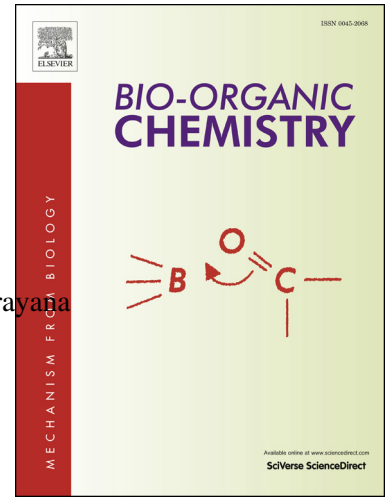

Design and synthesis of C3-tethered 1,2,3-triazolo-β-carboline derivatives: Anticancer activity, DNA-binding ability, viscosity and molecular modeling studies

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Design and synthesis of C3-tethered 1,2,3-triazolo-β-carboline derivatives: Anticancer activity, DNA-binding ability, viscosity and molecular modeling studies

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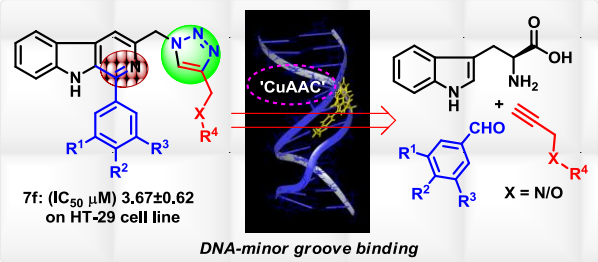
Nagula Shankaraiah[a,*], Chetna Jadala[a], Shalini Nekkanti[a], Kishna Ram Senwar[a], Narayana Nagesh[c,*], Shweta Shrivastava[d], V. G. M. Naidu[d], Manda Sathish[b], Ahmed Kamal[a,b*]



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ABSTRACT

A series of new DNA-interactive C3-tethered 1,2,3-triazolo-β-carboline derivatives have been synthesized via 'click' reaction and evaluated for their *in vitro* cytotoxicity as well as DNA binding affinity. Interestingly, these hybrids have displayed potent *in vitro* cytotoxicity in comparison to Harmine against the HT-29 (colon cancer) and HGC-27 (gastric cancer) cell lines. The compounds **7f**, **7k**, **7n** and **7s** appear to be more effective against the HGC-27 cell line, among which compound **7f** showed the highest cytotoxicity (5.44±0.58, IC_{50} μM). The compounds **7e** and **7f** appear to be more active against the HT-29 cell line, among which compound **7f** exhibited the highest cytotoxicity (3.67±0.62, IC_{50} μM). To gain more insight into the DNA-binding ability, spectroscopic techniques such as UV-visible, flourescence and circular dichroism studies were performed. Viscosity measurements and molecular docking studies substantiate that these compounds indeed bind to DNA via the minor groove.

1. Introduction

Anticancer drugs that target DNA are one of the most effective agents in clinical use and have enhanced the survival rates of patients when used alone or in combination with other drugs.[1] Lately, DNA interacting agents have been extensively studied with regard to cancer chemotherapy, which has led to the development of many novel heterocyclic compounds. These agents exert their biological profile by non-covalent and covalent interactions in either the minor or major groove or between the base pairs (intercalation) of the double helix. Anticancer drug discovery has focused mainly on the development of novel DNA interacting scaffolds like anthracyclines, acridines, anthraquinones, distamycins, β-carbolines and their derivatives.[2] Structural alteration of these bioactive scaffolds may result in the development of new drugs with improved therapeutic properties.

The β-carboline alkaloids display a broad spectrum of biological and pharmacological properties including DNA intercalation and minor grove binding, resulting in antitumor activity. Previous Structure-Activity Relationship (SAR) analysis demonstrated that the presence of appropriate groups at the C1 and C3-positions of the β-carboline core enhanced antitumor as well as DNA-binding ability.[3] It was also observed that β-carboline hybrids with substituted phenyl group and heterocyclics at the C1 and C3-positions respectively, have displayed potential antitumor properties.[4]

Over the past few years, several molecules possessing 1,2,3-triazole scaffold have been synthesized as useful chemotherapeutic agents[5] possessing antibacterial, antifungal, antihelmintic[6] and anticancer activities.[7] Among the diverse synthetic tools helpful for scaffold ligation, 1,2,3-triazole formation by Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reactions are significant owing to their reliability, greenness and atom economy.[8] 'Click' reaction has emerged as powerful modular reaction towards the synthesis of diverse compounds and have found widespread applications in combinatorial drug discovery,[9] material science,[10] and bio-conjugate chemistry.[11] In view of the biological importance of 1,2,3-triazoles and β-carbolines, it was of considerable interest to

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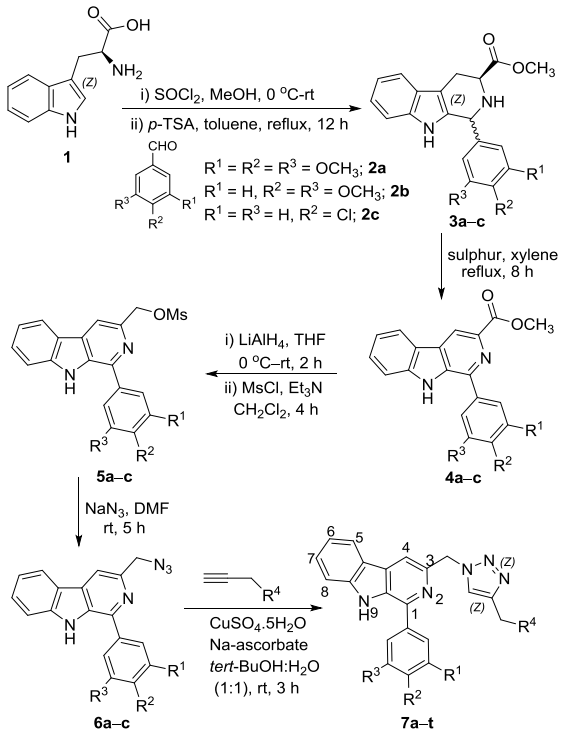
develop novel conjugates incorporating both the ring systems, such a combination could enhance the DNA interacting ability.



Scheme 1. Synthesis of various C3-tethered triazolo-β-carboline derivatives **7a**–**t**.

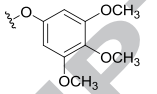
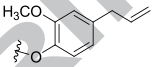
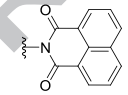
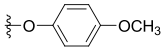
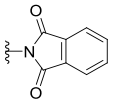
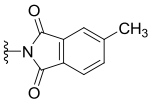
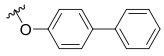
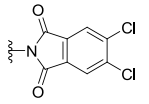
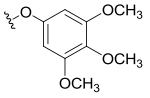
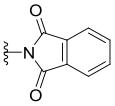
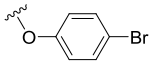
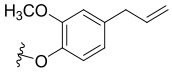
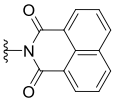
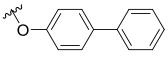
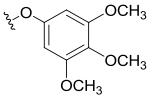
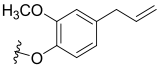
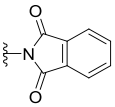
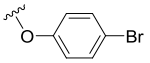
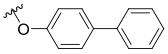
Moreover, the 1,4-substituted 1,2,3-triazole moiety built between the scaffolds can mimic the trans geometry of an amide bond, resulting in the conversion of a nonpeptidic molecule to a peptidomimetic with rigidity and protease stability, valuable in drug discovery. In this context, we have synthesized a library of hybrids that are diversified at positions C1 and C3 of the β-carboline scaffold, which possess potential antitumor activity and investigated their mode of DNA interaction. Since, 1,2,3-triazoles are one of the most flexible, easy to introduce and attractive connecting units, herein, we have designed and synthesized a series of novel C3-linked 1,2,3-triazolo-β-carboline hybrids by employing 'click' approach. The focus of this investigation was to explore the optimal structural requirements of the compounds for antitumor activities, and further development of more potent β-carboline hybrids.

