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Natural Product Research
Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: https://www.tandfonline.com/loi/gnpl20

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To cite this article: Srinivas Nerella, Shravankumar Kankala & Brahmeshwari Gavaji (2019): Synthesis of podophyllotoxin-glycosyl triazoles *via* click protocol mediated by silver (I)-*N*-heterocyclic carbenes and their anticancer evaluation as topoisomerase-II inhibitors, Natural Product Research, DOI: 10.1080/14786419.2019.1610958

To link to this article: https://doi.org/10.1080/14786419.2019.1610958

NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2019.1610958

Synthesis of podophyllotoxin-glycosyl triazoles *via* click protocol mediated by silver (I)-*N*-heterocyclic carbenes and their anticancer evaluation as topoisomerase-II inhibitors

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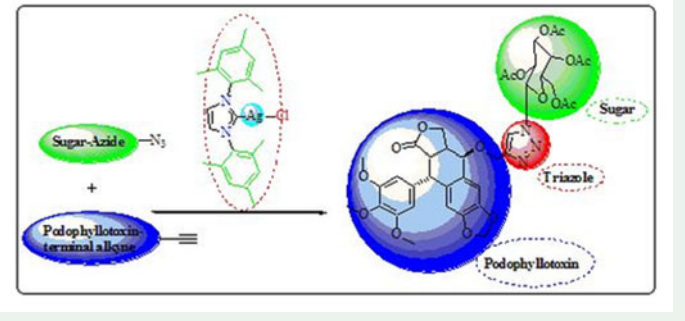
ABSTRACT

Herein we report the regioselective synthesis of podophyllotoxin-Glycosyl triazole hybrids catalysed by Ag(I)-*N*-heterocyclic carbene (Ag(I)-NHC) in a short reaction time (~30 min) at ambient conditions. In principle, it is the first report of Click alkyne-azide cycloaddition catalysed by Ag(I)-NHC catalyst and moreover, this new methodology yielded good results when compared with traditional CuAAC in terms of reaction time and selectivity. The synthesised compounds were further explored for *in vitro* anticancer activity against four human cancer cell lines Du145, HeLa, A-549, and MCF-7 and found that these synthesised compounds possess significant anticancer activity. Further, the compounds **5a** and **5e** were identified as promising leads due to their better activity across all cell lines than that of the standard drug etoposide. Molecular docking studies of 5a & 5e with DNA Topoisomerase-II were revealed that the free energy calculations of active compounds were in good agreement with observed IC_{50} values.



ARTICLE HISTORY
Received 10 January 2019
Accepted 10 April 2019

KEYWORDS
Podophyllotoxin; click chemistry; Ag (NHC) catalyst; podophyllotoxin-glycosyl triazoles; anticancer studies

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Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2019.1610958

1. Introduction

Plants of Podophyllum species were known for their curative properties for many centuries. In folklore medicine, the plant parts and its crude extracts were used as cathartic, purgative and also to treat genital warts and molluscum contagiosum (Teillac-Hamel et al. 1996). Later on it was identified that Podophyllotoxin (**1**), is the main component present in Podophyllum species which is responsible for its healing properties. Podophyllotoxin is a naturally occurring cyclolignan that can be extracted from roots and rhizomes of Podophyllum species such as Podophyllum peltatum and Podophyllum hexandrum (You 2005).

Podophyllotoxin and its analogues exhibit very interesting and diverse pharmacological activities such as insecticidal, antiviral, antifungal, antitumor, anti-inflammatory, antioxidative and antispasmogenic (Gordaliza et al. 2004; Lv and Xu 2011). However, its severe gastrointestinal side effects restricted its use as therapeutic agent (Pettit et al. 2016). To overcome these side effects, several analogues were synthesised and reported by various groups. Among all the activities, its antitumor activity (Cheng et al. 2014) attracted much attention after the success of its semi synthetic derivatives etoposide and teniposide as effective anticancer drugs (Gupta et al. 2006). Some of these semi synthetic derivatives differ significantly from their parent compound i.e., podophyllotoxin, in their mechanism of action. Podophyllotoxin is a Tubulin inhibiting agent (Kamal et al. 2014) whereas etoposide and its analogues are inhibitors of DNA Topoisomerase-II (Mariani et al. 2015).

