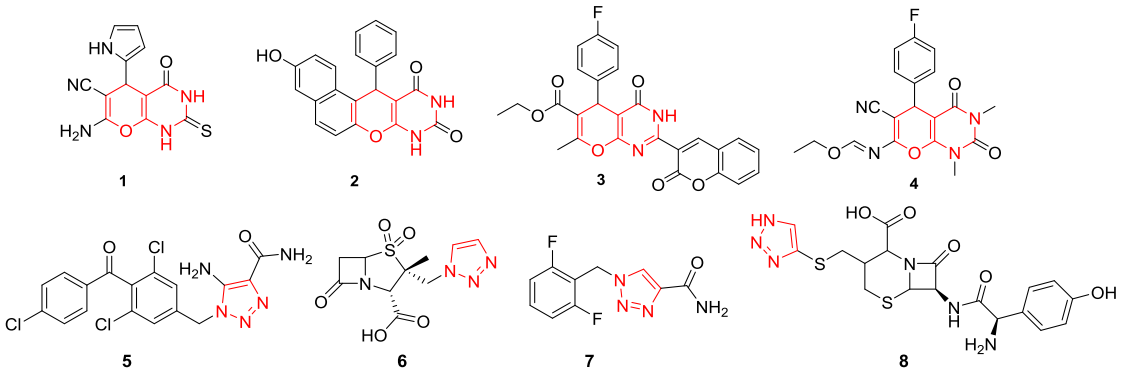


Figure 1. *Selected examples of pyranopyrimidine derivatives with pharmacological activity and structures of some 1,2,3-triazole drugs.*

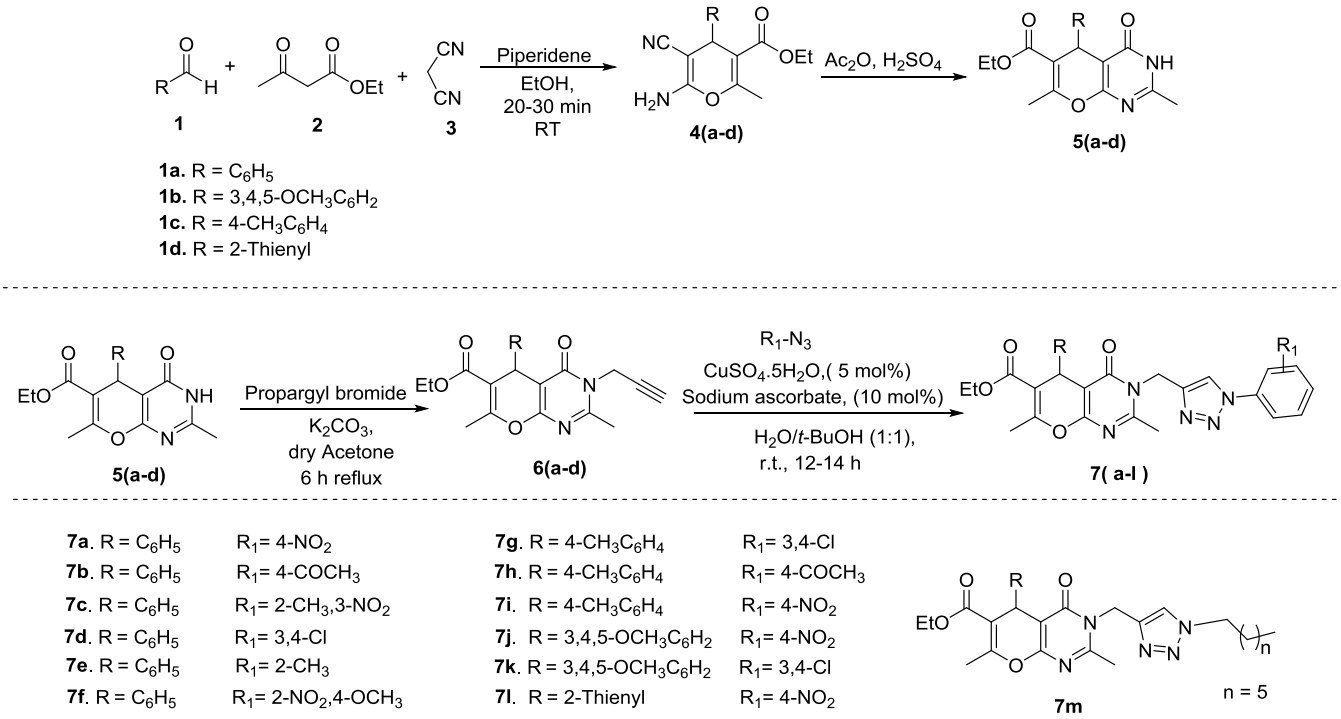
Considering the promising biological potencies of pyranopyrimidine and 1,2,3-triazole pharmacophores, we designed the synthesis of the title compounds by employing various substituents on both the pharmacophores with a presumption that their incorporation in a single structural entity could produce novel compounds. Herein, we report the synthesis of a series of novel ethyl 2,7-dimethyl-4-oxo-3-((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)-4,5-dihydro-3H-pyrano[2,3-d]pyrimidine-6-carboxylate derivatives 7(a-m). We further evaluated their in-vitro anticancer activity against four antitumor cell lines: A549 (human lung adenocarcenoma cell line), HepG2 (Human hematoma), MCF-7 (Human breast adenocarcinoma), and SKOV3 (human ovarian carcinoma cell line) and molecular docking studies with human DNA topoisomerase II.

Results and Discussion

A series of ethyl 2,7-dimethyl-4-oxo-3-((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)-4,5-dihydro-3H-pyrano[2,3-d]pyrimidine-6-carboxylate derivatives **7(a-m)** were synthesized in four steps (**Scheme 1**). In the first step, 4H-pyrans **4(a-d)** were easily obtained via one pot reaction of malononitrile **3**, ethyl aceto acetate **2** and various substituted aromatic aldehydes **1(a-d)** and catalytic amount of piperidine in ethanol stirring for 15-30 min. Compounds **4(a-d)** were used as precursors for the synthesis of pyrano[2,3-d]pyrimidine-4-one derivatives **5(a-d)** by using catalytic amount of sulphuric acid in acetic anhydride under reflux for 10 min. In the third step, pyrano[2,3-d] pyrimidine-4-one derivatives **5(a-d)** were reacted with propargyl bromide in the presence of anhydrous potassium carbonate in dry acetone under reflux for 4-5 h resulting in the formation of corresponding N-propargylated pyrano[2,3-d]pyrimidine-4-one derivatives **6(a-d).** These N-propargyl pyrano[2,3-d]pyridines-4-one derivatives **6(a-d)** were subjected to cycloaddition with substituted aromatic azides and 1-azidohexane under click chemistry reaction conditions in the presence of copper(I) as catalyst in 1:1 water/tert-butanol mixture for 12-14 h to result in novel ethyl 2,7-dimethyl-4-oxo-3-((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)-4,5-dihydro-3H-pyrano[2,3-d] pyrimidine-6-carboxylate derivatives **7(a-l)** and ethyl 3-((1-hexyl-1H-1,2,3-triazol-4-yl)methyl)-2,7-dimethyl-4-oxo-4,5-dihydro-3H-pyrano[2,3-d] pyrimidine-6-carboxylate 7m respectively in quantitative yields. The synthesized 3H-pyrano[2,3-d]pyrimidine-[1,2,3]triazole hybrids were well characterized by ^{1}H NMR, ^{13}C NMR, IR and ESI-MS spectra.