2. Results and discussion

2.1. Chemistry

The synthetic route for the novel C3-linked 1,2,3-triazolo-β-carboline hybrids was outlined in **Scheme 1**. The β-carboline ring system was obtained from the commercially available L-tryptophan (**1**), which was esterified with $SOCl_2$ in methanol to provide L-tryptophan methyl ester. Methyl 1-(3, 4, 5 substituted phenyl)-9*H*-pyrido[3,4-*b*] indole-3-carboxylates (**3a**–**c**) were prepared by the condensation of L-tryptophan methyl ester with 3,4,5-substituted benzaldehydes **2a**–**c** via Pictet-Spengler condensation followed by dehydrogenation with sulphur in refluxing xylene to afford the fully unsaturated compounds **4a**–**c**. Later, the ester group at C3-position was reduced into the corresponding alcohols by $LiAlH_4$ in dry THF. The alcohol was mesylated with mesyl chloride by using Et_3N. Then, the compounds **5a**–**c** were made to react with sodium azide in DMF to yield substituted 9*H*-pyrido indolyl azides **6a**–**c**. For the generation of 1,2,3-triazoles, different substituted alkynes i.e., O-linked and N-linked alkynes were used, which were prepared from substituted phenols and amines respectively. Finally, the Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction was performed to afford C3-linked 1,2,3-triazolo-β-carboline hybrids **7a**–**t** in 70–95% yields as shown in **Table 1**. The chemical

Table 1. Synthesis of C3-linked 1,2,3-triazolo-β-carboline hybrids **7a**–**t**

compound	R^1	R^2	R^3	R^4	yield (%)[a]
7a	OCH_3	OCH_3	OCH_3		95
7b	OCH_3	OCH_3	OCH_3		90
7c	OCH_3	OCH_3	OCH_3		88
7d	OCH_3	OCH_3	OCH_3		93
7e	OCH_3	OCH_3	OCH_3		80
7f	OCH_3	OCH_3	OCH_3		75
7g	OCH_3	OCH_3	OCH_3		70
7h	OCH_3	OCH_3	OCH_3		87
7i	OCH_3	OCH_3	OCH_3		90
7j	H	OCH_3	OCH_3		93
7k	H	OCH_3	OCH_3		84
7l	H	OCH_3	OCH_3		85
7m	H	OCH_3	OCH_3		85
7n	H	OCH_3	OCH_3		82
7o	H	OCH_3	OCH_3		90
7p	H	Cl	H		95
7q	H	Cl	H		88
7r	H	Cl	H		82
7s	H	Cl	H		85
7t	H	Cl	H		90

[a]Isolated yields.

structures of all the new compounds were characterized by ^{1}H, ^{13}C NMR, HRMS, and IR spectroscopic techniques.

2.2. MTT assay

The novel β-carbolines bearing substituted 1,2,3-triazole moieties at C3-position have been evaluated for their *in vitro* cytotoxicity against PC-3 (prostate cancer), HeLa (cervical carcinoma), MCF-7 (breast cancer), HT-29 (Colon cancer), and HGC-27 (Human gastric carcinoma) cell lines by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and compared with that of Harmine and Hoechst 33258. The results of this cytotoxicity data expressed as IC_{50} values were summarized in **Table 2**. These compounds have displayed significant *in vitro* cytotoxicity in comparison to Harmine against the HT-29 and HGC-27 cell lines. The compounds **7f**, **7k**, **7n** and **7s** appear to be more effective against the gastric carcinoma (HGC-27) cell line with IC_{50} values lower than 10 μM, among which compound **7f** displayed the highest cytotoxicity (5.44±0.58, IC_{50} μM). Compound **7f** was also potently cytotoxic against the colon cancer (HT-29) cell line **(**3.67±0.62, IC_{50} μM) compared to the standard drug Harmine (4.28±1.15, IC_{50} μM). Interestingly, the compounds **7f** and **7n** were found to be significantly cytotoxic against all the five human cancer cell lines investigated with IC_{50} values of lower than 27 µM. All these hybrids have shown lower activity against the breast cancer cell line with IC_{50} values greater than that of harmine (3.52±0.78, IC_{50} μM). In the prostate cancer (PC-3) cell line, only the hybrids **7f** and **7n** have displayed significant cytoxicities compared to that of harmine (24.45±3.93, IC_{50} μM) while the other hybrids have displayed no activity. Interestingly, compounds **7f**, **7h**, **7n** and **7s** were almost twice as cytotoxic as Hoechst 33258 in the MCF-7 cell line.

The most potent compound **7f** with a trimethoxy phenyl at C1-position and 1,2,3-triazolo methyl phthalimide at C3-position, has displayed good to moderate IC_{50} values on all the cancer cell lines while the compound **7n** with dimethoxy phenyl substituent at C1-position and 1,2,3-triazolo naphthalimide at C3-position exhibited cytotoxicities similar to that of **7f**. Similarly, compounds **7e** and **7k** possessing phthalimide groups at C3-position have shown significant activity on colon cancer (5.79±1.85, IC_{50} μM and 20.18±2.42, IC_{50} μM respectively**)** and gastric cancer (6.27±0.93, IC_{50} μM and 20.18±2.42, IC_{50} μM respectively) cell lines. From these results, we could infer that the presence of a phthalimide or naphthalimide pharmacophore linked through a 1,2,3-triazole at C3-position played a key role in the modulation of cytotoxic potencies.

2.3. Circular dichroism (CD) studies

Circular dichroism (CD) studies provide information on changes in the DNA conformation upon interaction with derivatives. DNA is a very important biological macromolecule which plays a major role in the cell proliferation and other bio logical activities. Hence, changes in the DNA topology will provide information on the nature of DNA-ligand interactions and conformational changes on its interaction with DNA. The CD spectrum of CT-DNA exhibits a positive band at 275 nm and a negative band at 245 nm due to π–π base stacking and right-hand helicity, the characteristic profile of B form DNA. In the present study, the positive CD exhibited hypochromocity initially and on increasing the ratio between CT-DNA and **7f** from 1:1 to 1:2, the positive band showed slight hyperchromicity. This indicates that **7f** unfolds DNA at lower DNA: derivative ratio and on increasing the derivative concentration, it tends to stabilize the DNA-derivative complex.[12] The negative band intensities have gradually increased indicating decrease in the DNA helicity on β-carboline's interaction with DNA. On the other hand CD spectra obtained on **7n** and **7s** interaction with DNA was different. The positive peak at 275 nm exhibited hyperchromicity. Hyperchromicity of the CD positive band indicates stabilization of CT-DNA on **7n** and **7s** interaction.[13] The positive CD band int-

Table 2. Cytotoxic (IC_{50} μM) evaluation of 1, 2, 3-triazolo-β-carboline derivatives **7a**–**t**

Compound	[a]PC-3	MCF-7	HeLa	HT-29	HGC-27
7a	>25	>25	>25	>25	>25
7b	>25	>25	>25	>25	>25
7c	>25	>25	>25	>25	>25
7d	>25	>25	>25	>25	>25
7e	>25	>25	>25	5.79±1.85	>25
7f	22.4±2.42	26.5±0.79	15.6±0.19	3.67±0.62	5.44±0.58
7g	>25	>25	>25	>25	>25
7h	>25	29.6±1.00	>25	>25	>25
7i	>25	>25	>25	>25	>25
7j	>25	>25	>25	>25	>25
7k	>25	>25	>25	20.18±2.42	6.27±0.93
7l	>25	>25	>25	>25	>25
7m	>25	>25	>25	>25	>25
7n	18.5±2.46	19.7±3.28	23.7±2.59	7.46±1.69	8.67±1.28
7o	>25	>25	>25	>25	>25
7p	>25	>25	>25	>25	>25
7q	>25	>25	>25	>25	>25
7r	>25	>25	>25	>25	>25
7s	>25	22.0±2.45	>25	15.55±2.32	9.95±1.52
7t	>25	>25	>25	>25	>25
Harmine	24.45±3.93	3.52±0.78	14.59±2.52	4.28±1.15	10.56±1.75
Hoechst 33258	N.D	52.2±2.1	0.02±0.005	N.D	N.D

[a]PC-3 (Prostate cancer), MCF-7 (Breast cancer), HeLa (Cervical cancer), HT-29 (Colon cancer), HGC-27 (Human gastric carcinoma). Half maximal inhibitory concentration values (IC_{50}) are the mean ±SD of three individual experiments performed in duplicate. Harmine (natural product) was included as reference standards. ND: not determined.

