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Design and synthesis of 1,2,3-triazolo-phenanthrene hybrids as cytotoxic agents

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ABSTRACT

A series of new 1,2,3-triazolo-phenanthrene hybrids has been synthesized by employing Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction. These compounds were evaluated for their *in vitro* cytotoxic potential against various human cancer cell lines viz. lung (A549), prostate (PC-3 and DU145), gastric (HGC-27), cervical (HeLa), triple negative breast (MDA-MB-231, MDA-MB-453) and breast (BT-549, 4T1) cells. Among the tested compounds, **7d** displayed highest cytotoxicity against DU145 cells with IC_{50} value of 1.5 ± 0.09 μM. Further, the cell cycle analysis shown that it blocks G0/G1 phase of the cell cycle in a dose dependent manner. In order to determine the effect of compound on cell viability, phase contrast microscopy, AO/EB, DAPI, DCFDA and JC-1 staining studies were performed. These studies clearly indicated that the compound **7d** inhibited the cell proliferation of DU145 cells. Relative viscosity measurements and molecular docking studies indicated that these compounds bind to DNA by intercalation.

Heterocyclic scaffolds capable of DNA targeting are the most promising agents in the anticancer drug discovery process.[1] Intercalation is one of the major modes of drug-DNA interaction that is exhibited by various potent natural and synthetic antitumor agents such as doxorubicin, acridines, anthraquinones, distamycins, and phenanthrene derivatives.[2] Combilexins are a class of DNA binding agents that exert their mode of action *via* both minor groove binding and intercalation. They are capable of strong binding with DNA than any other class of DNA binders due to their dual mode of binding.[3] Phenanthroindolizidine alkaloids were originally isolated from plants of the *Asclepiadaceae* family,[4] and are well-known to exhibit diverse pharmacological activities such as antitumor,[5] anti-inflammatory,[6] anti-microbial,[7] antifungal,[8] antiarthritis,[9] antilupus,[10] antiviral[11] and anti-angiogenic activities.[12] The most noteworthy biological property among these is the intense cytotoxic activity against various cancer cell lines, including multidrug-resistant cell lines.[13] This can be attributed to the inhibition of the enzymes involved in DNA synthesis[14] and intercalation of the phenanthrene core between the DNA base pairs.[15] Tylophorine and tylocrebrine (Fig. 1) are the representative natural alkaloids from this class.

On the other hand 1,2,3-triazoles are regarded as privileged building blocks for the synthesis of bioconjugates because of their high stability, selectivity and less adverse reactions.[16] They are highly stable under basic and acid hydrolysis including oxidative and reductive conditions. Moreover, this heterocycle is the bioisostere of amide and is capable of interacting with biomolecular targets through hydrogen-bonding.[17] This attractive chromophore displays a wide variety of activities like antibacterial, antifungal, antiallergic, anti-HIV, antitubercular, anticancer, antiviral, antimalarial and anticonvulsant profile.[18] Additionally, it can also interact with DNA and acts as a supporting motif for DNA targeting drugs.[19] The Cu(I)-catalyzed azide-alkyne cycloaddition or 'click reaction' can rapidly yield bioactive molecules linked through 1,2,3-triazoles[20] with high atom economy and has been found wide range of applications in combinatorial synthesis,[21] bio-conjugate chemistry[22] and material science.[23]

The Structure Activity Relationships (SARs) revealed that the substitution of a polar pharmacophore at C9-position of the phenanthrene ring is of prime importance for potential antitumor activity.[24] Hence, the incorporation of the polar 1,2,3-triazole ring at C9-position might effectively enhance the antitumor activity. As part of our scientific contributions toward bioactive scaffolds,[25] herein we have designed and synthesized a new series of 1,2,3-triazolo-phenanthrene hybrids by using diverse azide and alkyne building blocks. The newly synthesized compounds **7a–o** were fur-