In Vitro anticancer activity

All the synthesised pyrano[2,3-d]pyrimidine-6-carboxylate derivatives were evaluated for anticancer activity in in vitro mode using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay[32] on the A549 (human lung adenocarcenoma cell line), HepG2 (Human hematoma cell line), MCF-7 (Human breast adenocarcinoma cell line), and SKOV3 (human ovarian carcinoma cell line). The results of cytotoxic activity in vitro were expressed as the IC_{50} (μM) and doxorubicin was used as positive control (**Table 1**). The structural diversity in all the derivatives was introduced by varying substitution at 3rd position of the triazole ring and 4th position of pyran ring moiety intact. group. We observed that pyranopyrimidine, aromatic hydrocarbon and nitro group on triazole played an important role. As shown in **Table 1**, most of the compounds were moderately active but, compound **7a, 7b, 7f, 7l** and **7m** showed better inhibition against A549 (IC_{50} 0.69 μM), A549 (IC_{50} 6.74 μM), A549 (IC_{50} 5.76 μM), A549 (IC_{50} 1.85 μM) and A549 (IC_{50} 3.62 μM) cell lines respectively. Structure activity relationship studies revealed that the orientation of substituent on 1,2,3-triazole ring is not only crucial but also required 3H-pyrano[2,3-d]pyrimidine along with a phenyl.



1a. R = C_6H_5
1b. R = 3,4,5-$OCH_3C_6H_2$
1c. R = 4-$CH_3C_6H_4$
1d. R = 2-Thienyl

1 **2** **3** **4(a-d)** **5(a-d)** **6(a-d)** **7(a-l)**

7a. R = C_6H_5 R_1= 4-NO_2
7b. R = C_6H_5 R_1= 4-$COCH_3$
7c. R = C_6H_5 R_1= 2-CH_3,3-NO_2
7d. R = C_6H_5 R_1= 3,4-Cl
7e. R = C_6H_5 R_1= 2-CH_3
7f. R = C_6H_5 R_1= 2-NO_2,4-OCH_3
7g. R = 4-$CH_3C_6H_4$ R_1= 3,4-Cl
7h. R = 4-$CH_3C_6H_4$ R_1= 4-$COCH_3$
7i. R = 4-$CH_3C_6H_4$ R_1= 4-NO_2
7j. R = 3,4,5-$OCH_3C_6H_2$ R_1= 4-NO_2
7k. R = 3,4,5-$OCH_3C_6H_2$ R_1= 3,4-Cl
7l. R = 2-Thienyl R_1= 4-NO_2

7m

Scheme 1. Synthesis of of 3H-pyrano[2,3-d]pyrimidine-[1,2,3]triazole hybrids.

Table 1. Anticancer activity of compounds 7(a-m)

Compound	IC_{50} in µM			
	A549	**HepG2**	**MCF-7**	**SKOV3**
7a	0.69 ± 0.02	>100	71.41 ± 0.02	96.46 ±0.79
7b	6.74 ± 0.11	80.18 ± 0.02	>100	>100
7c	37.02 ± 0.09	>100	99.05 ± 0.01	>100
7d	>100	>100	>100	>100
7e	22.12 ± 0.03	98.71 ± 0.01	>100	81.06 ± 0.01
7f	5.76 ± 0.01	>100	>100	>100
7g	82.43± 0.02	>100	96.46 ± 0.79	90.16 ± 0.02
7h	16.83 ± 0.02	>100	>100	>100
7i	>100	>100	>100	>100
7j	76.74 ± 0.11	83.28 ± 0.08	>100	>100
7l	1.85 ± 0.01	>100	>100	86.46 ± 0.79
7m	3.62 ± 0.02	69.85 ± 0.02	>100	>100
Doxorubicin	0.14 ± 0.002	10.15 ± 0.003	5.73 ± 0.06	16.37 ± 0.02

Molecular docking studies

Exploration of molecular interaction of the title compounds was performed through molecular docking studies using Discovery Studio 2.1 software. To study the molecular interaction of compounds, crystallographic data of human DNA topoisomerase II (PDB: 3QX3) was retrieved from Protein Data Bank. The compounds used in the molecular docking studies were **7a-c, 7f, 7h, 7l, 7m** and Doxorubicin on human DNA topoisomerase II has shown good dock score (LibDock). Dock score and ligand interaction data of above compounds are shown in **Table 2** (Table S1 in supplementary material). The docking study of pyranopyrimidine-triazole derivative revealed the high docking scores and binding affinities in the range of 200.959 to 216.394, as compared to Doxorubicin (212.664) **Figure 2** and (Figure S1 in supplementary information). Compound **7a** was ranked with a docking score of 203.217 which also showed good hydrogen bonding interactions. It also showed some additional interactions with the binding site residues of target protein. The protein–ligand interaction visualization of the compound **7a** is shown in the **Figure 3**. This compound was docked into the active site region making three hydrogen bond interactions with the residues ARG503 and DG13. Two hydrogen bonds are formed with ARG503 and one with DG13. The NH1 of the residue arginine503 interacted with O3 and N7 of the compound 7a (ARG503:NH1–7a:O3 and ARG503:NH1–7a:N7) with hydrogen bond distances of 3.176 Å and 3.123 Å respectively. The N1 atom of the nucleotide residue DG13 interacted with the N21 atom of the compound 7a with a hydrogen bond distance of 3.101 Å. Also it formed some additional interactions with the nucleotide residue DG13. The compound 7l was docked into the active site region making two hydrogen bond interactions with the nucleotide residues DT9 and DC8 having dock score 200.959. One hydrogen bond was formed by the nitrogen atom of DT9 interacted with the oxygen atom of the compound 7l (DT9:N3–7l:O15) with a hydrogen bond distance of 2.942 Å. The second hydrogen bond interaction was formed with the N4 atom of the nucleotide DC8 interacted with the N7 atom of the compound **7l** (DC8:N4–7l:N7) with a hydrogen bond distance of 2.611 Å. These results indicate that most of the compounds bound within the binding site pocket of doxorubicin similar binding pattern with binding site amino acid residues. The significant docking score on the target infers that these compounds are promiscuous and can be potential leads against cancer.