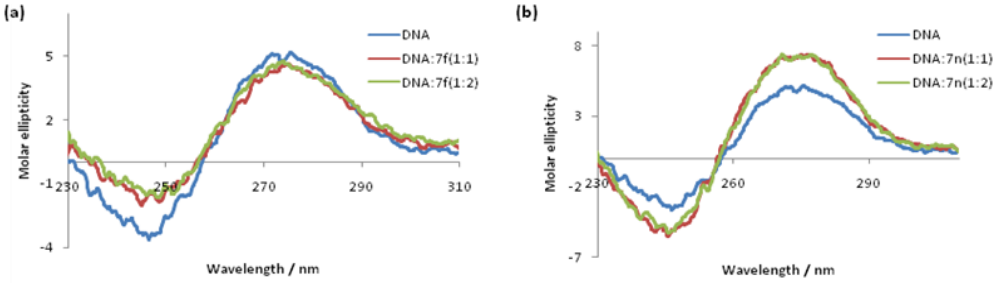


Figure 1. CD spectra of hybrids **7f** and **7n** (**a, b**) with CT-DNA in the absence or presence of increasing amounts of **7f** and **7n**.

ensities did not change even on increasing **7n** and **7s** concentration. The CD spectra of **7f** after interaction with CT-DNA exhibited couple of isodichroic points at 257 nm and 288 nm, manifesting that the interaction between the **7f** molecules and DNA occurs in multiple steps and more than one species co-exists in equilibrium with each other.[14] The CD spectra obtained when **7f** and **7n** interact with CT-DNA were shown in **Figure 1a** and **1b** respectively.

2.4. UV-Visible spectral studies

The results from the UV-Visible spectral studies indicate the mode of DNA-ligand interaction. In the present study, on addition of equal increments of CT-DNA to **7f** solution, the absorption band has showed continuous increase in its intensity and the hyperchromicity is more evident at 220 nm. Such continuous hyperchromicity of the absorption band is an indication of electrostatic binding to CT-DNA. Compounds **7n** and **7s** have also exhibited absorption bands at 875 nm and 570 nm respectively and the absorption band of these derivatives also exhibited hyperchromicity on interaction with CT-DNA, manifesting electrostatic binding or partial unfolding of CT-DNA exposing more bases to the incident light.[15,16] On the other hand, when CT-DNA was added to Harmine solution, the absorption band at 303 nm has shown hypochromisity and red shift of about 25 nm (shifed to 328 nm). This indicates that Harmine intercalates between the bases of CT-DNA. Since the UV-Visible spectral pattern of **7f**, **7s** and **7n** derivative was same, a representative UV-Visible spectral profile obtained when **7f** - interacts with CT-DNA was shown in **Figure 2**.

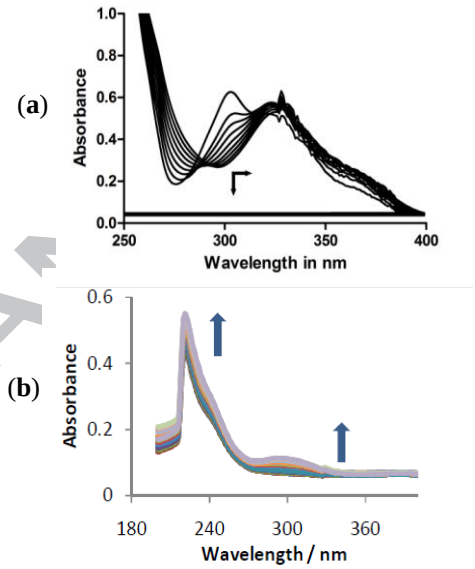


Figure 2. UV–visible spectra of harmine and **7f (a,b)**.

2.5. *Fluorescence emission spectra*

Fluorescence titration is yet another valuable technique for understanding the binding mode of small molecules with DNA and to study the electronic environment around the DNA-complex at comparatively lower concentrations. Since the complexes are fluorescent, their interaction with DNA can be monitored at low concentration with this study. Emission spectra of **7f**, **7n** and **7s** show prominent peaks at 512.5 nm, 875 nm and 573 nm respectively. On addition of equal increments of CT-DNA to all the β-carboline derivatives, the emission peak intensities decreased gradually, indicating that the derivative molecules bind externally to the surface of CT-DNA.[17] The fluorescence hypochromicity occurs due to the dissipation of energy by excited derivative molecules to the surrounding water molecules. Whereas when CT-DNA was added to Harmine solution, the fluorescence emission band at 418 nm exhibited hyperchromicity. This shows that Harmine will intercalate between the bases of CT-DNA. The representative fluorescence emission spectrum obtained after the addition of increasing concentrations of CT-DNA to **7f** is shown in **Figure 3**.

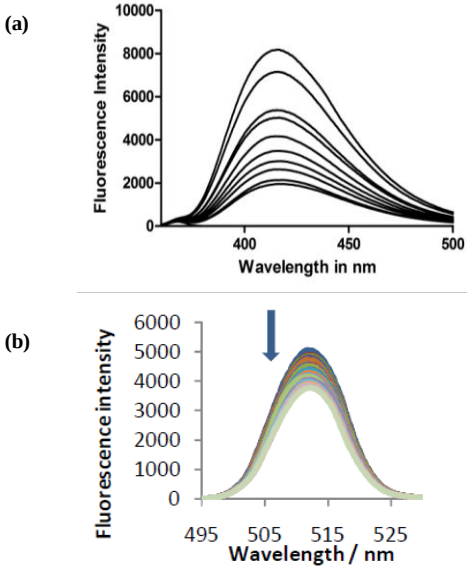


Figure 3. Fluorescence spectra of harmine and **7f (a,b)** in the presence of increasing amount of CT-DNA.

2.6. DNA interaction study by viscosity measurements

Spectroscopic studies provide information about the type of ligand binding to DNA. However, these studies alone cannot illustrate the nature of ligand binding to DNA. Hence, relative viscosity experiments were carried out to have a clear understanding on **7f, 7s** and **7n** derivative-DNA interaction. Intercalation of β-carboline derivative molecules between the two stakes of DNA results in an increase of the viscosity of DNA solutions.[18] Very small variation in viscosity is observed in case of molecules that bind to the surface of DNA or groove binders,[19] whereas a reduction in the relative viscosity is typically observed with covalent DNA-binding.[20]

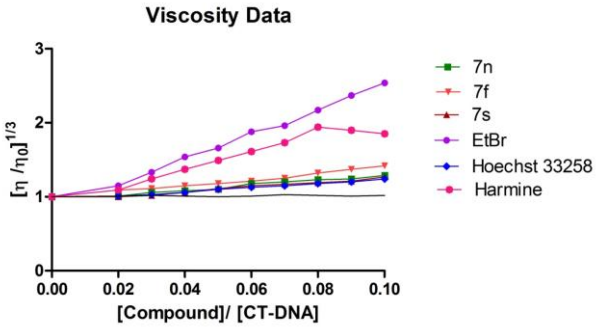


Figure 4. Relative viscosity experiment of hybrids **7f**, **7n** and **7s** with CT-DNA. EtBr, Harmine and Hoechst 33258 were used as controls.

