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Synthesis of hybrid Usnic Acid 1,2,3-triazole conjugate with cholesterol using Click Chemistry method

Bhupender S. Chhikara[1,2]*

[1]*Department of Chemistry, Aditi Mahavidyalaya, University of Delhi, Delhi, India.* [2]*Department of Pharmaceutical Science, School of Pharmacy, Lower College Road, Kingston, Rhode Island, US.*

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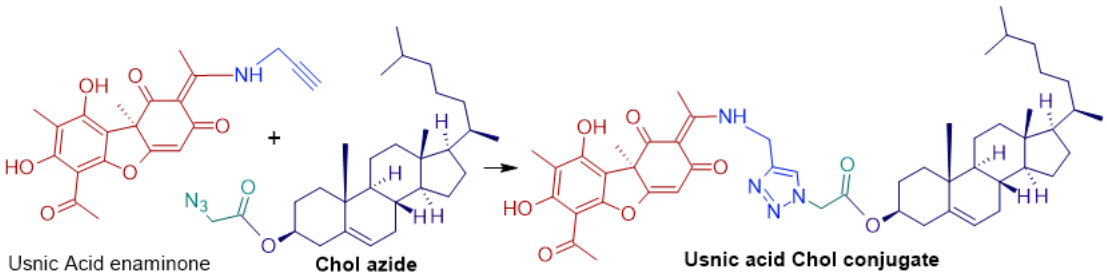
ABSTRACT



Usnic Acid, a natural product obtained from lichens, has been a lead of recent studies from natural products for possible pharmacological activities. Different derivatives, including hybrid molecules, of Usnic acid have been developed and evaluated for different biological activities. Here, in this study, the synthesis of hybrid conjugate molecule of Usnic acid and Cholesterol is reported. The synthesis of molecule was achieved by click chemistry reaction between enanminone of Usnic acid and azide of the cholesterol. Final 1,2,3-triazole conjugate have been characterized by 1HNMR, 13CNMR and mass spectra. The steroid-usnic acid conjugate could be explored for different medicinal and physical studies.

Keywords: Usnic Acid, Natural product, Hybrid compound, Cholesterol derivatives, Click Chemistry, steroid conjugate.

INTRODUCTION

The chemical world of natural products have high diversity of molecular structures, and have long history of exploration in chemistry research towards establishing the structures of different natural product. The diversity of natural products includes terpenes (terpenoids), flavanoids, steroids, carbohydrates, peptides, proteins, tannins, corticosteroids, nucleosides, anthocyanins and other variations. This diversity have brought their applications in different fields including drug discovery.[1] A number of natural products sourced from algae, lichens, fungi, bateria, crabs, plant parts, sea phytoplanktons and zooplanktons are being used as antibiotics, antifungal, anti-oxidants, anti-cancer,[2,3] anti-inflammatory,[4] anti-protozoal activity, anti-viral and a number of other pharmacological activities. Owing to the promise given by natural products, a number of derivatives of these molecules have been development to improve their drug efficiency or to reduce their side effects.

Usnic acid, (2,6-diacetyl-7,9-dihydroxy-8,9b-dimethyl-1,3(2H,9bH)-Dibenzofurandione) is a naturally occurring compound obtained from lichens, and it known to have antibacterial activities. Though Usnic acid is not having carboxylic acid group in its structure (figure 1), this molecule is acidic in nature with a pKa of 4.4, as it derives its acidity from phenolic hydroxyl groups (figure 1).

Discovered in the 1840s by German and Austrian scientists, usnic acid, characterized by its yellow color, was isolated from various lichen genera,[5] notably Usnea, which inspired its name. It naturally exists in (R)- and (S)-enantiomers as well as a racemate.[6]

Despite its health and environmental hazards, indicated in hazard information,[7] usnic acid is found in some over-the-counter dietary supplements, although there is advisory against its use.[8]

Usnic acid's potential medical applications,[9] particularly in cancer research,[10] have been explored. In 2012, Helga M. Ogmundsdottir and colleagues[11] observed its effects on

*Corresponding Author: Dr. B.S. Chhikara
Email: bschhikara@yahoo.com

mitochondrial and lysosomal function in cancer cells,[12] suggesting implications for therapeutic autophagy manipulation and pH-based drug distribution.[11]