Table 2. *Details of LibDock score, ligand interaction data and hydrogen bond distance revealed through molecular docking of compounds 7a and 7l on human DNA topoisomerase II (PDB: 3QX3).*

Compound	LibDock score	Interacting amino acids/ Nucleotides	Interacting atoms	donor	acceptor	Bond Distance (A°)
7a	*203.217*	*DG13, ARG503, DA12*	*F:DG13:N1 - 7a:N21*	*N1*	*N21*	*3.101000*
			A:ARG503:NH1 -7a:O3	*NH1*	*O3*	*3.176000*
			A:ARG503:NH1 -7a:N7	*NH1*	*N7*	*3.123000*
			7a:H45- F:DA12:C2'	*H45*	*C2'*	*2.207000*
7l	*200.959*	*DT9, DC8*	*D:DT9:N3 - 7l:O15*	*N3*	*O15*	*2.942000*
			C:DC8:N4 - 7l:N7	*N4*	*N7*	*2.611000*
			7l:H39 - D:DT9:O2	*H39*	*O2*	*2.040000*

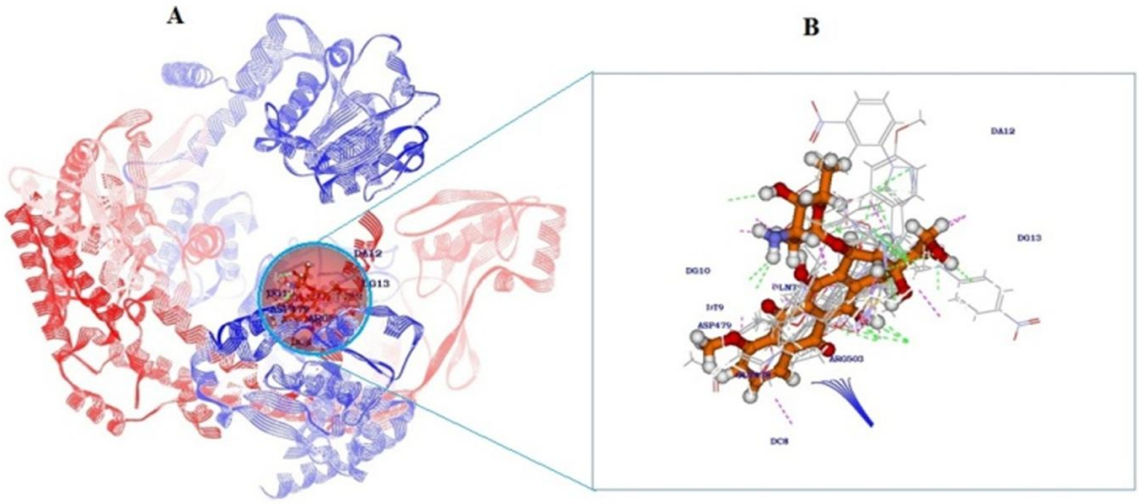


Figure 2. *(**A**) Structural model of DNA topoisomerase II (PDB: 3QX3) with Doxorubicin binding site (sphere); (**B**) Binding site and binding pattern of candidate compound along with control.*

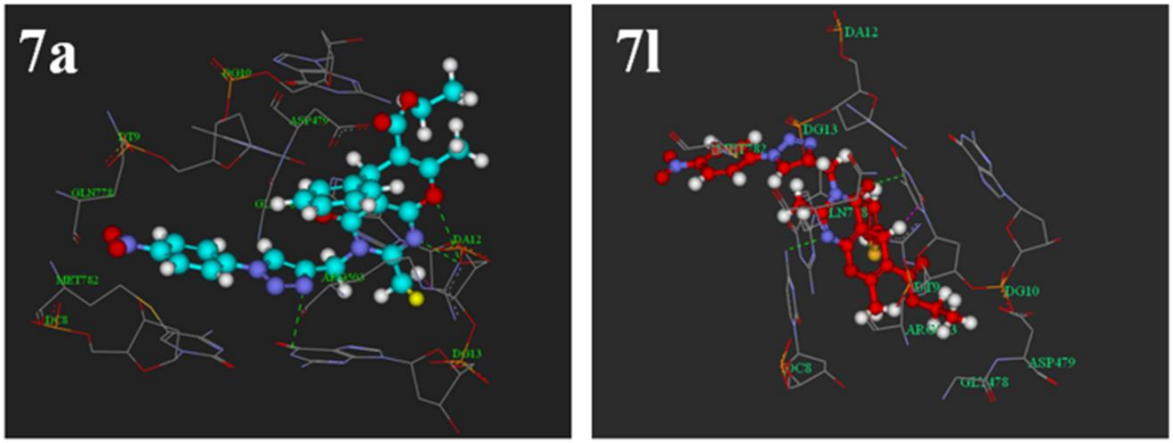


Figure 3. Receptor-ligand hydrogen bonds (green colour) and bumps (pink colour) of compounds **7a** and **7l** with active site residues of Human DNA topoisomerase II (PDB: 3QX3).

Bioavailability and drug likeness screening

Different chemical descriptors for the pharmacokinetics properties were calculated to check the compliance of studied compound with the standard range. For this the absorption level, blood-brain barrier penetration, aqueous solubility, hepatotoxicity, cytochrome P450 2D6 binding. The pharmacokinetic profiles of all the compounds under investigation were predicted by means of Discovery Studio 2.1 ADMET models and result provided in Table S2 in the supplementary

information. The aqueous solubility prediction (defined in water at 25 ^{0}C) indicated that all the compounds are soluble in water. All the compounds are found to be non- inhibitors of cytochrome P450 2D6 (CYP2D6). The CYP2D6 enzyme is one of the important enzymes involved in drug metabolism. During ADMET screening, the predictive hepatotoxicity was observed for the candidate compounds in comparison to doxorubicin (toxic).

DNA interaction studies

To further substantiate the anticancer activity data and evaluate the potential anti-tumor targets of the synthesized 3H-pyrano[2,3-d]pyrimidine-[1,2,3]triazole hybrids, UV-Vis absorption, viscosity studies were performed to ascertain the DNA binding properties of the compounds, **7a, 7l** *with CT-DNA. In order to investigate the DNA-binding properties of the synthesized compounds, the UV-Vis absorption spectra of the active compounds* **7a** *and* **7l** *were evaluated based on their interaction with CT-DNA. These studies were performed in the absence and presence of increasing concentrations of CT-DNA as shown in* **Figure 4**.