Ethidium bromide (EtBr), a well-known DNA intercalater increases the relative viscosity strongly by lengthening the DNA double helix. Whereas relative viscosity remains almost unaltered in case of well-known groove binders like Hoechst 33258. In the present experiment, EtBr was taken as a control and Hoechst 33258 was considered as positive control. On slowly increasing the concentration of the β-carboline derivatives, the relative viscosity of **7f, 7n** and **7s** complex solutions did not change much. However, when EtBr is added to CT-DNA, enhancement in viscosity of the solution was noticed. Similarly, when Harmine was added to CT-DNA solution, gradual enhancement of viscosity was noticed, indicating intercalation of Harmine with DNA (like EtBr). Further, on addition of Hoechst 33258 also not much change was observed. The lesser variation in viscosity suggests that these derivative molecules, bind to the surface of DNA through minor like Hoechst 33258. Among the β-carboline derivatives studied, **7f** showed marginal increase in viscosity after its interaction with DNA, indicating better interaction of **7f** hybrid with CT-DNA (**Figure 4**). The data obtained from viscosity studies supports the results obtained in spectroscopic studies.

2.7. Molecular Modelling

Molecular docking simulations were carried out using XP Glide 6.0[21] (Schrödinger 2013) with default settings into DNA duplex. The docked poses for the one of the best scored hybrids **7f**, shown in **Figure 5**, depict its binding along the minor groove. Interestingly, the 1,2,3-triazole ring forms a kink in the molecule akin to the peptide bond and shapes it in such a way that it orients itself in a curved manner along the minor groove. Further, the docked poses were stabilized electronically by hydrogen-bonds between the carbonyl oxygens of the phthalimide and the side chains of the DNA base pairs. The aromatic ring of the phthalimide was also observed to be involved in side wise π-π stacking with the base pairs. The results obtained from molecular docking studies were consistent with the DNA binding studies as well as *in vitro* cytotoxicity data.

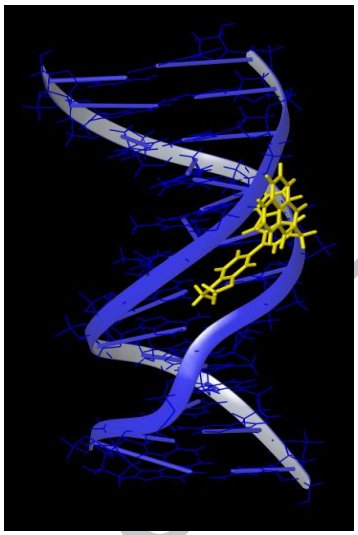


Figure 5. DNA minor groove binding of **7f** with DNA. DNA is shown in ribbon form and **7f** in yellow tube form.

3. Conclusion

In conclusion, an efficient access to the construction of a novel C3-tethered 1,2,3-triazolo-β-carboline hybrids with promising cytotoxicity and DNA interacting property has been accomplished. Derivatives **7f** and **7n** were found to be the most potent cytotoxic agents with IC_{50} values lower than 27 μM against the panel of human cancer cell lines investigated (PC-3, MCF, Hela, HT-29, HGC-27). Molecular docking studies have shown that these derivatives bind to DNA through minor groove binding. To further strengthen the DNA interacting ability of these congeners, DNA-binding assays were performed including circular dichroism, fluorescence titration, UV–visible and viscometric titration studies which indicated strong electrostatic binding of the derivatives into DNA. Based on the cytotoxicity and DNA-binding affinity, the Structure Activity Relationships (SARs) for this new class of derivatives were proposed and it was observed that the presence of a phthalimide or naphthalimide pharmacophore linked through a 1,2,3-triazole at the C3-position enhanced the cytotoxicity and DNA-binding potential.

4. Experimental

4.1. General

^{1}H NMR and ^{13}C NMR spectra are recorded on Varian Gemini 200 or Varian Unity 400 or Varian Inova 500 or Bruker Avance 300 MHz Making a solution of samples in $CDCl_3$ solvent using Tetramethylsilane (TMS) as the internal standard unless otherwise mentioned, and are given in the δ scale. The standard abbreviations s, d, t, m, dd refer to singlet, doublet, triplet, multiplet and doublet of a doublet respectively. Infrared (IR) spectra are recorded on Perkin-Elmer Infrared–683 or 1310 with NaCl optics. Spectra were calibrated against the polystyrene absorption at 1610cm^{-1}. Samples were scanned neat, KBr wafers or in chloroform as a thin film. Mass spectra recorded on CEC-21-110B, Finnegan Mat 1210 or MICROMASS-7070 spectrometers operating at 70eV using a direct inlet system. If necessary, FABMS is recorded. Melting points are determined on an Electro thermal melting point apparatus and are uncorrected. All reactions are monitored by thin layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254) with UV light, iodine as probing agents. Acme (India) silica gel (finer than 200 mesh) is used for flash chromatography. Column chromatography was performed by using silica gel 60-120, 100-

200 mesh. The reactions wherever anhydrous conditions needed are carried out under the positive pressure of nitrogen atmosphere using dry and freshly distilled solvents. All solvents and reagents were purified by standard techniques. All evaporation of solvents was carried out under reduced pressure on Heidolph rotary evaporator below 45 °C. Yield reported are isolated yields of material judged homogeneous by TLC and NMR spectroscopy. The names of all compounds given in the experimental section were taken from Chem. ultra, version 11.0. All computational modeling are performed on a desktop PC with a Red Hat Enterprise linux version 5.0 using Maestro software version 9.5.

4.2. Chemistry

4.2.1 General procedure for the synthesis of 7a–t.

To a stirred solution of alkyne (1.0 mmol) and *β*-carboline-azide **6a–c** (1.2 mmol) in *tert*-butanol-water (1:1) (20 mL), $CuSO_4{\cdot}5H_2O$ catalyst (1 mol%) and sodium ascorbate (5 mol%) were added and the mixture was stirred at room temperature for 12 h. The progress of the reaction was monitored by TLC. The resulting reaction mixture was washed with water (20 mL) and extracted with ethyl acetate (60 mL). The organic layer was dried with sodium sulfate and filtered. The filtrate was evaporated at reduced pressure, which was purified by silica gel column chromatography with a gradient elution of ethyl acetate/n-hexane to afford a desired products **7a–t**.

3-((4-((3, 4, 5-Trimethoxyphenoxy) methyl)-1H-1, 2, 3-triazol-1-yl) methyl)-1-(3, 4, 5-trimethoxyphenyl)-9H-pyrido [3, 4-b] indole (7a). 95%Yield; Mp: 143–145 ^oC; FT-IR (cm^{-1}) 3316, 3155, 2938, 2360, 1585, 1504, 1465, 1451, 1395, 1231, 1192, 1126, 812, 747, 611, 438; ^1H NMR (300 MHz, $CDCl_3$): δ 8.6 (brs, 1H), 8.12 (d, 1H, J = 7.5 Hz), 7.82 (d, 2H, J = 3.8 Hz), 7.46 (t, 2H), 7.34 (t, 1H), 7.12 (s, 2H), 6.23 (s, 2H), 5.90 (s, 2H), 5.17 (s, 2H), 3.90 (s, 18H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.6, 153.1, 152.9, 151.2, 148.2, 142.3, 141.3, 137.8, 132.4, 131.5, 130.5, 123.3, 122.9, 121.7, 121.4, 119.8, 111.1, 100.8, 93.7, 72.3, 60.8, 57.1, 56.1; HRMS (ESI): *m/z* calcd. for $C_{33}H_{34}N_5O_7$ 612.2458, found 612.2441 $[M+H]^+$

3-((4-((4-Allyl-2-methoxyphenoxy) methyl)-1H-1, 2, 3-triazol-1-yl) methyl)-1-(3, 4, 5 trimethoxyphenyl)-9H-pyrido [3, 4-b] indole (7b). 90% Yield; Mp: 150–153 ^oC; FT-IR (cm^{-1}) 3316, 3141, 2937, 2832, 2367, 1623, 1583, 1508, 1465, 1427, 1396, 1364, 1327, 914, 848; ^1H NMR (300 MHz, $CDCl_3$): δ 8.56 (brs, 1H), 8.02 (d, 1H, J = 8.1 Hz), 7.82 (s, 2H), 7.46 (s, 3H) 7.32 -7.16 (m, 1H), 7.04 (s, 1H), 6.90 (d, 1H, J = 8.4 Hz), 6.61 (d, 2H, J = 6.9 Hz), 5.81 (s, 2H), 5.91-5.72 (m, 1H), 5.22 (s, 2H), 4.95 (t, 2H), 3.87 (s, 12H), 3.27 (d, 2H, J = 6.6); ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.8, 153.1, 149.7, 148.2, 142.3, 141.3, 137.8, 136.5, 133.2, 132.4, 130.5, 123.3, 122.9, 122.3, 121.4, 115.9, 111.1, 100.8, 72.6, 60.8, 57.1, 56.1, 39.8; HRMS (ESI): *m/z* calcd. for $C_{34}H_{34}O_5N_5$ 592.2560, found 592.2537 $[M+H]^+$

