Short Note

**3′-[4-((3β,28-Bis(acetyloxy)lup-20(29)-en-30-yl)oxy)carbonyl]-1H,1,2,3-triazol-1-yl]-3′-deoxythymidine**

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Abstract: The reaction of the azidothymidine (AZT) with the 30-propynoylated derivative of 3,28-O, O′-diacetylbetulin gave a 1,4-disubstituted 1,2,3-triazole. The chemical structure of new derivative was characterized by 1H NMR, 13C NMR and HR-MS. The triterpene-AZT conjugate was tested against a human cancer cell lines such as glioblastoma (SNB-19), amelanotic melanoma (C-32), ovarian adenocarcinoma (SKOV-3) and breast cancer (T47D, and MCF-7). 3′-[4-((3β,28-Bis(acetyloxy)lup-20(29)-en-30-yl)oxy)carbonyl]-1H,1,2,3-triazol-1-yl]-3′-deoxythymidine shown significant activity against MCF-7 cells, with an IC₅₀ value of 4.37 µM.

Keywords: triterpene; 1,2,3-triazole; 1,3 dipolar cycloaddition (CuAAC)

1. Introduction

Natural pentacyclic lupane-type triterpenes are often the major constituents of medicinal plants. These compounds are characterized by a wide range of pharmacological activity and diversity in chemical structures [1]. The lupane family includes triterpenes that exhibit anticancer [2,3], antiviral [4,5], antibacterial [6,7], anti-inflammatory [8,9] and hepatoprotective properties [10]. Scientific research aimed at improving the activity of triterpenes is based on the modification of the triterpene scaffold or functionalization of the hydroxyl or carboxyl groups present in them. Improving bioactivity can also be achieved by introduction a triazole ring into the triterpene moiety. 1,2,3-Triazoles are prepared from the corresponding azides by copper catalyzed 1,3-dipolar cycloaddition (CuAAC) with alkyn derivatives of triterpenes [1,11,12].

One of the possibilities for the synthesis of new biologically active compounds is the combination of two bioactive moieties into hybrid molecule. It is assumed, that the obtained hybrid molecule should have more enhanced biological activity. Due to the anticancer effects of pentacyclic triterpenes on various types of cancer cells, the triterpene scaffold is an attractive moiety for the synthesis of such system. Another a bioactive substance that can be used in the preparation of hybrid compounds is azidothymidine (AZT, Figure 1). This nucleoside reverse transcriptase inhibitor applied in anti-HIV therapy also has an anticancer effect [13].

This study describes the synthesis and anticancer activity of the novel 30-substituted 3,28-O, O′-diacetylbeutelin derivative linked to AZT via a 1,2,3-triazole ring.
The 3,28- diacetyl-30-propynoylbetulin 1 was obtained by the Steglich reaction as previously described in the literature [14]. Propynyl-functionalized derivative 1 was used as starting compound in the 1,3-dipolar cycloaddition (CuAAC-Cooper Catalyzed Azide-Alkyne Cycloaddition). The CuAAC reaction of alkyne derivative 1 with azidothymidine in the presence of copper(I) iodide in refluxing toluene led to the formation of triterpene-AZT conjugate at a 54% yield. The synthesis of 1,2,3-triazole derivative 2 is outlined in Scheme 1.

The chemical structure of compound 2 was confirmed by NMR, IR and HR-MS analysis. The new 1,2,3-triazole derivative 2 and cisplatin (reference drug) were evaluated in vitro for cytotoxic activity against the following human cancer cell lines such as glioblastoma (SNB-19), amelanotic melanoma (C-32), ovarian adenocarcinoma (SKOV-3) and breast cancer (T47D, and MCF-7). The obtained cytotoxicity results expressed as IC$_{50}$ values (half-maximum inhibitory concentrations) are presented in Table 1.