More recent research from 2018 by W. Pan et.al.[13] demonstrated usnic acid's induction of cell cycle arrest, apoptosis, and autophagy in gastric cancer cells.[13]

Based on potential shown by different studies using Usnic Acid in cancer, the Usnic acid has garnered extensive development of its different synthetic derivatives towards possible pharmacological activities.[14]

There is need of synthesis of Usnic acid derivates to mitigation of toxic effects. The development of hybrid and novel heterocyclic compounds of the Usnic acid would pave path towards improved or new pharmacological activities.[15] The synthesized derivatives need to be explored for applications in materials development besides pharmacological activities.

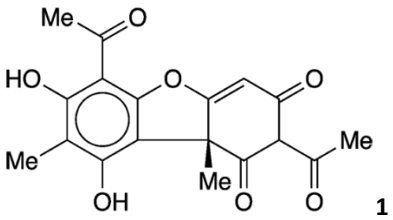
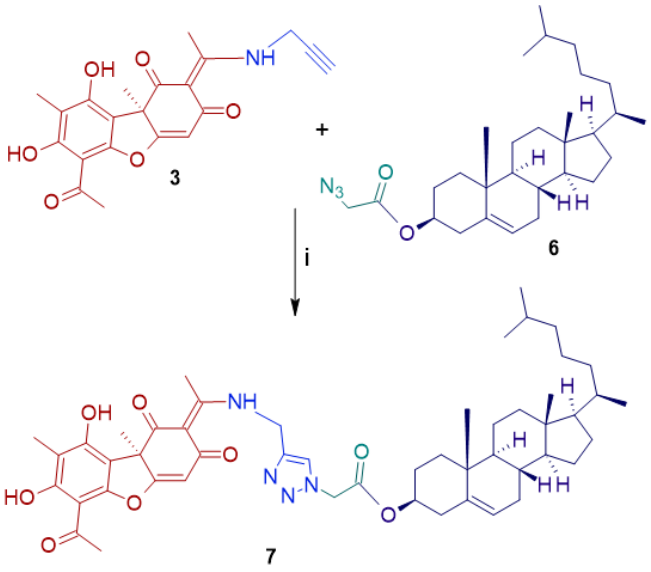


Figure 1. Chemical structure of Usnic acid (**1**).

The cholesterol molecule, a naturally occurring steroid, has been extensively studied for its effects on various aspects of cellular function. It is widely recognized that cholesterol influences properties like fluidity, permeability, strength, elasticity, and stiffness of membranes, as well as parameters such as transition temperature (Tm), drug retention, phospholipid packing, and plasma stability.[16] Its significance extends to crucial roles in cellular membranes and as a precursor for the synthesis of important steroid hormones. Within cell membranes, composed primarily of a double layer of phospholipids, cholesterol profoundly affects membrane fluidity, the structure of microdomains (lipid rafts), and permeability by interacting with both the hydrophilic headgroups and hydrophobic tails of phospholipids. Furthermore, alterations in cholesterol's stereochemistry, oxidation states of its fused rings, side chain, and functional groups give rise to various biologically significant molecules, including bile acids, vitamin D, and numerous steroid hormones. In recent years, cholesterol has become a favored starting material or model system for organic synthesis due to its readily modifiable functional groups, abundance, and affordability. Based on all these properties, the derivatization of molecules with the Cholestrol moiety has been a wide area of explorations for applications of synthesized derivatives in drug discovery as well as in materials sciences.[16,17]

Considering the need of synthesis of new derivatives of the Usnic acid and interesting properties of cholestrol molecules, herein, we report the synthesis of 1,2,3-triazolo linked cholesterol hybrid conjugate of Usnic acid (Scheme 1).



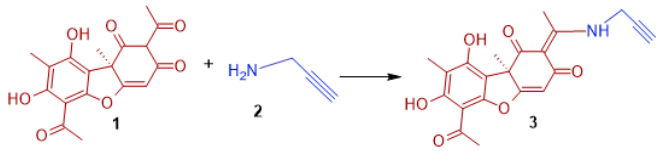
Scheme 1. Chemical synthesis of cholesterol-usnic acid hybrid.