2-((1-((1-(3, 4, 5 Trimethoxyphenyl)-9H-pyrido [3, 4-b] indol-3-yl) methyl)-1H-1, 2, 3-triazol-4-yl) methyl)-1H benzo [de]isoquinoline-1, 3(2H)-dione (7c). 88% Yield; Mp: 199–203 ^oC; FT-IR (cm^{-1}) 3320, 2927, 2362, 1695, 1656, 1625, 1586, 1502, 1384, 1326, 1236, 848; ^1H NMR (300 MHz, $CDCl_3$): δ 8.58 (brs, 1H), 8.54 (d, 2H, J = 6.4 Hz), 8.18 (d, 2H, J = 7.6 Hz), 8.03 (d, 1H, J = 8.3 Hz) 7.73 (d, 2H, J = 15.6 Hz), 7.70 (d, 2H, J = 8.30 Hz), 7.67 (s, 2H), 7.46 (d, 2H, J = 7.6 Hz), 7.25(s, 1H), 7.04 (d, 1H, J = 8.30 Hz), 5.82 (s, 2H), 5.52 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): 159.6, 157.8, 153.1,141.3, 140.2, 137.5, 137.9, 137.8, 132.4, 130.7, 130.5, 129.5, 128.1, 125.6, 123.6, 123.3, 122.9, 121.4, 111.1, 107.9, 103, 60.8, 57.1, 56.1, 44.5; HRMS (ESI): *m/z* calcd. for $C_{36}H_{29}O_5N_6$ 625.2199, found 625.2184 $[M+H]^+$

3-((4-((4-Methoxyphenoxy) methyl)-1H-1, 2, 3-triazol-1-yl) methyl)-1-(3, 4, 5-trimethoxyphenyl)-9H-pyrido [3, 4-b] indole (7d). 93% Yield; Mp: 176–179 ^oC; FT-IR (cm^{-1}) 3368, 3152, 2990, 2960, 2931, 2827, 2361, 1717, 1625, 1581, 1508, 1453, 771, 721, 612, 521; ^1H NMR (300 MHz, $CDCl_3$): δ 8.6 (brs, 1H), 8.12 (d, 1H, J = 7.55 Hz), 7.82 (d, 2H, J = 3.77 Hz), 7.46 (t, 2H) 7.34(t, 1H), 7.12 (s, 2H), 6.23 (s, 2H), 5.90 (s, 2H), 5.17 (s, 2H),3.90(s, 18H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.8, 153.1, 152.9, 151.3, 148.2, 142.3, 141.3, 137.8, 132.4, 130.5, 123.3, 122.9, 121.4, 115.3, 111.1, 107.9, 103.0, 72.3, 60.8, 56.1, 55.8, 57.1; HRMS (ESI): *m/z* calcd. for $C_{31}H_{30}O_5N_5$ 552.2247, found 552.2297 $[M+H]^+$

2-((1-((1-(3, 4, 5-Trimethoxyphenyl)-9H-pyrido [3, 4-b] indol-3-yl) methyl)-1H-1, 2, 3-triazol-4-yl) methyl) isoindoline-1, 3-dione (7e). 80% Yield; Mp:168–171 ^oC; FT-IR (cm^{-1}) 3354, 3157, 3061, 2941, 2834, 2361, 1947, 1763, 1678, 1585, 1505, 1423, 935, 785, 691; ^1H NMR (300 MHz, $CDCl_3$): δ 8.6 (brs, 1H), 7.92 (s, 2H), 7.84 (s, 2H), 7.52 (t, 1H, J = 7.6 Hz), 7.56 (s, 1H), 7.25 (s, 1H), 7.32 (t, 1H, J = 6.7 Hz) 5.84 (s, 2H), 4.99 (s, 2H), 3.96(s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.2, 157.8, 153.1, 148.2, 141.9, 141.3, 137.8, 132.4, 132.5, 131.9, 130.7, 130.5, 129, 127.8, 127.5, 123.3, 122.9, 121.4, 121.7, 119.8, 111.1, 107.9, 100.8, 60.8, 57.1, 56.1; HRMS (ESI): *m/z* calcd. for $C_{32}H_{27}O_5N_6$ 575.2043, found 575.2022 $[M+H]^+$

5-Methyl-2-((1-((1-(3, 4, 5-trimethoxyphenyl)-9H-pyrido [3, 4-b] indol-3-yl) methyl)-1H-1, 2, 3-triazol-4-yl) methyl) isoindoline-1, 3-dione (7f). 75% Yield; Mp: 143–145 ^oC; FT-IR (cm^{-1}) 3354, 3157, 3061, 2941, 2834, 2361, 1947, 1763, 1678, 1585, 1505, 1423, 935, 785, 691; ^1H NMR (300 MHz, $CDCl_3$): δ 8.6 (brs, 1H), 7.92 (s, 2H), 7.84 (s, 2H), 7.52 (t, 1H, J = 6.3 Hz), 7.56 (s, 1H), 7.25 (s, 1H), 7.32 (t, 1H, J = 7.5 Hz) 5.84 (s, 2H), 4.99 (s, 2H), 3.96 (s, 9H), 2.43 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.2, 157.8, 153.1, 148.2, 141.9, 141.3, 137.8, 132.4, 132.5, 131.9, 130.7, 130.5, 129, 127.8, 127.5, 123.3, 122.9, 121.4, 121.7, 119.8, 111.1, 107.9, 100.8, 60.8, 57.1, 56.1, 20.9; HRMS (ESI): *m/z* calcd. for $C_{33}H_{29}O_5N_6$ 589.2199, found 589.2180 $[M+H]^+$

3-((4-((3, 4-Dihydroquinolin-1(2H)-yl) methyl)-1H-1, 2, 3-triazol-1-yl) methyl)-1-(3, 4, 5-trimethoxyphenyl)-9H-pyrido [3, 4-b] indole (7g). 70% Yield; Mp: 183–185 ^oC; FT-IR (cm^{-1}) 3384, 2925, 2366, 1219, 1043, 772; ^1H NMR (300 MHz, $CDCl_3$): δ 8.63 (brs, 1H), 8.07 (d, 1H, J = 7.9 Hz), 7.96 (d, 1H, J = 7.5 Hz), 7.59 (t, 1H, J = 8.2 Hz), 7.53 (s, 1H), 7.46 (t, 1H, J = 8.0 Hz), 7.30 (t, 1H, J = 7.52 Hz), 7.09 (s, 1H), 6.95 (t, 1H, J = 7.2 Hz), 6.90 (d, 1H, J = 7.5 Hz), 6.64 (d, 1H, J = 8.2 Hz), 6.54 (t, 1H, J = 7.52 Hz), 5.84 (s,2H), 4.58 (s, 2H), 3.93 (s, 9H), 3.35 (t, 2H, J = 5.7 Hz), 2.72 (t, 2H, J = 6.4 Hz), 1.90-1.94 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 154.3, 146.3, 144.3, 141.8, 133.8, 133.3, 133.2, 131.0, 129.3, 129.0, 128.7, 128.5, 127.2, 123.0, 122.3, 122.1, 121.8, 120.7, 116.5, 112.0, 111.3, 105.7, 76.8, 61.1, 56.5, 49.8, 47.5, 29.9, 28.1, 27.9, 22.4; HRMS (ESI): *m/z* calcd. for $C_{33}H_{33}O_3N_6$ 561.2614, found 561.25893 $[M+H]^+$

