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Podophyllotoxin-1,2,3-triazole-thiazolidinedione hybrids: Design, synthesis and biological evaluation

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Abstract

A series of 4β-substituted-1,2,3-triazole-thiazolidinedione hybrids of podophyllotoxin were synthesized by employing click chemistry approach. The terminal alkynes were prepared in two steps, the first step employs Knoevenagel condensation and the second step involves propargylation. These terminal alkynes were then condensed with azidopodophyllotoxin under click chemistry reaction conditions to get a series of target compounds. All these compounds were screened for their anticancer activity against two human cancer cell lines using sulforhodamine B assay and showed significant activities against both cancer cell lines.

Key words: Podophyllotoxin, Knoevengal condensation, click reaction, Copper catalyses.

1. Introduction

Podophyllotoxin is a well known naturally occurring cyclolignin isolated from the root of *Podophyllum hexandrum* and has cathartic, antirheumatic and antiviral properties[1]. It is known to be an antimicrotubule agent acting at the colchicine binding site of tubulin[2-4]. Since podophyllotoxin is highly toxic and thus itself is not used as anticancer drug but its 4β-congeners like etoposide and teniposide are clinically used against several cancers including small cell lung cancer, testicular carcinoma, lymphoma, Kaposi's sarcoma, glioblastoma multiforme and leukemia[5-8]. Unlike podophyllotoxin which is antimicrotubule agent, its 4β-congeners such as etoposide[3], teniposide[9] and etopophos are potent DNA topoisomerse II inhibitors. The podophyllotoxin causes cell death by interfering with the function of the mitotic spindle and induces cell apoptosis by promoting mitotic arrest while the topoisomerase II inhibitor etoposide induces cell death by enhancing topoisomerase II-mediated DNA cleavage through the stabilization of the transient DNA-TOP II cleavage complex[10]. In this complex DNA is cleaved on both strands and covalently linked to the enzyme

which prevents dissociation of the complex[11].

Keeping in view the potentiality of 4β-congeners of podophyllotoxin against various types of cancers we synthesized a library of podophyllotoxin-1,2,3-triazole-thiazoli dinedione hybrids and then evaluated them for anticancer activity against two human cancer cell lines *i.e.* DU-145 (prostate) and HCT-15 (colon).

2. Experimental

2.1. General procedure for the synthesis of terminal alkynes :

A mixture of substituted aldehyde **2** (4 mmol) and thiazolidine-2,4-dione **1** (0.47g, 4 mmol) with catalytic activity of piperidine was refluxed in toluene with continuous removal of water using dean stark apparatus for 4 hours. The solid precipitate was obtained after cooling the mixture to room temperature and recrystallized from water to get pure compound **3.** The (0.65g, 2.3 mmol) of compound **3** and (0.32g, 2.3 mmol) of potassium carbonate was dissolved in 2 ml dry acetone and then propargyl bromide (0.27g, 2.3 mmol) was added slowly while stirring. The reaction mixture was stirred under under reflux conditions for 4 h. After completion the reaction mixture was extracted with ethyl acetate to get crude compound **4.** That was purified by column chromatography to get compound **4** in pure state.

2.2. General procedure for the synthesis of target compounds :

To a solution of **4** (1 mmol) in t-butyl alcohol and water (1:2, 8 ml) was added $CuSO_4.5H_2O$ (1 mmol), sodium ascorbate (2 mmol) followed by 4β-azido- podophyllotoxin **5** (44mg, 0.1mmol). The reaction mixture was stirred at room temperature for 8 hours. After completion, the reaction mixture was diluted with 80 ml of water and extracted with ethylacetate (2 x 20 mL). The combined extracts were washed with brine, dried over Na_2SO_4 and evaporated *in vaccuo*. The crude product obtained was precipitated in diethyl ether to yield the pure product **6**. Spectral details of some of the representative are mentioned below.

*2.2.1. 5-Benzylidene-3-{1-[8-oxo-9-(3,4,5-trimethoxy-phenyl)-5,5a,6,8,8a, 9-hexahydro-furo[3',4':6,7] naphtha [2,3-d][1,3]dioxol-5-yl]-1H-[1,2,3] triazol-4-ylmethyl}-thiazolidine-2,4- dione **(6a)***

^{1}HNMR (200 MHz, $CDCl_3$): δ 2.79 (m, 1H), 3.18 (m, 2H), 3.65 (s, 6H), 3.89 (s, 3H), 3.90 (d, 1H, J=2.28 Hz), 4.40-4.45 (m,3H), 4.69 (d, 1H, J = 4.67 Hz), 6.10 (m, 3H), 6.23 (s, 2H), 6.47-6.69 (m, 5H), 7.10-7.21 (m,3H); ^{13}C NMR (125 MHz, CDCl3):δ 37.1, 42.1, 42.9, 46.0, 56.3, 59.9, 62.3, 104.1, 109.09, 110.4, 113.2, 115.8, 120.0, 123.5, 125.4,132.2, 134.7, 133.2, 145.4, 149.1, 151.4, 157.2,173.3; IR (KBr): 3415.4, 1780.6, 1611.4, 1494.2, 1485.1, 1237.5, 1136.7, 752.2 cm^{-1}; Mass (ESI-MS): 705 (M + Na); Anal Calcd. for $C_{35}H_{30}N_4O_9S$: C, 61.58; H, 4.43; N, 8.21. Found: C, 61.36; H, 4.50; N, 8.27.