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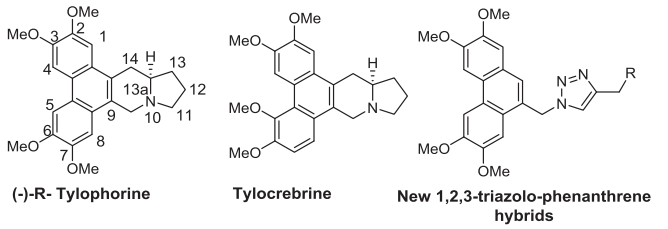
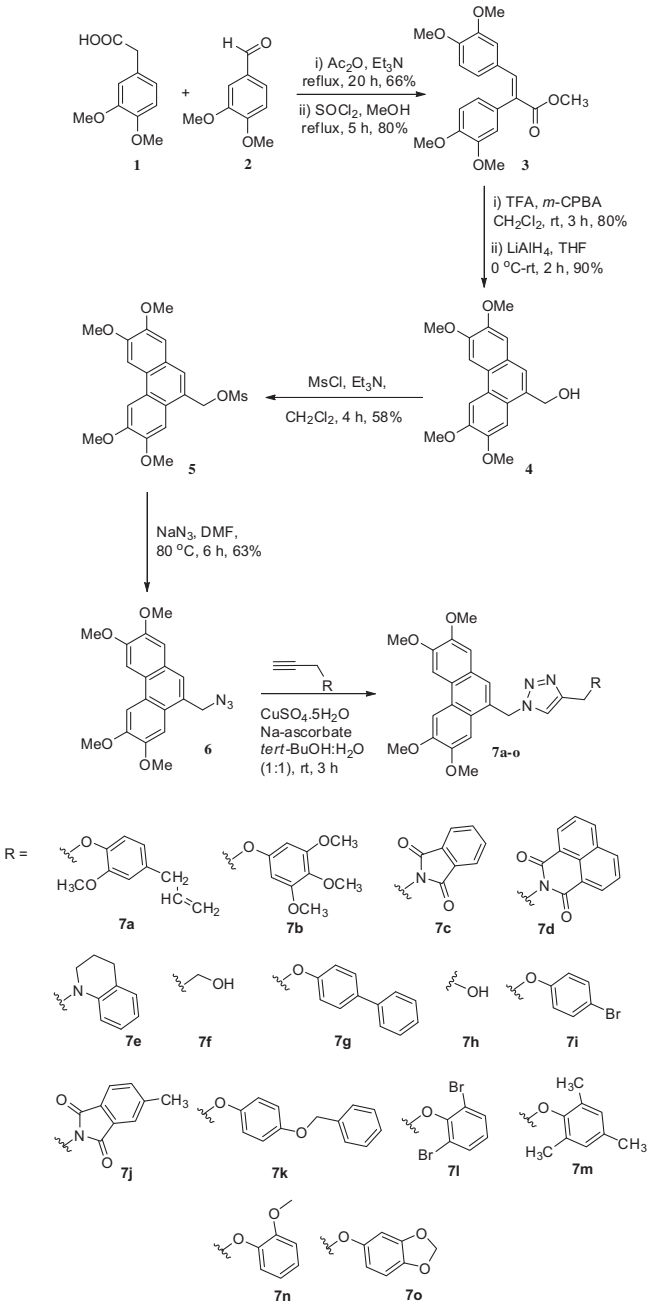


Fig. 1. Structures of Tylophorine, Tylocrebrine and the newly synthesized C-9 linked 1,2,3-triazolo-phenanthrene hybrids.

ther evaluated for their *in vitro* cytotoxicity, cell growth inhibition and DNA interaction.