3-((4-((Biphenyl-4-yloxy) methyl) -1H-1, 2, 3 -triazol-1-yl) methyl) -1- (3, 4, 5-trimethoxyphenyl) -9H-pyrido [3, 4-b] indole (7h). 87% Yield; Mp: 198–201 ^oC; FT-IR (cm^{-1}) 3312, 3057, 3061, 2934, 2874, 2361, 1897, 1628, 1486, 1467, 1450, 1384, 1287, 1270, 1243, 1112, 861, 792; ^1H NMR (300 MHz, $CDCl_3$): δ 8.66 (brs, 1H), 8.10 (d, 2H, J = 8.30 Hz), 7.72 (s, 2H), 7.51(s, 2H), 7.54 (t, 1H, J = 8.30 Hz), 7.40 (t, 1H,J = 6.79 Hz), 7.29 (s, 2H), 7.15 (s, 2H), 7.11(s, 1H), 7.04 (d, 1H, J = 8.30 Hz), 5.90 (s, 2H), 5.25 (s, 2H), 3.94(s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.9, 153.1, 142.3, 141.3, 140.8, 137.8, 133.1, 132.4, 130.5, 130.1, 129.2, 127.9, 127.6, 123.3, 122.9, 121.7, 121.4,

114.8, 119.8, 111.1, 107.9, 72.3, 60.8, 57.1, 56.1; HRMS (ESI): *m/z* calcd. for $C_{36}H_{32}O_4N_5$ 598.2454, found 598.24693 $[M+H]^+$

5, 6-Dichloro-2-((1-((1-(3, 4, 5-trimethoxyphenyl)-9H-pyrido [3, 4-b] indol-3-yl) methyl)-1H-1, 2, 3-triazol-4-yl) methyl) isoindoline-1, 3-dione (7i). 90% Yield; Mp: 193–195 ^oC; FT-IR (cm^{-1}) 3128, 2962, 2360, 1780, 1719, 1622, 1585, 1506, 1447, 1425, 1396, 1327, 1223, 858, 800, 749, 727, 601; ^1H NMR (300 MHz, $CDCl_3$): δ 8.6 (brs, 1H), 8.07 (s,2H), 7.92 (s, 2H), 7.84 (s, 2H), 7.52 (t, 1H, *J* = 8.30 Hz), 7.56(s, 1H), 7.25 (s, 1H), 7.32 (t, 1H, *J* = 7.0 Hz) 5.84(s, 2H), 4.99 (s, 2H), 3.96 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.2, 157.8, 153.1, 148.2, 141.9, 141.3, 137.8, 132.4, 132.5, 131.9, 130.7, 130.5, 129, 127.8, 127.5, 123.3, 122.9, 121.4, 121.7, 119.8, 111.1, 107.9, 100.8, 60.8, 57.1, 56.1; HRMS (ESI): *m/z* calcd. for $C_{32}H_{25}Cl_2O_5N_6$ 643.1263, found 643.1258 $[M+H]^+$

1-(3, 4-Dimethoxyphenyl)-3-((4-((3, 4, 5 trimethoxyphenoxy) methyl)-1H-1, 2, 3-triazol-1-yl) methyl)-9H-pyrido [3, 4-b] indole (7j). 93% Yield; Mp: 143–145 ^oC; FT-IR (cm^{-1}) 3313, 3139, 2928, 1601, 1507, 1450, 1401, 1317, 1222, 811, 745, 607, 438; ^1H NMR (300 MHz, $CDCl_3$): δ 8.6 (brs, 1H), 8.12 (d, 1H, *J* = 7.55 Hz), 7.82 (d, 2H, *J* = 3.77 Hz), 7.46 (t, 2H, *J* = 7.5 Hz) 7.34 (t, 1H), 7.12 (s, 2H), 6.23 (s, 2H), 5.90 (s, 2H), 5.17 (s, 2H), 4.02 (s, 9H), 3.90 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 57.6, 153.1, 152.9, 151.2, 148.2, 142.3, 141.3, 137.8, 132.4, 131.5, 130.5, 123.3, 122.9, 121.7, 121.4, 119.8, 111.1, 100.8, 93.7, 72.3, 60.8, 57.1, 56.1; HRMS (ESI): *m/z* calcd. for $C_{32}H_{32}O_6N_5$ 582.2353, found 582.2453 $[M+H]^+$

2-((1-((1-(3, 4-Dimethoxyphenyl)-9H-pyrido [3, 4-b] indol-3-yl) methyl)-1H-1, 2, 3-triazol-4-yl) methyl) isoindoline-1, 3-dione (7k). 84% Yield; Mp: 165–168 ^oC; FT-IR (cm^{-1}) 3354, 3157, 3061, 2941, 2834, 2361, 1947, 1763, 1678, 1585, 1505, 1423, 935, 785, 691; ^1H NMR (300 MHz, $CDCl_3$): δ 8.6 (brs, 1H), 7.92 (s, 2H), 7.84 (s, 2H), 7.52 (t, 1H, *J* = 8.3 Hz), 7.56 (s, 1H), 7.25 (s, 1H), 7.32 (t, 1H, *J* = 7.5 Hz) 5.84(s, 2H), 4.99 (s, 2H), 3.96 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.2, 157.8, 153.1, 148.2, 141.9, 141.3, 137.8, 132.4, 132.5, 131.9, 130.7, 130.5, 129, 127.8, 127.5, 123.3, 122.9, 121.4, 121.7, 119.8, 111.1, 107.9, 100.8, 60.8, 57.1, 56.1; HRMS (ESI): *m/z* calcd. for $C_{31}H_{25}O_4N_6$ 545.1937, found 545.1987 $[M+H]^+$

3-((4-((4-Bromophenoxy) methyl)-1H-1, 2, 3-triazol-1-yl) methyl)-1-(3, 4-dimethoxyphenyl)-9H-pyrido [3, 4-b] indole (7l). 85% Yield; Mp: 148–150 ^oC; FT-IR (cm^{-1}) 3338, 3140, 2928, 1624, 1578, 1560, 1513, 1487, 1450, 1425, 1400, 1274, 814, 759, 605, 503; ^1H NMR (300 MHz, $CDCl_3$): δ 8.66 (brs, 1H), 8.10 (d, 2H, *J* = 8.30 Hz), 7.72 (s, 2H), 7.62(t, 1H, *J* = 8.3 Hz), 7.51(s, 2H), 7.54 (t, 1H, *J* = 8.30 Hz), 7.40 (t, 1H, *J* = 6.79 Hz), 7.29 (s, 2H), 7.15 (s, 2H), 7.11(s, 1H), 7.04 (d, 1H, *J* = 8.30 Hz), 6.82 (t, 1H, *J* = 8.3 Hz), 5.90 (s, 2H), 5.25 (s, 2H), 3.94(s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.9, 153.1, 142.3, 141.3, 140.8, 137.8, 133.1, 132.4, 130.5, 130.1, 129.2, 127.9, 127.6, 123.3, 122.9, 121.7, 121.4, 114.8, 119.8, 111.1, 107.9, 72.3, 60.8, 57.1, 56.1; HRMS (ESI): *m/z* calcd. for $C_{29}H_{25}BrO_3N_5$ 570.1141, found 570.1187 $[M+H]^+$

3-((4-((4-Allyl-2-methoxyphenoxy) methyl)-1H-1, 2, 3-triazol-1-yl) methyl)-1-(3, 4-dimethoxyphenyl)-9H-pyrido [3, 4-b] indole (7m). 85% Yield; Mp: 152–155 ^oC; FT-IR (cm^{-1}) 3317, 3145, 3002, 2923, 2853, 2837, 2360, 1729, 1671, 1625, 1589, 1468, 1514, 1029, 1024, 758, 619, 469, 438; ^1H NMR (300 MHz, $CDCl_3$): δ 8.58 (brs, 1H), 8.54 (d, 2H, *J* = 6.04 Hz), 8.18 (d, 2H, *J* = 7.55 Hz), 8.03 (d, 1H, *J* = 8.30 Hz) 7.73 (d, 2H, *J* = 15.6 Hz), 7.70 (d, 2H,*J* = 8.30 Hz), 7.67 (s, 2H), 7.46 (d, 2H, *J* = 7.55 Hz), 7.25(s, 1H), 7.04 (d, 1H, *J* = 8.30 Hz), 5.82 (s, 2H), 5.52 (s, 6H), 3.87(s,6H);^{13}C NMR (75 MHz, $CDCl_3$) δ 182.5, 181.1, 148.4, 148.1, 146.5, 137.2, 135.8, 135.0, 130.0, 129, 128, 120.2, 115.6, 115.4, 109.2, 109.1, 108.0, 107.0, 106.3, 102.1, 68.5, 61.1, 58.1, 56.2, 53.0, 46.3, 44.2, 42.1, 36.7, 30.0; HRMS (ESI): *m/z* calcd. for $C_{33}H_{32}O_4N_5$ 562.2454, found 562.24331 $[M+H]^+$