EXPERIMENTAL

Materials and methods

(+)-Usnic acid was procured from the Sigma-Adrich. The reagents and solvents were used as supplied. The chemical structures of final products were characterized by nuclear magnetic resonance spectra (^{1}H NMR, ^{13}C NMR) determined on a Varian NMR spectrometer (500 MHz). Chemical shifts are reported in parts per millions (ppm). ^{13}C NMR spectra are fully decoupled. Chemical shifts were reported in parts per millions (ppm) using deuterated solvent peak or tetramethylsilane (internal) as the internal standards. The chemical structures of final products were confirmed by a high-resolution Biosystems QStar Elite time-of-flight electrospray mass spectrometer.

The compound N-Propargyl Usnic Acid Enaminone (**3**) was synthesized essentially following the procedure reported by P.K. Bangalore et.al.[18] (Scheme 2).

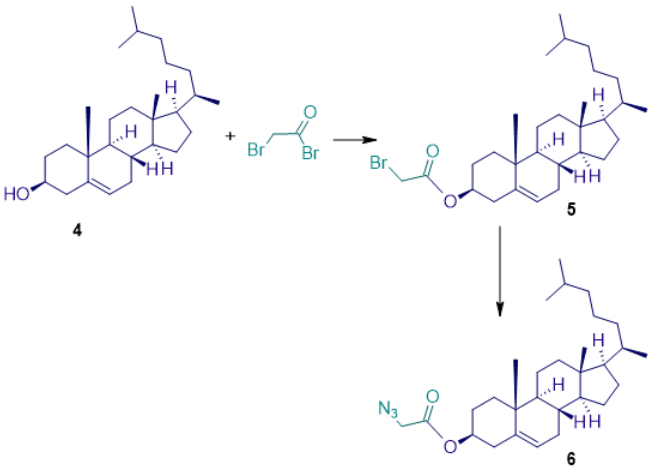


Scheme 2. Synthesis of N-Propargyl Usnic Acid Enaminone (**3**)

(E)-6-acetyl-7,9-dihydroxy-8,9b-dimethyl-2-(1-(prop-2-yn-1-ylamino)ethylidene)dibenzo[b,d]furan-1,3(2H,9bH)-dione (3): Usnic acid (344mg, 1.0mmol) was suspended in absolute ethanol (200mL) in a clean dried 500mL round bottomed flask. Triethylamine (300mg, 3mmol) was added to the reaction mixture and heated to refluxed on oil bath (85 °C) for 5mins. Propargylamine(**2**)(60mg, 1.10mmol) was added slowly to the reaction mixture with stirring and the contents were further refluxed for 4h. The reaction was monitored with TLC. On completion of reaction, the solvent was evaporated under reduced

pressure and contents were extracted into ethylacetate (100 mL x 3). The combined organic layers were washed with brine solution, organic layer collected and dried over sodium sulphate and evaporated under reduced pressure to obtain crude product. The crude was then purified by column chromatography using n-hexane-ethylacetate (7:3), to yield the desired product **3** as light yellow solid. Yield: 307mg (62%); M.p.: 155-158°C (reported:156-158°C); IR spectrum, (ν, cm–1): 3424, 3277, 3072, 2926, 2698, 1691, 1617, 1549, 1463, 1417, 1374, 1279, 1188, 1135, 1065; ^{1}H NMR(300 MHz, $CDCl_3$): δ 13.68 (s, 1H), 13.34 (s, 1H), 11.80 (s, 1H), 5.80 (s, 1H), 4.23 (d, J=2.9 Hz, 2H), 2.69 (s, 3H), 2.66 (s, 3H), 2.44 (s, 1H), 2.08 (s, 3H), 1.70 (s, 3H); 13C NMR (126MHz, $CDCl_3$): δ 200.78, 198.78, 190.89, 175.64, 174.52, 163.62, 158.28, 155.90, 108.20, 105.04, 102.69, 102.36, 101.48, 76.50, 74.33, 57.59, 33.34, 31.95, 31.40, 18.48, 7.61; ESI-MS: (C21H19NO6) 381.37 m/z: calcd: 382.12[M+H]+, found: m/z 382.20 [M+H]+.