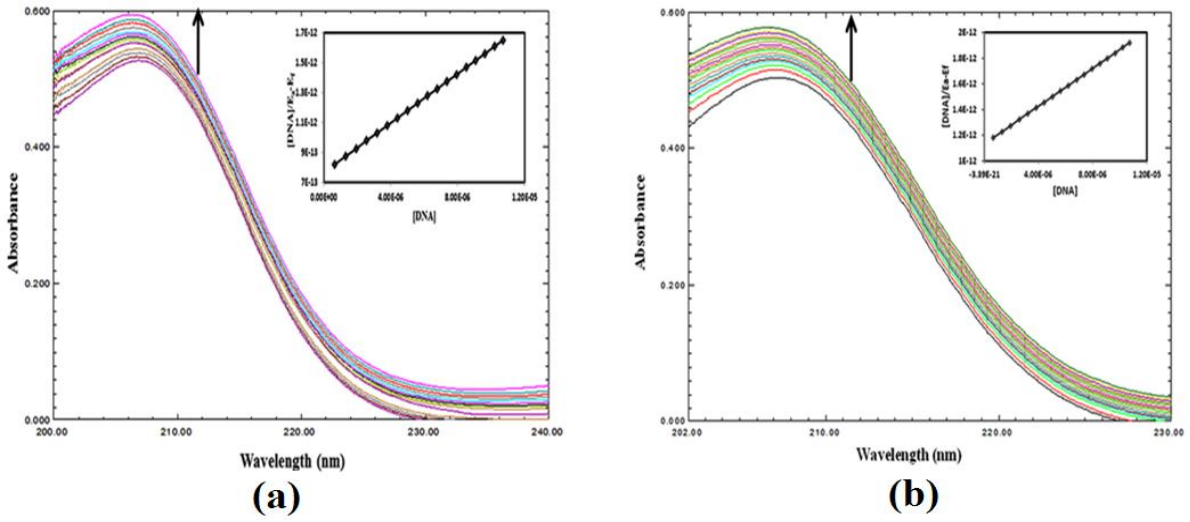


Figure 4. *Absorption spectra of 7a (a), 7l (b) in tris-buffer upon addition of CT-DNA in absence (lower) and presence of CT-DNA (top) the [compound] = 2 µM. [DNA] = 0–10 µM. Inset plot: of [DNA]/(Ea- Ef) versus [DNA]. Arrow shows change in absorption with increasing DNA concentrations.*

7a *and* **7l** *exhibited maximum absorption at 207.15 and 206.35 nm respectively. Addition of increasing amounts of CT-DNA shows hyperchromism effect, suggesting that there exists a strong interaction between the compound and DNA which is different from the classical intercalation binding and can be rationalised in terms of groove binding[33]. From UV-Visible titration data, the* **7a** *compound-CT-DNA and* **7l** *compound-CT-DNA binding constants, k_b, were found to be, 10.77 x 10^4, 6.48 x 10^4 respectively, comparable with that of the well-established groove binding agent spermine.[34] A classical intercalation model demands that the DNA helix must lengthen as base pairs are separated to accommodate the binding ligand, leading to increase in DNA viscosity.[35] In contrast, a partial and/or non-classical intercalation could bend (or kink) the DNA helix, reduce its effective length and concomitantly its viscosity, while compounds that bind exclusively in the DNA grooves (e.g., netropsin, distamycin), under the same conditions, typically cause less pronounced changes (positive or negative) or no changes in DNA solution viscosity.[36] The slow increase in relative viscosity of CT-DNA with increasing amount of* **7a, 7l** (**Figure 5**) *proves the groove binding.[37] In principle, this could be explained by changes in conformation, flexibility or solvation of the DNA molecule. This behavior is similar to the Metformin-DNA binding studies reported by Nahid Shahabadi et.al.[33]*

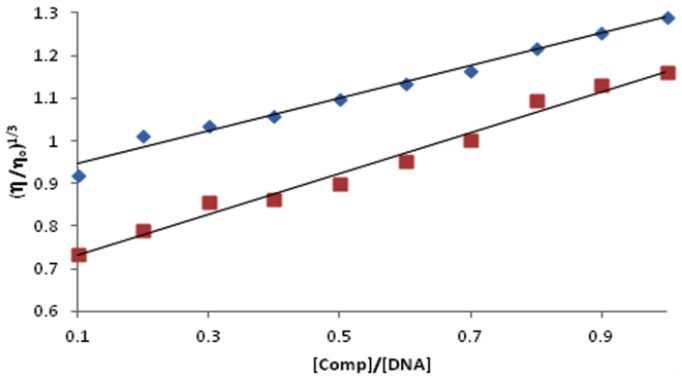


Figure 5. *Effect of increasing amount of compounds 7a (blue), 7l (red) on the relative viscosities of CT-DNA at room temperature in 5 mM Tris–HCl buffer.*

The photo induced cleavage of super coiled pBR322 DNA by **7a, 7l** was studied upon irradiation with UV light at 365nm. The extent of cleavage was monitored by agarose gel electrophoresis. When circular plasmid DNA is subjected to electrophoresis, relatively fast migration will be observed for the intact super coiled form (Form I). If scission occurs on one strand (nicked), the super coiled will relax to generate a slower-moving open circular form (Form II)[38]. If both strands are cleaved, a linear form (Form III) that migrates between Form I and II will be generated. It was observed that the compounds **7a, 7l** promoted DNA cleavage upon irradiation. At 50 µM concentration, both were able to partially transform supercoiled DNA (Form I) into open circular DNA (Form II) and to a much lesser extent to linear DNA (Form III). At 100 µM concentration, both were able to completely transform supercoiled DNA (Form I) into open circular DNA (Form II) and to a much lesser extent to linear DNA (Form III). Both compounds behave as DNA photocleavers (**Figure 6**).

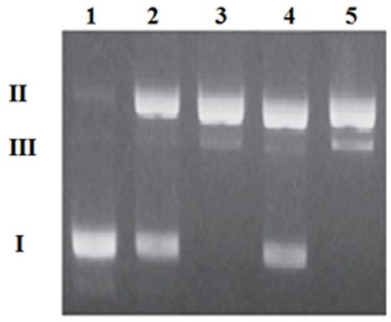


Figure 6. *Photo cleavages of DNA by 1,2,3-triazole incorporating substituted pyranopyrimidine derivatives were irradiated with UV light at 365 nm. Lane 1: control DNA (without compound), Lane 2: 50 µM (7a) and Lane 3: 100 µM (7a), Lane 4: 50 µM (7l), Line 5: 100 µM (7l). Supercoiled DNA runs at position I, nicked coiled DNA at position II, and linear coiled DNA at position III.*

Conclusions

In conclusion, we have synthesized a novel series of 1,2,3-triazole tagged 3H-pyrano[2,3-d]pyrimidine-6-carboxylate derivatives by adopting click chemistry approach. In addition, all the synthesized compounds were tested for their anticancer activity. Most of these compounds exhibited significant cytotoxicity in the micromolar range against the human lung adenocarcenoma cell line. Among those, 7a, 7b, 7f, 7l and 7m compounds have exhibited cytotoxicity with IC50 value ranging from 0.69 to 6.74 µM in tested cell lines. Further, the DNA binding potentiality of these active compounds 7a and 7l were evaluated through UV-vis spectroscopic titration studies. The photo induced cleavage of super coiled pBR322 DNA by 7a, 7l were also studied. In addition, Molecular docking of synthesized derivatives 7a-7c, 7f, 7h, 7l and 7m with human DNA topoisomerase II revealed the LibDock score in the range of 200.959 to 216.394 showed compliance with stranded parameters of drug likeness. Based on the above experimental results it is evident that these compounds have the potential to be developed as a new class of cancer therapeutics.