2-((1-((1-(3, 4-Dimethoxyphenyl)-9H-pyrido [3, 4-b] indol-3-yl) methyl)-1H-1, 2, 3-triazol-4-yl) methyl)-1H-benzo [de]isoquinoline-1, 3(2H) - dione (7n). 82% Yield; Mp:182–185 ^oC; FT-IR (cm^{-1}): 3376, 3157, 2922, 2851, 2361, 1701, 1662, 1627, 1590, 1515, 1438, 1402, 1385, 1358, 1235, 1140, 1029, 961, 846, 777, 739; ^1H NMR (300 MHz ,$CDCl_3$,) δ 8.58 (brs, 1H), 8.54 (d, 2H, *J* = 6.0 Hz), 8.18 (d, 2H, *J* = 7.5 Hz), 8.03 (d, 1H, *J* = 8.3 Hz) 7.73 (d, 2H, *J* = 15.6 Hz), 7.70 (d, 2H, *J* = 8.3 Hz), 7.67 (s, 2H), 7.46 (d, 2H, *J* = 7.5 Hz), 7.25(s, 1H), 7.04 (d, 1H, *J* = 8.30 Hz), 5.82 (s, 2H), 5.52 (s, 6H); HRMS (ESI): *m/z* calcd. for $C_{35}H_{27}O_4N_6$ 595.2094, found 595.20767$[M+H]^+$

3-((4-((Biphenyl-4-yloxy) methyl)-1H-1, 2, 3-triazol-1-yl) methyl)-1-(3, 4-dimethoxyphenyl)-9H-pyrido [3, 4-b] indole (7o). 90% Yield; Mp: 175–178 ^oC; FT-IR (cm^{-1}) 3319, 3140, 2929, 1606, 1514, 1449, 1401, 1113, 832, 744, 697, 606; ^1H NMR (300 MHz, $CDCl_3$): δ 8.66 (brs, 1H), 8.10 (d, 2H, *J* = 8.30 Hz), 7.72 (s, 2H), 7.51(s, 2H), 7.54 (t, 1H, *J* = 8.30 Hz), 7.40 (t, 1H,*J* = 6.79 Hz), 7.29 (s, 2H), 7.15 (s, 2H), 7.11(s, 1H), 7.04 (d, 1H, *J* = 8.30 Hz), 5.90 (s, 2H), 5.25 (s, 2H), 3.94(s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.9, 153.1, 142.3, 141.3, 140.8, 137.8, 133.1, 132.4, 130.5, 130.1, 129.2, 127.9, 127.6, 123.3, 122.9, 121.7, 121.4, 114.8, 119.8, 111.1, 107.9, 72.3, 60.8, 57.1, 56.1; HRMS(ESI): *m/z* calcd. for $C_{35}H_{27}O_4N_6$ 568.2349, found 568.2403$[M+H]^+$

1-(4-Chlorophenyl)-3-((4-((3, 4, 5-trimethoxyphenoxy) methyl)-1H-1, 2, 3-triazol-1-yl) methyl)-9H-pyrido [3, 4-b] indole (7p). 95% Yield; Mp: 135–138 ^oC; FT-IR (cm^{-1}) 3314, 3140, 2961, 2364, 1773, 1628, 1595, 1505, 1503, 1449, 1406, 1321, 1224, 1123, 1049, 802, 738, 699,610; ^1H NMR (300 MHz, $CDCl_3$): δ 8.66 (brs, 1H), 8.02 (d, 2H, *J* = 7.5 Hz), 7.20 (s, 1H), 7.65 (d, 1H, *J* = 8.3 Hz) 7.63 (s, 2H), 7.60 (t, 2H, *J* = 8.3 Hz), 7.50 (s, 1H), 7.21 (s, 2H), 6.12 (s, 2H), 5.32 (s,2H), 5.20(s, 2H), 3.83 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.8, 152.9, 151.3, 148.3, 140.8, 139.5, 134.1, 134.4, 129.5, 129.0, 123.2, 122.9, 111.6, 111.1, 107.9, 72.3, 60.8, 57.1, 56.1; HRMS (ESI): *m/z* calcd. for $C_{35}H_{27}O_4N_6$ 556.1752, found 556.1792$[M+H]^+$

3-((4-((4-Allyl-2-methoxyphenoxy) methyl)-1H-1, 2, 3-triazol-1-yl) methyl)-1-(4-chlorophenyl)-9H-pyrido [3, 4-b] indole (7q). 88% Yield; Mp: 146–149 ^oC; FT-IR (cm^{-1}) 3144, 2924, 2853, 2352, 1625, 1451,1465, 1429, 1397, 1383, 1221, 1135, 913, 733, 602, 453; ^1H NMR (300 MHz, $CDCl_3$): δ 8.66 (brs, 1H), 8.02 (d, 2H, *J* = 7.5 Hz), 7.20 (s, 1H), 7.65 (d, 1H, *J* = 8.3 Hz) 7.63 (s, 2H), 7.60 (t, 2H, *J* = 8.3 Hz), 7.50 (s, 1H), 7.21 (s, 2H), 6.12 (s, 2H), 5.32 (s,2H), 5.20(s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.8, 149.7, 148.2, 146.8, 142.3, 141.3, 139.5, 136.5, 133.2, 132.9, 132.4, 129.3, 129.0, 123.3, 122.9, 122.3, 121.4, 119.8, 115.9, 114.1, 112.3, 111.1, 107.9, 56.1, 39.8; HRMS (ESI): *m/z* calcd. for $C_{35}H_{27}O_4N_6$ 536.1853, found 536.1820$[M+H]^+$

2-((1-((1-(4-Chlorophenyl)-9H-pyrido [3, 4-b] indol-3-yl) methyl)-1H-1, 2, 3-triazol-4-yl) methyl) isoindoline-1, 3-dione (7r). 82% Yield; Mp: 150–153 ^oC; FT-IR (cm^{-1}) 3317, 3143, 2360, 1770, 1713, 1625, 1563, 1396, 1321,1111, 742, 713, 609, 530; ^1H NMR (300 MHz, $CDCl_3$,): δ 8.6 (brs, 1H), 7.92 (s, 2H), 7.84 (s, 2H), 7.52 (t, 1H, *J* = 8.3 Hz), 7.56 (s, 1H), 7.25 (s, 1H), 7.32 (t, 1H, *J* = 7.5 Hz) 5.84(s, 2H), 4.99 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.9, 153.1, 142.3, 141.3, 140.8, 137.8, 133.1,

132.4, 130.5, 130.1, 129.2, 127.9, 127.6, 123.3, 122.9, 121.7, 121.4, 114.8, 119.8, 111.1, 107.9, 72.3, 60.8, 57.1, 56.1; HRMS (ESI): *m/z* calcd. for $C_{35}H_{27}O_4N_6$ 519.1336, found 519.1296$[M+H]^+$