The Cholestrolacyl azide (6) was obtained as shown in Scheme 3.



Scheme 3. Chemical synthesis of Cholest-5-en-3β-yl-2-azidoacetate (**6**). Protocol step 1: Br-acetyl-Br, K_2CO_3, DCM, 6hr, step 2: NaN_2, K_2CO_3, DCM, 3hr.

Cholest-5-en-3β-yl-2-bromoacetate (5): Cholesterol (3.86 g, 10.0 mmol) was taken in anhydrous CH_2Cl_2 (100 ml) in round bottom flask, added K_2CO_3 (1.65g, 12 mmol) and reaction mixture was cooled to 4°C in ice bath. The contents were stirred and added dropwise a solution of Bromoacetylbromide (2.02gm, 10.0 mmol) in anhydrous CH_2Cl_2 (50 ml). The reaction was maintained at 4 °C for 1hr, warmed to room temperature, and stirred for 6 hr. The reaction was monitored by TLC (eluent: hexanes / ethyl acetate, 9:1). On completion of reaction, 50ml of distilled water was added and product was extracted using CH_2Cl_2 (50 ml x 3) and carried forward for further reaction. A fraction was purified for analysis. Yield (crude): (4.02 g, 79%) as a white solid, ^{1}H NMR (300 MHz, $CDCl_3$) δ5.42 (s, 1H), 4.52 (m, 1H), 3.02 (s, 2H), 2.50 (m, 2H), 2.04-0.85 (m, 38H), 0.68 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.2, 138.6, 123.7, 81.9, 56.5, 56.0, 49.9, 42.2, 39.6, 39.4, 39.1, 38.7, 36.8, 36.3, 36.1, 35.7, 31.8, 31.7, 28.9, 28.1, 27.9, 24.2, 23.8, 22.8, 22.5, 20.9, 19.1, 18.6, 11.8; ESI-MS (ESI+) cald: 507.5 found: m/z 507.2.

Cholest-5-en-3β-yl-2-Azidoacetate (6). To a solution of Bromoacetyl-Cholest-5-en-3β-ol (**5**, 507mg, 1.0 mmol) in anhydrous CH_2Cl_2 (100 mL) was added K2CO3 (138mg, 1.0mmol) followed by slow addition of NaN_3 (65mg, 1.0 mmol) in small fraction with continuous stirring of reaction mixture. The reaction mixture was further stirred at room temperature for 3 hr. The reaction was monitored by TLC and on completion of reaction, added 50ml of distilled water. The organic layer was collected and further, the aqueous layer was extracted with CH_2Cl_2 (50 ml x2). The combined organic extracts were washed with saturated aqueous NaCl solution (100 ml), dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to afford crude **6** as a yellowish white solid. The crude product was purified by column chromatography (eluent heaxane: ethylacetate 90:10). Yield: 407mg (80%) as a puff-white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.38 (d, 1H), 3.20 (m, 1H), 2.54 (s, 2H), 2.29 (d, *J* = 7.9 Hz, 2H), 2.04-0.85 (m, 38H), 0.68 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.3; 139.8, 122.5, 72.1, 56.7, 56.1, 50.1, 42.3, 39.7, 39.5, 38.1, 37.6, 36.6, 36.2, 35.8, 31.8, 31.7, 28.2, 28.0, 27.9, 24.2, 23.8, 22.8, 22.5, 21.0, 19.2, 18.7, 11.8; ESI-MS: cald: 469.7 found: m/z 470.65 ($M+H^+$).