Experimental Section

Chemistry

Melting points of all the compounds were recorded on Casia-Siamia (VMP-AM) melting point apparatus and uncorrected. IR spectra were recorded on a Perkin –Elmer FT-IR 240-C spectrometer using KBr optics. ^{1}H-NMR spectra were recorded on Burker Avance 400 MHz in $CDCl_3$ using TMS as an internal standard. Electron impact (EI) and chemical ionization mass spectra were recorded on a VG 7070 H instrument at 70 ev. All the reactions were monitored by thin layer chromatography (TLC) on precoated M/s Merck silica gel 60 F254 (mesh); spots were visualized with UV light. Silica gel (100-200 mesh) used for column chromatography was procured from Merck.

*General procedure for the synthesis of 4H-pyrans **4(a-d)***

A mixture of aromatic aldehyde 1(a-d) (5 mmol), ethyl acetoacetate 2 (5 mmol), malononitrile 3 (5 mmol) and the catalytic quantity of piperidine in ethanol (5 ml) was stirred at room temperature for 20-30 min. After completion of reaction (monitored by TLC) the separated product was filtered off, washed with methanol.

*General procedure for the synthesis of pyrano[2,3-d]pyrimidines **5(a-d)***

4H-pyran 4(a-d) (5 mmol), acetic anhydride (5 ml), and concentrated sulfuric acid (0.5 mmol) were mixed. The reaction mixture obtained was refluxed for 10 min, cooled to room temperature and kept for 1 day. A precipitate formed was filtered off, washed with water (3×5 ml), ethanol (5 ml), and light petroleum (5 ml). The product obtained was recrystallized from ethanol and dried at 120 ^{0}C until the weight became constant.

General procedure for Synthesis of N-propargyl pyrano[2,3-d]pyrimidine-4-one derivatives ***6(a-d)***

Compound 5(a-d) (0.02 mol) was dissolved in dry acetone (25 ml), anhydrous potassium carbonate (0.04 mol) was added. Then, propargyl bromide (0.03 mol) was added slowly while stirring. The reaction mixture was refluxed for 1-2 hours. After completion of reaction (monitored by TLC), the solvent was removed under reduced pressure and the residue was diluted with ice cold distilled water and extracted with chloroform, the organic layer was dried over anhydrous Na_2SO_4 and concentrated to get the product 6(a-d). The crude product was purified by column chromatography.

Ethyl 2,7-dimethyl-4-oxo-3-(prop-2-yn-1-yl)-5-(p-tolyl)-4,5-dihydro-3H-pyrano[2,3-d]pyrimidine-6-carboxylate ***(6c)***

White solid, Yield: 90%, M.p. 140–142 ^{0}C, ^{1}H NMR (400 MHz, $CDCl_3$): 7.22 (d, J = 8.1 Hz, 2H, Ar-H); 7.06 (d, J = 7.8 Hz, 2H, Ar-H); 4.98 (dd, J = 16.8, 3.2 Hz, 2H, CH_2); 4.49 (s, 1H, H of pyran ring); 4.06 (q, J = 7.1 Hz, 2H, CH_2); 2.63 (s, 3H, CH_3); 2.49 (s, 3H, CH_3); 2.27 (s, 3H, CH3); 2.26 (s, 1H, ≡CH); 1.18 (t, J = 7.1 Hz, 3H, CH_3); 13C NMR (100 MHz, CDCl3): 166.2; 160.9; 158.6; 158.4; 158.1; 140.7; 136.4; 128.9; 128.4; 108.6; 101.9; 76.5; 73.0; 60.5; 36.6; 33.2; 22.5; 21.1; 18.8; 14.0.

General procedure for synthesis of ethyl 2,7-dimethyl-4-oxo-3-((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)-4,5-dihydro-3H-pyrano[2,3-d] pyrimidine-6-carboxylate derivatives ***7(a-m)***

Compound 6(a-d) (3 mmol) and substituted aromatic azides/1-azidohexane (3 mmol) were suspended in 12 ml of a 1:1 water/tert-butanol mixture. Aqueous solution of Sodium ascorbate (0.3 mmol) was added, followed by the addition of aqueous solution of copper (II) sulfate pentahydrate (0.03 mmol). The heterogeneous mixture was stirred vigorously overnight, at which point it cleared and TLC analysis indicated complete consumption of the reactants. The reaction mixture was diluted with 50 ml of water and cooled in ice, and the precipitate was collected by filtration. After being washed with cold water (2- 25 ml), the precipitate was dried under vacuum to afford pure product as a powder.

Ethyl 2,7-dimethyl-3-((1-(4-nitrophenyl)-1H-1,2,3-triazole-4-yl)methyl)-4-oxo-5phenyl-4,5-dihydro-3H pyrano [2,3-d]pyrimidine-6-carboxylate ***(7a)***

Yellow solid, Yield: 91%, M.p.135–137 ^{0}C, IR (KBr) cm^{-1} 3088, 2980, 1709, 1672, 1559, 1428, 1244. ^{1}H NMR (400 MHz, $CDCl_3$): 8.41 (d, J = 4.4 Hz, 2H, Ar-H); 8.16 (s, 1H, =CH of triazole ring); 7.88 (d, J = 8.6 Hz, 2H, Ar-H); 7.34 (d, J = 8.6 Hz, 2H, Ar-H); 7.30 – 7.21 (m, 2H, Ar-H); 7.18 (t, J = 7.3 Hz, 1H, Ar-H); 5.35 (s, 2H, N-CH_2); 4.99 (s, 1H, H of pyran ring); 4.07 (q, J = 7.1 Hz, 2H, CH_2); 2.84 (s, 3H, CH_3); 2.49 (s, 3H, CH_3); 1.17 (t, J = 7.1 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): 166.1; 161.9; 161.7; 158.5; 147.4; 146.9; 143.7; 143.1; 140.8; 128.6; 128.2; 125.5; 122.4; 122.2; 120.6; 108.3; 101.9; 60.6; 39.9; 37.1; 23.3; 18.8; 14.1. MS (ESI) (m/z) (M+1) calculated for $C_{27}H_{24}N_6O_6$, 528.18, found 529.30.

Ethyl 3-((1-(4-acetylphenyl)-1H-1,2,3-triazol-4-yl)methyl)-2,7-dimethyl-4-oxo-5-phenyl-4,5-dihydro-3H-pyrano[2,3-d]pyrimidine-6-carboxylate ***(7b)***

Yellow solid, Yield: 86%, M.p. 140–141 ^{0}C, IR(KBr) cm^{-1} 3025, 2976, 1712, 1676, 1559, 1428, 1242. ^{1}H NMR (400 MHz, $CDCl_3$): 8.11 (d, J = 4.4 Hz, 2H, Ar-H); 8.10 (s, 1H, =CH of triazole ring); 7.78 (d, J = 8.6 Hz, 2H, Ar-H); 7.38 – 7.26 (m, 4H, Ar-H); 7.18 (t, J = 7.3 Hz, 1H, Ar-H); 5.35 (s, 2H, N-CH_2); 4.99 (s, 1H, H of pyran ring); 4.07 (q, J = 7.1 Hz, 2H, CH_2); 2.84 (s, 3H, CH_3); 2.66 (s, 3H, CH_3); 2.49 (s, 3H, CH_3); 1.17 (t, J = 7.1 Hz, 3H, CH_3). MS (ES+) (m/z) (M+1) calculated for $C_{29}H_{27}N_5O_5$, 525.20, found 526.