### Table 1. Cytotoxic activity of 1,2,3-triazole derivative 2 and cisplatin against SNB-19, C-32, SKOV-3, MCF-7, and T47D cells (IC$_{50}$ in μM).

| Compound | Human Cancer Cell Lines [IC$_{50}$ ± SD] |
|----------|----------------------------------------|
|          | SNB-19  | C-32  | SKOV-3 | MCF-7  | T47D   |
| 2        | 19.42 ± 1.74 | 10.15 ± 1.03 | 11.97 ± 1.37 | 4.37 ± 0.52 | 32.62 ± 2.48 |
| Cisplatin| 13.10 ± 4.66 | 14.39 ± 2.33 | 36.77 ± 6.66 | 17.53 ± 2.66 | 31.50 ± 9.83 |
The compound 2 showed highest activity against the C-32, SKOV-3 and MCF-7 cell lines higher than cisplatin used as reference drug. For derivative 2, the rank order of the anticancer activity against tested cancer cell lines is as follows: MCF-7 > C-32 > SKOV-3 > SNB-19 > T47D. Comparison of the cytotoxicity of substances 1 and 2 against MCF-7 and C-32 cells indicates that the introduction of the triazole moiety increases the activity. The literature describes betulin-AZT hybrids containing AZT moiety at C-28 position, and acetyl or hydroxyl group at C-3 position of betulin molecule [15,16]. The results of biological studies of these hybrids and compound 2 showed that the position of the AZT moiety influences on anticancer activity against T47D, MCF-7 and SNB-19 cells as follows: 28-O-[1-(3′-deoxythymidine-5′-yl)-1H-1,2,3-triazol-4-yl]carbonylbetulin < 3-acetyl-28-[1-(3′-deoxythymidine-5′-yl)-1H-1,2,3-triazol-4-yl]carbonylbetulin < compound 2.

3. Materials and Methods

3.1. Experimental Procedures

Melting point was detected on an Electrothermal IA 9300 apparatus (Bibby Scientific Limited, Stone, Southampton, UK), and was uncorrected. 1H and 13C NMR spectra were recorded in DMSO-d6 on a Bruker Avance III 600 spectrometer (Bruker, Billerica, MA, USA) at 600 MHz and 150 MHz, respectively. High resolution mass data was performed on a Bruker Impact II instrument (Bruker) using an APCI method (negative mode). Infrared spectrum (KBr, pellet) was measured on an IRAffinity-1 Shimadzu spectrometer (Shimadzu Corporation, Kyoto, Japan). The progress of a reaction and the purity of the compound were monitored by TLC method on silica gel 60 254F plates (Merck, Darmstadt, Germany). The developed plates were sprayed with an ethanolic solution of sulfuric acid and heated to 100 °C. Purification of derivative 2 was carried out by column chromatography [the stationary phase—silica gel 60 (0.063–0.200 mm), the mobile phase—chloroform: ethanol 15:1, v/v]. The reagents and solvents were purchased from the Sigma-Aldrich (Sigma-Aldrich, Saint Louis, MO, USA).

3.2. Synthesis of 3′-[4-[(3β,28-Bis(acetyloxy)lup-20(29)-en-30-yl]oxy}carbonyl)-1H-1,2,3-triazol-1-yl]-3′-deoxythymidine 2

Preparation of Compound 2:

The 3,28-O,28-O′-diacetyl-30-propynoylbetulin 1 was applied as starting material to the synthesis of compound 2. A mixture of compound 1 (0.12 g, 0.21 mmol), azidothymidine (0.06g, 0.22 mmol), and copper(I) iodide (0.0033 g, 0.0011 mmol) in toluene (4.0 mL) was heated at reflux for 72 h. The completeness of the 1,3-dipolar cycloaddition was monitored by TLC. Next, the solvent was removed under reduced pressure. The residue was purified by column chromatography (SiO2, chloroform: ethanol 15:1, v/v).