Usnic acid -triazoyl-chol conjugate (**7**): N-Propargyl Usnic Acid Enaminone (**3**) (382mg, 1.0 mmol) was taken in acetonitrile (20ml) in a 100ml round bottomed flask, added $CuSO_45H_2O$ (500mg, 2.0 mmol), sodium-L-ascorbate (350mg, 2.0 mmol) to the reaction mixture. Compound **6** (469mg, 1.0 mmol) in methanol (20ml) was added slowly to the reaction mixture with continuous stirring at room temperature over 30 min. Reaction mixture was further stirred for 2hr. On completion of reaction as monitored by TLC, the solvent was evaporated under reduced pressure, added 50ml water and extracted with ethylacetate (50 ml x 3). Combined organic organic layers were washed with brine solution, organic layer collected and dried over sodium sulphate and evaporated under reduced pressure to obtain crude product. The crude was then purified by column chromatography using n-hexane-ethylacetate (7:3), to yield the desired product **7** as a yellow solid. Yield: 654mg (77%); ^{1}H NMR(300 MHz, $CDCl_3$): δ 13.86 (s, 1H), 11.80 (s, 1H), 7.8 (s, 1H), 5.90 (s, 2H), 5.76 (s, 1H), 5.38 (d, 1H), 4.87 (s, 1H), 3.20 (m, 1H), 2.69 (s, 3H), 2.66 (s, 3H), 2.54 (s, 2H), 2.44 (s, 1H), 2.04-0.85 (m, 44H), 0.68 (s, 3H); ^{13}C NMR (100MHz, $CDCl_3$): δ 200.80, 198.64, 190.09, 165.64, 163.62, 158.28, 155.90, 139.8, 122.5, 108.20, 105.04, 102.69, 102.36, 101.48, 72.1, 57.59, 56.7, 56.1, 50.1, 42.3, 39.7, 39.5, 38.1, 37.6, 36.6, 36.2, 35.8, 33.34, 31.95, 31.8, 31.7, 31.40, 28.2, 28.0, 27.9, 24.2, 23.8, 22.8, 22.5, 21.0, 19.2, 18.7, 18.48, 11.8, 7.61; ESI-MS: (C50H66N4O8) 851.08 m/z: calcd: 851.495[M+H]+, found: m/z 852.01 [M+H]+

RESULTS AND DISCUSSIONS

Considering the emerging need of new derivatives of the Usnic acid, the hybrid conjugate with cholesterol molecule have been designed and synthesized. The conjugate linkage involved the 1,2,3-triazole heterocyclic moiety achieved through copper salt catalyzed click chemistry reaction.[19–21] For the conjugation, the alkyne group was introduced on the Usnic acid by formation of

the compound N-Propargyl Usnic Acid Enaminone (**3**) synthesized by following the reported procedure by P.K. Bangalore et.al.[18] (Scheme 2). Representatively, for formation of **3**, (+)-Usnic acid (**1**) and propargyl amine (2) were refluxed at 85 °C in absolute EtOH in the presence of Et3N for 4 h. The crude product obtained on removal of solvent was purified by normal phase chromatography using a silica gel column eluted with n-hexane–EtOAc (7:3) to give N-propargyl usnic acid enaminone **3** in good yield. The structure of compound **3** was established using NMR and MS data that matched with the reported data. As Bangalore et.al.[18] has already established that the enaminone selectively formed at the C-14 acetyl group of usnic acid confirmed by Single-crystal X-ray diffraction analysis of compound **3** [crystallized in CHCl3–MeOH (1:9 v/v)], it was carried further for conjugation with azide of cholesterol. The azide on the cholesterol molecules was developed on the -OH group of the cholesterol, first by reacting the Cholesterol with bromoacetyl bromide in presence of potassium carbonate as base and further substitution of bromide group using sodium azide in presence of K_2CO_3 gave the 2-azidoacetylcholesterol (**6**).[22] The final conjugate was formed by click chemistry reaction between N-propargyl usnic acid enaminone **3** and 2-azidoacetylcholesterol (**6**) catalyzed by copper sulphate and sodium-L-ascorbate. The crude product **7**, purified by colum chromatography (silica gel, eluent ethyl acetate and hexane) was obtained as yellow solid in good yield.

Conclusions

In conclusion, the synthesis of usnic acid and cholestrol hybrid conjugate molecule is reported. The two moieties have been connected via 1,2,3-triazole linkage achieved through click chemistry protocol. Further, evaluation of physico-chemical properties of this conjugate hybrid molecule would provide their usability in different fields including polarity (polar type usnic acid and lipophilic type cholesterol moiety) based applications and other gel or surfactant like behaviour of the conjugate. The simple synthesis protocol of this molecule provide the easy accessibility of the molecule for diiferent applications.

Conflict of Interest

Authors declare there is no academic or financial conflict of interest for this work.

Acknowledgments

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