Ethyl 2,7-dimethyl-3-((1-(2-methyl-3-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)-4-oxo-5-phenyl-4,5-dihydro-3H-pyrano[2,3-d]pyrimidine-6-carboxylate ***(7c)***

Yellow solid, Yield: 88%, M.p. 122–124 ^{0}C, IR (KBr) cm^{-1} 2981, 2928, 1707, 1672, 1558, 1429, 1245. ^{1}H NMR (400 MHz, $CDCl_3$): 8.03 (t, J = 7.1 Hz, 1H, Ar-H); 7.86 (s, 1H, =CH of triazole ring); 7.51 (d, J = 4.6 Hz, 2H, Ar-H); 7.38 – 7.33 (m, 1H, Ar-H); 7.32 (d, J = 7.3 Hz, 2H, Ar-H); 7.23 (d, J = 7.6 Hz, 1H, Ar-H); 7.16 (d, J = 7.1 Hz, 1H, Ar-H); 5.33 (s, 2H, N-CH2); 4.96 (s, 1H, H of pyran ring); 4.06 (q, J = 7.1 Hz, 2H, CH2); 2.87 (s, 3H, CH3); 2.49 (s, 3H, CH3); 2.18 (s, 3H, CH3); 1.15 (t, J = 7.1 Hz, 3H, CH3). ^{13}C NMR (100 MHz, $CDCl_3$): 166.1; 161.8; 158.7; 158.6; 151.8; 143.7; 143.3; 142.6; 137.8; 130.5; 129.4; 128.5; 128.2; 127.3; 126.9; 126.3; 125.9; 108.4; 101.8; 60.6; 39.8; 37.1; 23.4; 18.8; 14.5; 14.1. MS (ES+) (m/z) (M+1) calculated for $C_{28}H_{26}N_6O_6$, 542.19, found 543.47.

Ethyl 3-((1-(3,4-dichlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-2,7-dimethyl-4-oxo-5-phenyl-4,5-dihydro-3H-pyrano[2,3-d]pyrimidine-6-carboxylate ***(7d)***

Yellow solid, Yield: 85%, M.p. 143–145 ^{0}C, IR (KBr) cm^{-1} 3085, 2930, 1710, 1672, 1559, 1429, 1245, ^{1}H NMR (400 MHz, $CDCl_3$): 8.00 (s, 1H, =CH of triazole ring); 7.75 (d, J = 7.3 Hz, 1H, Ar-H); 7.57 (d, J = 7.3 Hz, 1H, Ar-H); 7.55 – 7.25 (m, 5H, Ar-H); 7.20 (s, 1H, Ar-H); 5.32 (s, 2H, N-CH_2); 4.99 (s, 1H, H of pyran ring); 4.07 (q, J = 7.1 Hz, 2H, CH_2); 2.82 (s, 3H, CH_3); 2.49 (s, 3H, CH_3); 1.17 (t, J = 7.1 Hz, 3H, CH_3). MS (ES+) (m/z) (M+1) calculated for $C_{27}H_{23}Cl_2N_5O_4$, 551.11, found 552.

Ethyl 2,7-dimethyl-4-oxo-5-phenyl-3-((1-(o-tolyl)-1H-1,2,3-triazol-4-yl)methyl)-4,5-dihydro-3H-pyrano[2,3-d] pyrimidine-6-carboxylate ***(7e)***

Yellow solid, Yield: 82 %, M.p.120–122 ^{0}C, IR (KBr) cm^{-1} 3072, 2929, 1705, 1670, 1557, 1423, 1238. ^{1}H NMR (400 MHz, $CDCl_3$): 7.99 (s, 1H, =CH of triazole ring); 7.51 (d, J = 8.4 Hz, 2H, Ar-H); 7.34 (d, J = 7.2 Hz, 2H, Ar-H); 7.30 – 7.30 (m, 3H, Ar-H); 7.23 (d, J = 10.9 Hz, 1H, Ar-H); 7.18 (d, J = 7.2 Hz, 1H, Ar-H); 5.35 (s, 2H, N-CH_2); 4.99 (s, 1H, H of pyran ring); 4.07 (q, J = 7.1 Hz, 2H, CH_2); 2.83 (s, 3H, CH_3); 2.49 (s, 3H, CH_3); 2.42 (s, 3H, CH_3); 1.17 (t, J = 7.1 Hz, 3H, CH_3). MS (ESI) (m/z) (M+1) calculated for $C_{28}H_{27}N_5O_4$, 497.21, found 498.10.

Ethyl 3-((1-(4-methoxy-2-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)-2,7-dimethyl-4-oxo-5-phenyl-4,5-dihydro-3H-pyrano[2,3-d]pyrimidine-6-carboxylate ***(7f)***

Yellow solid, Yield: 86%, M.p.125–126 ^{0}C, IR (KBr) cm^{-1} 3023, 2977, 1709, 1672, 1541, 1428, 1244. ^{1}H NMR (400 MHz, $CDCl_3$): 8.05 (t, J = 7.1 Hz, 1H, Ar-H); 7.86 (s, 1H, =CH of triazole ring); 7.51 (d, J = 4.6 Hz, 1H, Ar-H); 7.38 – 7.33 (m, 1H, Ar-H); 7.32 (d, J = 7.3 Hz, 2H, Ar-H); 7.23 (d, J = 7.6 Hz, 2H, Ar-H); 7.16 (t, J = 7.1 Hz, 1H, Ar-H); 5.18 (s, 2H, N-CH_2); 4.96 (s, 1H, H of pyran ring); 4.06 (q, J = 7.1 Hz, 2H, CH_2); 3.59 (s, 3H, OCH3); 2.87 (s, 3H, CH_3); 2.49 (s, 3H, CH_3); 1.15 (t, J = 7.1 Hz, 3H, CH_3). MS (ESI) (m/z) (M+1) calculated for $C_{28}H_{26}N_6O_7$, 558.19, found 559.