Yield 54%; mp 163–168 °C; Rf 0.21 (chloroform/ethanol, 15:1, v/v); 1H NMR (600 MHz, CDCl3) δ ppm: 0.80 (s, 3H, CH3), 0.85 (s, 3H, CH3), 0.86 (s, 3H, CH3), 0.96 (s, 3H, CH3), 1.04 (s, 3H, CH3), 1.97 (s, 3H, CH3-AZT), 1.07–2.02 (m, 25H, CH, CH2), 2.07 (s, 3H, CH3C=O), 2.09 (s, 3H, CH3C=O), 2.46 (m, 1H, H-19), 3.88 (m, 1H, AZT), 4.07 (d, J = 12.0 Hz, 1H, H-28), 4.28 (m, 1H, AZT), 4.46 (m, 2H, H-3, AZT), 4.86 (m, 2H, C-30), 5.07 (s, 1H, H-29), 5.09 (s, 1H, H-29), 5.56 (m, 1H, AZT), 6.22 (m, 1H, AZT), 7.45 (m, 1H, AZT), 8.23 (s, 1H, CH-triazol), 8.52 (s, 1H, NH-AZT) (Figure S1 Supplementary Materials); 13C NMR (150 MHz, CDCl3) δ ppm: 12.48, 14.80, 16.47, 18.14, 18.45, 21.09, 23.67, 26.69, 26.97, 27.92, 29.68, 30.97, 31.20, 34.13, 34.39, 37.54, 37.76, 38.44, 40.90, 42.68, 46.37, 49.69, 50.28, 55.30, 58.53, 59.65, 61.41, 62.45, 81.21, 85.35, 89.04, 111.48, 127.58, 137.84, 140.26, 150.03, 160.04, 163.07, 171.09, 172.02 (Figure S2 Supplementary Materials); IR (νmax cm−1, KBr): 1246, 1265, 1367, 1471, 1541, 1685, 1707, 1720, 2947, 3528 (Figure S3 Supplementary Materials); HR-MS (APCI) m/z: C47H67N5O10; Calcd. 861.4888; Found 860.4792[(M-H)−]; (Figure S4 Supplementary Materials).
3.3. Experimental Procedures

3.3.1. Cell Culture

The compound 2 and cisplatin were evaluated for cytotoxic activity in vitro against five human cancer cell lines such as glioblastoma (SNB-19, DSMZ, Braunschweig, Germany), amelanotic melanoma (C-32, ATCC, Rockville, MD, USA), ovarian adenocarcinoma (SKOV-3, ATCC, Rockville, MD, USA) and breast cancer (T47D, and MCF-7, ATCC, Rockville, MD, USA). Cell cultures were maintained using DMEM (Lonza, Basel, Switzerland) supplemented with 10% fetal bovine serum (FBS) (Biological Industries Cromwell, CT, USA) and two antibiotics—penicillin (10,000 U/mL) and streptomycin (10 mg/mL) (Lonza, Basel, Switzerland). The cells were seeded at 5 × 10^4/well in 96-well plates (Nunc Thermo Fisher Scientific, Waltham, MA, USA) and incubated under the conditions recommended by the manufacturer (24 h, 37 °C, 5% CO_2, constant humidity).

3.3.2. WST-1 Assay

The WST-1 test (Roche Molecular Biochemicals, Mannheim, Germany) was applied to evaluate the cytotoxic activity. This colorimetric test determines the ability of viable cells to cleavage of tetrazolium salt of WST-1 to dark red formazan. The cells with compound 2 (concentrations ranging from 1 to 100 µg/mL) and WST-1 were incubated for 72 h. The amount of formazan was quantified by measuring the absorbance at λ = 450 nm with a UVM340 microplate reader (Biogenet, Józefów, Poland).

Supplementary Materials: The following supporting information can be downloaded at: Figure S1: 1H NMR spectrum of derivative 2; Figure S2: 13C NMR spectrum of derivative 2; Figure S3: IR spectrum of derivative 2; Figure S4: HRMS spectrum of derivative 2.

Author Contributions: E.B. conceptualization, writing—original draft preparation, formal analysis, writing—review and editing, investigation; M.K.-T. formal analysis, methodology; E.C. methodology, writing—review and editing; M.L. validation, formal analysis. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by The Medical University of Silesia in Katowice Grant nos. PCN-1-009/N/1/F and PCN-1-010/N/1/F.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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