The synthetic route for the preparation of 1,2,3-triazolo-phenanthrene hybrids **7a–o** was outlined in Scheme 1. The azide partner **6** was synthesized from 2,3,6,7-tetramethoxy phenanthren-9-yl methanol (**4**). The intermediate **4** was prepared from the commercially available starting materials **1** and **2** *via* the conventional four step sequence according to the reported procedure.[26] Initially, Perkin condensation of 3,4-dimethoxybenzaldehyde (**2**) and 3,4-dimethoxy phenyl acetic acid (**1**) resulted in the formation of 2,3-bis(3,4-dimethoxyphenyl)acrylic acid. The acid was converted into its methyl ester **3** followed by *m*-CPBA/TFA mediated intramolecular oxidative cyclization to give the tricyclic phenanthrene ester. The ester functionality was reduced to alcohol **4** by $LiAlH_4$. The alcohol **4** was mesylated by using mesyl chloride in the presence of Et_3N to give **5**. As this intermediate is highly unstable, then it was immediately taken-up for azidation reaction by using NaN_3, refluxing in DMF to afford 9-(azidomethyl)-2, 3, 6, 7-tetramethoxyphenanthrene (**6**). Next for the synthesis of new 1,2,3-triazolo-phenanthrene hybrids, different substituted alkyne building blocks *i.e., N*- and *O*-linked alkynes were prepared from propargylation of substituted amines and phenols respectively. Finally, we tailored these azides and alkynes by employing 'CuAAC' reaction to obtain the new hybrids **7a–o** in overall 63–90% yields. All the newly synthesized compounds **7a–o** were characterized by IR, HRMS, ^1H and ^{13}C NMR spectroscopy.

The newly synthesized 1,2,3-triazolo-phenanthrene hybrids **7a–o** were evaluated for their *in vitro* cytotoxicity on nine different cancer cell lines such as lung (A549), prostate (PC-3 and DU145), breast (BT-549 and 4T1), gastric (HGC-27), cervical (HeLa) and triple negative breast cancer (MDA-MB-231 and MDA-MB-453) by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[27] The IC_{50} (μM) values (concentration required to inhibit 50% of the tumor cells) of the tested compounds and the reference drug 5-fluorouracil (5-FU) are listed in Table 1. It is noticeable from the initial screening that the hybrids **7c**, **7d**, **7j** and **7o** displayed broad range of activity against majority of the tested human cancer cell lines with IC_{50} values ranging from 1.5 ± 0.09 μM to 18.9 ± 0.81 μM (Table 1). Among them, one of the compounds **7d** with a napthalimide substitution at C9-position linked through 1,2,3-triazolo moiety was found to be more active on all the cancer cell lines except A549 and exhibited highest potency on DU145 cell line (IC_{50} 1.5 ± 0.09 μM). It was also observed that, the derivatives **7j** and **7o** were active against A549 cells with IC_{50} values of 12.3 ± 0.42 and 14.5 ± 0.61 μM respectively. Compounds **7c**, **7d**, **7e**, **7g, 7j, 7l** and **7o** displayed activity less than 20 μM in PC-3 cells, among which the compound **7j** possessing 1,2,3-triazolo methyl phthalimide substitution proved to be best with an IC_{50} of 7.12 ± 0.73 μM, followed by compound **7c** with a 1,2,3-triazolo pthalimide substitution (IC_{50} 7.6 ± 0.32 μM) and then compound **7d** having 1,2,3-triazolo napthalimide substitution (IC_{50} 8.5 ± 0.82 μM). Except **7e**, **7g**, **7h**, and **7m**, all other derivatives were found to be active on HGC-27 cell line and **7d**



Scheme 1. Synthesis of various C9-linked 1,2,3-triazolo-phenanthrene hybrids **7a–o**.

being the most active (IC_{50} 3.5 ± 0.24 μM) followed by **7i** with the substitution of 4-bromo phenyloxy group (IC_{50} 3.9 ± 0.16 μM). Derivatives **7b**, **7c**, **7h**, and **7o** showed moderate activity on MDA-MB-231 triple negative breast cancer cells with IC_{50} values ranging from 6.8 ± 0.13 to 19.2 ± 0.46 μM. Interestingly, compound **7o** with a triazolo-benzodioxolo substitution was found to be active on most of the tested cancer cell lines. Compound **7b** with a trimethoxy phenyl substitution proved to be selectively cytotoxic against HGC-27 and MDA-MB-231 cell lines with IC_{50} values of 14.8 ± 0.37 μM and 19.2 ± 0.46 μM respectively. It could be easily observed from the *in vitro* cytotoxicity data that the hybrids with napthalimide and phthalimide groups at C9-position of triazolo-phenanthrene scaffold elevated the biological response. On the other hand, compound **7o** with a triazolo-benzodioxolo substitu-