Ethyl 3-((1-(3,4-dichlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-2,7-dimethyl-4-oxo-5-(p-tolyl)-4,5-dihydro-3H-pyrano[2,3-d]pyrimidine-6-carboxylate ***(7g)***

Yellow solid, Yield: 81%, M.p.135–136 ^{0}C, IR (KBr) cm^{-1} 3050, 2977, 1710, 1674, 1559, 1428, 1241. ^{1}H NMR (400 MHz, $CDCl_3$): 8.07 (s, 1H, =CH of triazole ring); 7.84 (s, 1H, Ar-H); 7.58 (t, J = 11.5 Hz, 1H, Ar-H); 7.41 (d, J = 8.8 Hz, 1H, Ar-H); 7.22 (d, J = 7.9 Hz, 2H, Ar-H); 7.07 (d, J = 7.7 Hz, 2H, Ar-H); 5.23 (d, J = 10.2 Hz, 2H, N-CH_2); 4.95 (s, 1H, H of pyran ring); 4.07 (q, J = 7.1 Hz, 2H, CH_2); 2.82 (s, 3H, CH_3); 2.47 (s, 3H, CH_3); 2.28 (s, 3H, CH_3); 1.19 (t, J = 5.6 Hz, 3H, CH_3). MS (ESI) (m/z) (M+1) calculated for $C_{28}H_{25}Cl_2N_5O_4$, 565.13, found 566.42.

Ethyl 3-((1-(4-acetylphenyl)-1H-1,2,3-triazol-4-yl)methyl)-2,7-dimethyl-4-oxo-5-(p-tolyl)-4,5-dihydro-3H-pyrano[2,3-d]pyrimidine-6-carboxylate ***(7h)***

Yellow solid, Yield: 85%, M.p.142–143 ^{0}C, IR (KBr) cm^{-1} 2977, 2927, 1710, 1674, 1558, 1428, 1241. ^{1}H NMR (400 MHz, $CDCl_3$): 8.16 (s, 1H, =CH of triazole ring); 8.11 (d, J = 6.4 Hz, 2H, Ar-H); 7.81 (d, J = 8.6 Hz, 2H, Ar-H); 7.27 (d, J = 8.1 Hz, 2H, Ar-H); 7.08 (d, J = 8.1 Hz, 2H, Ar-H); 5.38 (s, 2H, N-CH_2); 4.95 (s, 1H, H of pyran ring); 4.07 (q, J = 7.1 Hz, 2H, CH_2); 2.83 (s, 3H, CH_3); 2.66 (s, 3H, CH_3); 2.47 (s, 3H, CH_3); 2.28 (s, 3H, CH_3); 1.17 (t, J = 7.1 Hz, 3H, CH_3). MS (ESI) (m/z) (M+1) calculated for $C_{30}H_{29}N_5O_5$, 539.22, found 540.10.

Ethyl 2,7-dimethyl-3-((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)-4-oxo-5-(p-tolyl)-4,5-dihydro-3H-pyrano[2,3-d]pyrimidine-6-carboxylate **(7i)**

Yellow solid, Yield: 90%, M.p.132–133 ^{0}C, IR (KBr) cm^{-1} 3093, 2981, 1712, 1671, 1558, 1429, 1241. ^{1}H NMR (400 MHz, $CDCl_3$): 8.41 (d, J = 8.9 Hz, 2H, Ar-H); 8.20 (s, 1H, =CH of triazole ring); 7.91 (d, J = 8.9 Hz, 2H, Ar-H); 7.22 (d, J = 7.8 Hz, 2H, Ar-H); 7.08 (d, J = 7.5 Hz, 2H, Ar-H); 5.38 (s, 2H, N-CH2); 4.95 (s, 1H, H of pyran ring); 4.07 (q, J = 7.1 Hz, 2H, CH_2); 2.83 (s, 3H, CH_3); 2.47 (s, 3H, CH_3); 2.28 (s, 3H, CH_3); 1.19 (t, J = 5.8 Hz, 3H, CH_3). MS (ESI) (m/z) (M+1) calculated for $C_{28}H_{26}N_6O_6$, 542.19, found 543.47.

*Ethyl 2,7-dimethyl-3-((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)-4-oxo-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-3H-pyrano[2,3-d]pyrimidine-6-carboxylate (**7j**)*

Yellow solid, Yield: 88%, M.p.202–203 ^{0}C, IR (KBr) cm^{-1} 3095, 2971, 1716, 1672, 1559, 1425, 1233. ^{1}H NMR (400 MHz, $CDCl_3$): 8.42 (d, J = 9.1 Hz, 2H, Ar-H); 8.16 (s, 1H, =CH of triazole ring); 7.89 (d, J = 9.1 Hz, 2H, Ar-H); 6.55 (s, 2H, Ar-H); 5.39 (s, 2H, N-CH_2); 4.96 (s, 1H, H of pyran ring); 4.13 (q, J = 7.1 Hz, 2H, CH_2); 3.81 (s, 6H, OCH_3); 3.79 (s, 3H, OCH_3); 2.84 (s, 3H, CH_3); 2.49 (s, 3H, CH_3); 1.22 (t, J = 7.1 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): 166.1; 161.9; 158.9; 158.5; 152.9; 147.4; 143.7; 140.8; 139.3; 137.0; 125.6; 122.3; 120.6; 108.4; 105.6; 101.7; 60.7; 60.7; 56.1; 39.9; 37.2; 23.3; 18.8; 14.2. MS (ESI) (m/z) (M+1) calculated for $C_{30}H_{30}N_6O_9$, 618.21, found 619.54.

Ethyl 3-((1-(3,4-dichlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-2,7-dimethyl-4-oxo-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-3H-pyrano[2,3-d]pyrimidine-6-carboxylate **(7k)**

Yellow solid, Yield: 85%, M.p.199–200 ^{0}C, IR (KBr) cm^{-1} 2975, 2942, 1711, 1672, 1559, 1424, 1236. ^{1}H NMR (400 MHz, $CDCl_3$): 8.84 (s, 1H, Ar-H); 8.25 (s, 1H, =CH of triazole ring); 7.95 (t, J = 9.1 Hz, 2H, Ar-H); 7.88 (t, J = 9.1 Hz, 2H, Ar-H); 6.45 (s, 2H, Ar-H); 5.38 (s, 2H, N-CH_2); 4.81 (s, 1H, H of pyran ring); 4.07 (q, J = 7.1 Hz, 2H, CH_2); 3.68 (s, 6H, OCH_3); 3.34 (s, 3H, OCH_3); 2.65 (s, 3H, CH_3); 2.41 (s, 3H, CH_3); 1.15 (t, J = 7.1 Hz, 3H, CH_3). MS (ESI) (m/z) (M+1) calculated for $C_{30}H_{29}Cl_2N_5O_7$, 641.14, found 642.