DNA Topoisomerases are set of enzymes which operate on topology of DNA and essential for unwinding of DNA strands during replication (Rybenkov et al. 1997). DNA strands must be separated during replication process, but super coiled nature of DNA doesn't allow this. At this juncture, Topoisomerase–II binds to DNA and causes breaks and seals to strands to ease the torsional strain leading to separation of DNA strands. Drugs which target Topoisomerase-II inhibit this action leading to stoppage of DNA replication and eventually leading to arrest of cell proliferation. Etoposide, a glycoside of podophyllotoxin also works on these lines (Byl et al. 2001). Hence we would like to synthesise etoposide like glycosides by reacting *O*-propargyl podophyllotoxin with glycosyl azides. On the other hand, triazoles also exhibit a broad spectrum of biological activities like Podophyllotoxin (Yempala et al. 2014). Triazole ring is a dynamic pharmacophore containing high dipole moment and thus able to participate actively in hydrogen bond formation as well as in dipole-dipole interactions with biological targets (Whiting et al. 2006). Especially Triazole moiety is prevalent in natural products and also in potential pharmaceutical compounds like carboxyamidotriazole (CAI), Tazobactum and cefatrizine. In addition to this, even glycosyl triazoles have their own significance in medicinal chemistry as chemical therapeutics (Zhang et al. 2005).

N-Heterocyclic Carbenes (NHCs) were already well established as versatile organo and organometallic catalysts and found diverse and myriad applications in organic transformations (Rafet and Naim 2017). Amidst diverse properties of NHCs, their catalytic property is the most significant and studied elaborately. We reported previously cooperative effect between Ag (I) and organo-NHCs in catalyzing a multicomponent one-pot reaction (Kankala et al. 2015). Literature survey revealed that the organo and organometallic NHCs have not yet been explored extensively for alkyne-azide

R=Tetracetylglucosyl (**5a**), Glucosyl (**5b**), Tetracetyl C2-glucosyl (**5c**), C2-Glucosyl (**5d**), Tetracetylgalactosyl (**5e**), galactosyl (**5f**), Tetracetyl mannosyl (**5g**), Mannosyl (**5h**), Tetracetylarabinosyl (**5i**), arabinosyl (**5j**), Tetracetylxylosyl (**5k**), xylosyl (**5l**).

Figure 1. Synthesis of Podophyllotoxin-glycosyl triazoles **(5a–l)**.

cycloaddition. Therefore we would like to employ diverse NHCs for the click 1, 3-dipolar cyclisation reaction to find out the efficacy of these catalysts and the best catalyst among them.

2. Results and discussion

This work describes a facile route for the regioselective synthesis of 1, 4- disubstituted podophyllotoxin-glycosyl triazoles *via* catalytic 1, 3-dipolar cycloaddition of sugar azides and 4β-*O*-propargyl podophyllotoxin. We have made 4β-*O*-propargylated podophyllotoxin to react with pre-synthesised sugar azides in cycloaddition to obtain podophyllotoxin-glycosyl triazole hybrids. The 4β-*O*-propargyl podophyllotoxin (**3**) was obtained from podophyllotoxin (**1**) and propargyl alcohol (**2**) in presence of Boron trifloride etherate in a single step synthesis (Figure 1). The synthesised compound (**3**) was then treated with sugar azides (**4a–l**) in the presence of Ag (I)-NHC catalyst to obtain podophyllotoxin-glycosyl triazole hybrids (**5a–l**).

Initially we chose to study 1, 3-dipolar cycloaddition reaction between 4β-*O*-propargyl podophyllotoxin (**3**) and galactose azide (**4e**) in ethanol. We would also like to explore the efficacy of a variety of potential catalysts in catalyzing 1, 3-dipolar cycloaddition as depicted in Table S1. There was no cycloaddition observed in the absence of catalyst (Table S1, entry 1). We are also interested to know the progress of click reaction employing widely used copper salts like CuI, $CuSO_4$ and $Cu(OAc)_2$, but obtained products in trace amounts after 24 h at reflux conditions (Table S1, entries 2–4). When silver salts (AgOAc and AgOTf) and zinc salts ($Zn(OAc)_2$ and $Zn(OTf)_2$) were employed as catalysts there was also no progress in the above cycloaddition reactions at reflux temperature (Table S1, entries 5–8).

However, when Ag(I)-NHC (NHC precursor = 1,3-Bis (2,4,6-trimesitylphenyl) imidazolium chloride) was employed as catalyst, the results were remarkable yielding podophyllotoxin-glycosyl triazole hybrid (**5e**) selectively just in 30 minutes with better yields (94%, Table S1, entry 9). On the other hand, previous reports revealed that alkyne-azide cycloaddition reaction employing catalysed and uncatalysed methodlogies was accomplished only under refluxing conditions with longer reaction times. The out come of these results clearly demonstarted the efficiency of Ag(I)-NHC as a potential catalyst for 1,3-dipolar cycloaddition.