2-((1-((1-(4-Chlorophenyl)-9H-pyrido [3, 4-b] indol-3-yl) methyl)-1H-1, 2, 3-triazol-4-yl) methyl) isoindoline-1, 3-dione (7s). 85% Yield; Mp: 145–148 ^oC; FT-IR:(cm^{-1}) 3311, 3131, 2925, 2854, 2359, 1401, 1122, 736, 603, 504; ^1H NMR (300 MHz, $CDCl_3$): δ 8.66 (brs, 1H), 8.10 (d, 2H, *J* = 8.30 Hz), 7.72 (s, 2H), 7.62(t, 1H, *J* = 8.3 Hz), 7.51(s, 2H), 7.54 (t, 1H, *J* = 8.30 Hz), 7.40 (t, 1H, *J* = 6.79 Hz), 7.29 (s, 2H), 7.15 (s, 2H), 7.11(s, 1H), 7.04 (d, 1H, *J* = 8.30 Hz), 6.82 (t, 1H, *J* = 8.3 Hz), 5.90 (s, 2H), 5.25 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.4, 144.3, 143.8, 142.9, 140.9, 138.1, 132.4, 131.0, 129.5, 129.4, 129.1, 128.3, 123.6, 122.1, 122.7, 120.8, 116.8, 113.5, 113.2, 111.9, 110.5, 105.2 107.9, 72.3, 60.8, 57.1, 56.1; HRMS (ESI): *m/z* calcd. for $C_{35}H_{27}O_4N_6$ 544.0539, found 544.05276$[M+H]^+$

3-((4-((Biphenyl-4-yloxy) methyl)-1H-1, 2, 3-triazol-1-yl) methyl)-1-(4-chlorophenyl)-9H-pyrido [3, 4-b] indole (7t). 90% Yield; Mp: 160-163 ^0C; FT-IR (cm^{-1}) 3153, 2938, 2362, 1583, 1486, 1465, 1393, 1365, 1326, 1237, 1177, 1127, 1005, 831, 745, 724, 692, 434; ^1H NMR (300 MHz, $CDCl_3$): δ 8.66 (brs, 1H), 8.10 (d, 2H, *J* = 8.30 Hz), 7.72 (s, 2H), 7.51(s, 2H), 7.54 (t, 1H, *J* = 8.30 Hz), 7.40 (t, 1H, *J* = 6.79 Hz), 7.29 (s, 2H), 7.15 (s, 2H), 7.11(s, 1H), 7.04 (d, 1H, *J* = 8.30 Hz), 5.90 (s, 2H), 5.25 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.9,157.8, 148.2, 142.8, 141.9, 140.9, 139.5,134.0, 133.1, 132.4, 131.0, 129.5, 129.4, 129.1, 127.3, 127.3, 123.6, 122.1, 121.7, 120.8, 116.8, 113.5, 113.2, 111.9, 110.5, 105.2 107.9, 72.3, 60.8, 56.1; HRMS (ESI): *m/z* calcd. for $C_{35}H_{27}O_4N_6$ 542.1746, found 542.1703$[M+H]^+$

4.3. MTT assay

Cytotoxicity of formulations was determined by MTT assay based on mitochondrial reduction of yellow MTT tetrazolium dye to a highly colored blue Formosan product. $1x10^4$ Cells (counted by Trypan blue exclusion dye method)) in 96 - well plates were incubated with formulations and standard Erlotinib with series of concentrations for 48 hrs at 37 ^oC in MEM with 10% PBS medium. Then the above media was replaced with 90 µl of fresh serum free media and 10 µl of MTT reagent (5mg/ml) and plates were incubated at 37 ^oC for 4h, there after the above media was replaced with 200 µl of DMSO and incubated at 37 ^oC for 10 min. Human tumor cell line consisted of PC-3 (Prostate cancer), MCF-7 (Breast cancer), Hela (Cervical cancer), HT-29 (colon cancer), HGC (Human gastric carcinoma). In all these experiments, triplicates were used to determine each point.

4.4. Circular dichroism (CD) studies

Circular dichroism experiments were carried out using JASCO 815 CD spectropolarimeter (Jasco, Tokyo, Japan). CD spectrum was recorded from 230 to 310 nm to find out the conformational changes in the CT DNA after derivatives interaction. For each CD experiment, $15x10^{-6}$ M of CT DNA was used initially. Further, for the characterization of derivatives –DNA interaction and find the conformational changes in CT DNA. CD spectra were recorded in 1:1 and 1:2 molar ratios of CT DNA and derivative respectively. CD titrations were performed in 100 mM KBPES buffer (pH 7.0) at 25^oC. Each spectrum was recorded three times and the average of three scans was taken.

4.5. UV-Visible spectral studies

UV-Visible spectroscopic titrations were performed using ABI Lambda 40 UV-Vis spectrophotometer (Foster City, USA) at 25 ^oC using 1 cm path length quartz cuvette. A stock solution of 25 µM of derivatives was prepared in DMSO and 25 µM CT DNA were prepared in 100 mM KBPES buffer (30 mM Potassium Phosphate with 100 mM KCl, pH 7.0). UV-visible absorption titrations were done by adding CT-DNA stock solution (25 µM) in 100 mM KBPES buffer to the quartz cuvette containing approximately 25 µM complex solution. Preparation of CT-DNA and complexes were done on the same day of performing the experiment. Titrations were carried out until the derivatives absorption band remains at a fixed wavelength upon successive additions of CT-DNA. In the present experiment, a well-known β-carboline molecule namely Harmine was considered as control. UV-visible titration was carried out between Harmine-CT-DNA also under the same experimental conditions. 25 µM of Harmine was dissolved in 1:1 water:methanol solution. The stock solution was heated to 60 ^oC to increase the solubility of Harmine. UV-visible spectra was recorded from 250 nm to 400 nm.

4.6. Fluorescence emission spectra

Fluorescence emission spectra were measured at 25 ^oC using a Hitachi F4500 spectrofluorimeter (Maryland, USA) using a 1 cm path length quartz cuvette. Quartz cuvettes was thoroughly washed with distilled water and dilute nitric acid (approximately 0.1 N) to minimize non-specific binding of the derivative's molecules to the surface of the cuvette. Throughout the fluorescence experiment, concentration of the derivatives were kept constant (10 µM) and titrated with increasing concentrations of CT-DNA (multiples of 0.5 µM). Fluorescence spectra were recorded after each addition of CT-DNA to the fluorescent cuvette. Emission spectra of 7f, 7n and 7s show prominent peaks at 512.5 nm, 875 nm and 573 nm respectively. Titration with Harmine was considered as a control and for 2 µM of Harmine in the fluorescence cuvette, each time, 0.5 µM of CT-DNA was added. Harmine was excited at 327 nm and emission spectra were recorded from 360 nm to 500 nm. Each spectrum was recorded three times and the average of three scans was taken.

4.7. Viscosity measurements

Viscosity experiments were conducted on Ostwald's viscometer, immersed in a water bath maintained at 25 ^oC. Titrations were performed for each derivative (5µM), while it was added to CT-DNA solution (50 µM) present in the viscometer. 5µM of Ethidium bromide, Harmine and Hoechst 33258 solution was used as controls in the experiment. DNA solution was prepared in 100 mM *Tris*-HCl (pH 7.0). Data were presented as $(\eta/\eta_o)^{1/3}$ versus the ratio of the concentration of the hybrid to CT-DNA, where η is the viscosity of CT-DNA in the presence of hybrid and η_o is the viscosity of CT-DNA alone.

4.8. Molecular Modelling

The hybrids **7a**–**t** were constructed using molecular builder of Maestro 9.5, prepared using Ligprep 2.7 and geometrically minimized with Macromodel 10.1 based on OPLS-2005 force field followed by their Macromodel conformational analysis. Truncated Newton Conjugate Gradient (TNCG) minimization method was used with 500 iterations and a convergence threshold of 0.05 kJ/mol was applied. The $d(GAAGCTTC)_2$ duplex was prepared and optimized using protein preparation tool. The Glide XP 6.0 algorithm was employed using a grid box volume of 10x10x10 Å for ligands docking.

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Highlights

- A series of C3-tethered 1,2,3-triazolo-β-carboline derivatives were synthesized.
- Cytotoxicity tested on HeLa, HT-29, MCF, PC-3 and HGC-27 cell lines.
- DNA-binding affinity was evaluated by spectroscopic studies.
- Compounds showed potent DNA electrostatic binding and *in vitro* cytotoxicity.
- Viscometric titration and molecular docking established DNA minor groove binding.