Ethyl 2,7-dimethyl-3-((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)-4-oxo-5-(thiophen-2-yl)-4,5-dihydro-3H-pyrano[2,3-d]pyrimidine-6-carboxylate **(7l)**

Yellow solid, Yield: 89%, M.p.136–137 ^{0}C, IR (KBr) cm^{-1} 3087, 2973, 1715, 1672, 1557, 1429, 1224. ^{1}H NMR (400 MHz, $CDCl_3$): 8.42 (d, J = 8.8 Hz, 2H, Ar-H); 8.26 (s, 1H, =CH of triazole ring); 7.93 (d, J = 8.9 Hz, 2H, Ar-H); 7.12 (d, J = 4.8 Hz, 1H, Ar-H); 6.93 (dd, J = 14.3, 10.4 Hz, 1H, Ar-H); 6.87 (d, J = 4.8 Hz, 1H, Ar-H); 5.40 (s, 2H, N-CH_2); 5.23 (s, 1H, H of pyran ring); 4.16 (q, J = 7.1 Hz, 2H, CH_2); 2.86 (s, 3H, CH_3); 2.48 (s, 3H, CH_3); 1.23 (t, J = 7.1 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): 165.9; 161.8; 158.9; 158.8; 155.9; 147.5; 143.7; 142.9; 140.8; 126.6; 125.5; 125.1; 124.2; 122.6; 120.6; 108.1; 101.3; 60.8; 39.9; 31.7; 23.4; 18.8; 14.1. MS (ESI) (m/z) (M+1) calculated for $C_{25}H_{22}N_6O_6S$, 534.13, found 535.20.

*Ethyl 3-((1-hexyl-1H-1,2,3-triazol-4-yl)methyl)-2,7-dimethyl-4-oxo-5-phenyl-4,5-dihydro-3H-pyrano[2,3-d] pyrimidine-6-carboxylate (**7m**)*

Yellow solid, Yield: 81%, M.p.114–116 ^{0}C, IR (KBr) cm^{-1} 3021, 2927, 1713, 1665, 1555, 1433, 1216. ^{1}H NMR (400 MHz, $CDCl_3$): 7.57 (s, 1H, =CH of triazole ring); 7.33 (d, J = 7.3 Hz, 2H, Ar-H); 7.22 (d, J = 13.0 Hz, 2H, Ar-H); 7.17 (t, J = 7.2 Hz, 1H, Ar-H); 5.27 (s, 2H, N-CH_2); 4.97 (s, 1H, H of pyran ring); 4.26 (dt, J = 11.4, 6.6 Hz, 2H, CH_2); 4.07 (q, J = 7.1 Hz, 2H, CH_2); 2.80 (s, 3H, CH3); 2.48 (s, 3H, CH_3); 1.86 – 1.78 (m, 2H); 1.32 – 1.22 (m, 10H); 1.17 (t, J = 7.1 Hz, 3H, CH_3); 0.88 (t, J = 6.8 Hz, 3H, CH_3). MS (ESI) (m/z) (M+1) calculated for $C_{29}H_{37}N_5O_4$, 519.20, found 520.20.

Biology

Anticancer activity

Cytotoxicity of formulations was assessed using MTT assay to determine the cell viability according to a method described by Hansen et al. The assay is based on the reduction of MTT by the mitochondrial dehydrogenase of viable cells into purple formazan crystals which gets dissolved in DMSO and read at 570 nm. Briefly, 1x10^4 exponentially growing cells were seeded into each 96 well plate (counted by Trypan blue exclusion dye method) allowed to grow to 60-70% confluence then compounds were added to the culture medium with the final concentrations ranging from of 0.1, 1, 5 and 10 μM and along with controls (negative without compound) and positive (Doxorubicin) incubated for 24 hours CO_2 incubator at 37 ^{0}C with a 90% humidified atmosphere and 5% CO_2. Then

the media of the wells were replaced with 90µl of fresh serum free media and 10 µl of MTT (5mg/mL of PBS), plates were incubated at 37 ^{0}C for 2 h, thereafter the above media was discarded allow to dry for 30minutes. Add 100 µl of DMSO in each well at 37 ^{0}C for 5 min. The purple formazan crystals were dissolved and immediately read absorbance at 570 nm was measured using Spectra Max plus 384 UV-Visible plate readers (Molecular Devices, Sunnyvale, CA, USA). IC_{50} values were determined by the probit analysis software package of MS-excel. % Cell viability (from control) versus concentration.

DNA interaction studies

Concentrated CT-DNA stock solution was prepared in Tris HCl buffer (5mM Tris HCl/50 mM NaCl in water at pH = 7.5) and the concentration of DNA solution was determined by UV absorbance at 260 nm. The molar absorption coefficient was taken as 6600 M^{-1} cm^{-1}. Solution of CT-DNA in Tris HCl buffer gave a ratio of UV absorption at 260 nm and 280 nm A_{260}/A_{280} of ca. 1.8-1.9, indicating that the DNA was sufficiently free of protein. All stock solutions were stored at 4 ^{0}C and wre used within four days. The DNA binding experiments were done in Tris-HCl buffer using 1mM 5% v/v DMSO/Water solution of active compounds 7a, 7l. Absorption titration experiments were performed by maintaining a constant compound concentration (2µM) and varying the DNA concentration (0–10 µM) in the buffer. The active compounds 7a, 7l exhibited maximum absorption at around 210 nm. After each addition of DNA to the respected compound, the resulting solution was allowed to equilibrate at 25^{0}C for 5min, after which, the change in absorbance was recorded. The intrinsic binding constant, K_b, was determined from the spectral data using the Wolfe–Shimmer Equation[39], [DNA]/(εa–εf) = [DNA]/(εb–εf) + 1/Kb (εb–εf), where [DNA] = Concentration of DNA in base pairs, εa = the apparent extinction coefficient = (Aobsd/[Compound]), εf = the extinction coefficient for free compound, εb = the extinction coefficient for free compound in the fully bound form. In plots of [DNA]/(εa−εf) versus [DNA] Kb is given by the ratio of slope to the intercept.

Viscosity measurements were performed with an Ostwald Viscometer at room temperature. The concentration of DNA was 20µM, compounds concentration varied from 0 to 100 µM and the flow times were measured with a digital timer and each sample was measured three times for accuracy, and an average flow time was calculated. Data was presented as $(\eta/\eta o)_{1/3}$ versus [compound]/ [DNA], where η is the viscosity of DNA in the presence of complex and ηo that of DNA alone. Viscosity values were calculated from the observed flow time of DNA containing solutions (t) corrected for that buffer alone (5 mM Tris–HCl/50 mM NaCl) (to), η = (t – to).

For gel electrophoresis experiments TE-buffer (10mMTris-HCl and 1 mM Na2EDTA), TAE-buffer (pH 8.0; 40 mM Tris-base, 20 mM acetic acid, 1 mM EDTA) were used. TE-buffer (10mMTris-HCl and 1 mM Na2EDTA) used for dilution of pBR322 DNA. Super coiled pBR322 DNA (0.1 g/µL) was treated with 50 µM and 100 µM of title derivatives were irradiated at 365 nm for 1 h. A loading buffer containing 25% bromophenol blue, 0.25% xylene cyanol, and 30% glycerol (2 µL) was added. The samples were then analyzed by 0.8% agarose gel electrophoresis at 50 V for 2 h. The gel was stained with 2 µL (from 1 mg/100 µL) of ethidium bromide[40] and photographed under UV light. The gels were viewed with a gel documentation system and photographed using a CCD camera.

Supplementary Material

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/MS-numbe

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