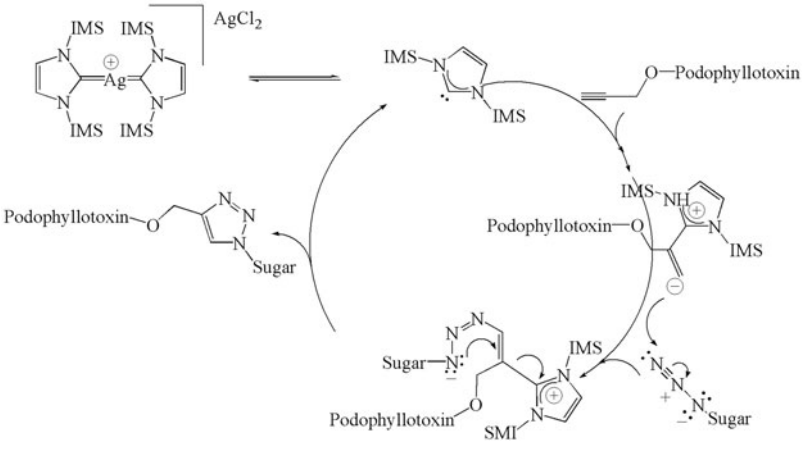

Figure 2. A plausible mechanism for the formation of 1, 4-disubstituted triazoles catalyzed by Ag (I)-NHC.

We have also interested to investigate the above cycloaddition reaction using other co-catalysts (CuI & $Zn(OTf)_2$) along with Ag(I)-NHC and the results were comparable with Ag(I)-NHC (Table S1, entries 10 and 11). We have also studied the same reaction using simple organo-NHC as a catalyst and found that the reaction was relatively slow and the yields of triazoles were moderate (Table S1, entry 12). However, we have got better yields when Silver salt (AgOTf) was introduced during the reaction course into the organo-NHC mediated cycloaddition (Table S1, entry 13) than the organo-NHC alone mediated 1,3-dipolar cycloaddition.

Employing the same optimised conditions (RT, 5 mol% Ag(I)-NHC, 30 min, ethanol), we investigated the scope of the reaction with a variety of structurally diverse sugar azides (**4a**–**l**) for the cycloaddition reaction and the results are depicted in Table S2. The structures of the podophyllotoxin-glycosyl triazole hybrids (**5a**–**l**) were established on the basis of elemental analysis and spectral data (^{1}H NMR, ^{13}C NMR and Mass).

The literature reports reveal that the synthesis of regioselective Podophyllotoxin-triazole hybrids from 4β-azidopodophyllotoxin and terminal alkynes was easy to achieve. However, the selective synthesis of these hybrids from 4β-*O*-propargylpodophyllotoxin and azides (simple or sugar) is still remain as a challenge to accomplish. All of the earlier efforts were not so successful and produced mixture of regioisomers (Hong et al. 2011; Mahesh et al. 2014). The results and conditions depicted in Table S2 for the regioselective synthesis of podophyllotoxin-glycosyl triazole hybrids (**5a**–**l**) suggest that our yields and reaction time are comparable or even better than those for the closely related synthesis of podophyllotoxin-triazole hybrids in earlier reports (Bhat et al. 2008; Reddy et al. 2011).

Based on the results obtained in the regioselective synthesis of 1, 4-disubstituted-1, 2, 3-triazoles a plausible mechanism has been deduced as depicted in Figure 2. The formation of triazole could occur in a domino fashion in cycloaddition. According to Figure 2, the organo-NHC catalyst will interact first with the 4β-*O*-Propargylated podophyllotoxin to form a zwitterion. The reactive zwitterionic species will now interact with sugar azide through nucleophilic attack and form another zwitterion *via* C-N

bond formation. This undergoes finally to C-N heterocyclisation to produce regioselectively the corresponding 1, 4-disubstituted-1, 2, 3-triazole.

2.1. In vitro anticancer activity

The anticancer activity of podophyllotoxin-glycosyl triazole hybrids (**5a–l**) was evaluated by MTT assay against human tumor cell lines Du145, HeLa, A-549 and MCF-7 and compared with etoposide as working standard, and the final inhibitory concentration to an extent of 50% was calculated using the reported methods (Mosmann 1983). The *in vitro* cytotoxicity values depicted in Table S3 indicate that the podophyllotoxin-glycosyl triazole hybrids (**5a**–**l**) show promising anticancer activity against all the tested cancer cell lines.

Among the synthesised compounds, **5a** and **5e** have shown more potent antitumor activity across all cell lines than the standard drug etoposide. The compound **5a** has shown the best activity against Prostate cancer cell lines (Du 145) with IC_{50} value $1.02 \pm 0.02\,\mu M$ whereas **5e** has shown the best activity against breast cancer cell lines (MCF-7) with IC50 value $1.25 \pm 0.02\,\mu M$. As far as the structure–activity relationship is concerned, some correlations can be drawn from the data to support the observed cytotoxic activity based on the type of the sugar substituents present on the triazole ring. The podophyllotoxin-glycosyl triazole hybrids bearing both protected and unprotected sugars have shown potential anticancer activity. However, the compounds with acetyl protected sugars particularly **5a** and **5e** have exhibited better anticancer activity than the unprotected sugars. This can be attributed to the presence of acetyl groups which have high binding affinity with the enzyme as supported by the docking studies. This effective drug-receptor interaction might be one of the promising reasons for the improvement in the activity of acetyl protected podophyllotoxin-glycosyl triazole hybrids.

2.2. Molecular docking studies

Docking studies of **5a** and **5e** were performed with Auto Dock software to reveal the interactions of these compounds with the active sites of Topoisomerase-II enzyme with PBD ID 1ZXN (Figure S1). The results were impressive showing high binding affinity of these compounds towards the enzyme and a free energy of −9.2 and −9.3 was observed for **5a** and **5e** respectively.

Docking studies of **5a** revealed that the molecule bound well with the enzyme through various types of interactions such as Hydrogen bonding, alkyl, pi-alkyl and carbon hydrogen interactions. Glycosyl moiety was shown strong binding affinity with the enzyme through hydrogen bonding with amino acids like Aspargine C92, Threonine C184, Arginine C-70, and Serine C-120. Triazole ring was interacted with Arginine C70 *via* pi-alkyl interactions. Other notable interactions of **5a** include the pi-alkyl interaction between aromatic rings (B and E) of Podophyllotoxin and Isoleucine D5.

The compound **5e** has shown strong interactions with the enzyme and formed hydrogen bonds with amino acid residues like ARG C70, SER C121, SER C120 and ASN

C122. It was clearly shown that the sugar moiety of the compound was actively participated in hydrogen bonding. The aromatic rings of Podophyllotoxin were interacted with amino acid residues such as ILE D5 and ARG C70 through pi-alkyl attractions. Other prominent interactions include pi-donor hydrogen between methyl hydrogens of E-ring of podophyllotoxin and ILE D5, ARG D4 and ASP C124. The docking studies and scores of **5a** and **5e** were in good agreement with observed IC_{50} values.

3. Experimental

3.1. General procedure for the synthesis of 4β-(prop-2-ynyloxy) epipodophyllotoxin (3)

Podophyllotoxin (**1**) and propargyl alcohol (**2**) was dissolved in dichloromethane (10 ml) and added Boron trifloride etherate slowly drop wise over a period of 10 minutes while maintaining the temperature at −20 °C and stirring was continued for 2 hours at the same temperature. The combined organic layers were washed consecutively with cold dilute HCl and brine solution. The extracted organic solution was dried under reduced pressure to afford a crude product which was subjected to column to afford pure product (**3**).

3.2. General procedure for the synthesis of sugar azides (4a-l)

Sugar azides were synthesised by the reported procedures in the literature based on Bertho's method. Firstly, Sugars are acetylated, then brominated with HBr in AcOH (33%) at 0 °C and finally made to react with NaN_3 under reflux conditions for 2 h in the presence of PTC to yield corresponding glycosyl azides, which were subjected to column chromatography to afford pure products (**4a**–**l**)

3.3. General procedure for the synthesis of podophyllotoxin-triazole hybrids (5a-l)

O-Propargylated podophyllotoxin (**3**) and Ag (I)-NHC was dissolved in dry EtOH and added sugar azide (**4a**–**l**). The reaction mass was stirred for 30 minutes. The organic layer was separated and the aqueous layer extracted with dichloromethane. The combined organic layers were dried and evaporated under reduced pressure to afford a crude product which was subjected to column chromatography to afford pure products (**5a**–**l**).

4. Conclusion

In conclusion, we have developed a simplistic catalytic approach for the regioselective synthesis of 1, 4-disubstiututed podophyllotoxin-glycosyl triazole hybrids in high yields and in short period of time. To our knowledge, this is the first methodology developed, optimised and extensively studied with Ag (I)-NHCs as catalyst for click reaction. These advantages make this methodology facile, appropriate and can be put into practice to create diverse compound libraries in future. The synthesised hybrid

molecules **5a** and **5e** have shown impressive *in vitro* anticancer activity and thus identified as promising lead compounds for future studies such as *in vivo* studies. The success of Ag (I)-NHCs as versatile catalysts for click reaction opens up entirely new vistas in this field and paves the way for further design and chemical modification of podophyllotoxin as anticancer agents. These promising findings and the computer modelling studies indicate that potent activities may be found in many related podophyllotoxin derivatives in the future.

Acknowledgement

SN would like to thank UGC for financial assistance and study leave through MRP & Faculty Development Programme respectively.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the University Grants Commission, Government of India under Grant number UGC-MRP-5732/15/620.

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