*2.2.2. 5-(2-Methyl-benzylidene)-3-{1-[8-oxo-9-(3,4,5-trimethoxy-phenyl)-5, 5a,6,8,8a,9-hexahydro-furo[3',4':6,7] naphtho[2,3-d][1,3]dioxol-5-yl]-1H-[1,2,3]triazol-4-ylmethyl}-thiazolidine-2,4-dione **(6b)***

^{1}HNMR (200 MHz, $CDCl_3$):δ 2.89 (m, 1H), 3.06 (m, 2H), 3.36 (s, 6H), 3.84(s, 3H), 3.96 (d, 1H, J=2.28 Hz), 4.25-4.32 (m,3H), 4.69 (d, 1H, J = 4.67 Hz), 6.08 (m, 3H), 6.31 (s, 2H), 6.47-6.81 (m, 5H), 7.20-7.25 (m,3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 18.0, 34.1, 40.6, 43.1, 44.3, 57.1, 58.3, 59.6, 67.7, 103.6,108.9, 110.1, 117.7, 123.5, 127.4,131.2, 133.7, 134.9, 145.4, 149.5, 151.4, 157.2,173.3; IR (KBr): 3425.4, 1777.6, 1562.4, 1504.2, 1237.5,1125.7, 752.2 cm^{-1}; Mass (ESI-MS): 719 (M + Na); Anal Calcd. for $C_{36}H_{32}N_4O_9S$:C, 62.06; H, 4.63; N, 8.04. Found: C, 62.00; H, 4.57; N, 8.17.

2.2.3. 5-(2-Chloro-benzylidene)-3-{1-[8-oxo-9-(3,4,5-trimethoxy-phenyl)-5, 5a,6,8,8a,9-hexahydro-furo[3',4':6,7] naphtho[2,3-d][1,3]dioxol-5-yl]-1H-[1,2,3]triazol-4-ylmethyl}-thiazolidine-2,4-dione ***(6e)***

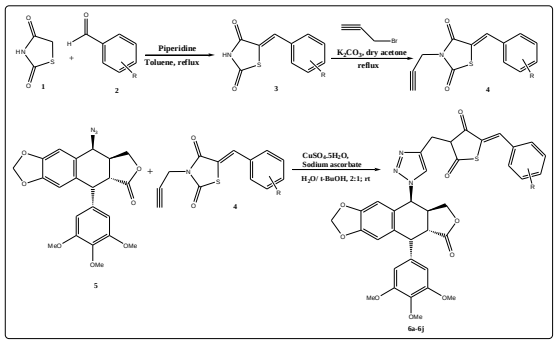
^{1}HNMR (200 MHz, $CDCl_3$): δ 3.04 (m, 1H), 3.25 (m, 2H), 3.66 (s, 6H), 3.78(s, 3H), 4.12-4.21 (m, 1H), 4.38- 4.51 (m, 3H), 4.68 (d, 1H, J = 4.84 Hz), 5.97-6.04 (m, 3H), 6.51 (s, 2H), 6.50-6.62 (m, 5H), 7.11 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 36.86, 39.3, 40.6, 43.3, 55.3, 57.8, 61.2, 66.3, 103.0, 108.3, 110.9, 112.7, 113.0, 113.2, 119.2, 120.0, 124.5, 131.2, 133.2, 135.2, 137.7, 148.0, 149.5, 150.1, 152.8, 174.1; IR (KBr): 3399.2, 1780.6, 1588.5, 1515.3, 1485.2, 1238.0, 1000.6, 765.7 cm^{-1}; Mass (ESI-MS): 739 (M + Na); Anal Calcd. for $C_{35}H_{29}ClN_4O_9S$: C, 58.62; H, 4.08; N, 7.81. Found: C, 58.761; H, 4.04; N, 7.89.

3. Results and Discussion

The [3+2] cycloaddition between a terminal alkyne and an azide results in the synthesis of a mixture of 1,4- and 1,5-disubstituted 1,2,3-triazoles[12] and was regarded as a "cream of crop" of click chemistry by Sharpless[13] and has become most popular click reaction to date[14]. Click chemistry is a reliable synthetic approach first proposed by Sharpless and who utilized copper as a catalyst to generate regioselectively 1,4-disbstituted triazoles[15]. However when ruthenium is used in place of copper as a catalyst then only generation of 1,5-disubstituted triazoles gets formed[16]. Novel pharmacophores have been synthesized by applying this reliable approach of click chemistry[13].

Using the same approach we synthesized a novel series of podophyllotoxin-1,2,3-triazole-thiazoli dindione hybrids in very good yields. The terminal alkynes **4** were prepared in two steps, the first step employs Knoevenagel condensation between substituted aldehydes **2** and thiazolidine-2,4-dione **1** and the second step involves propargylation of compound **3** (**Scheme 1**). These alkynes **4** were then condensed with azidopodophyllotoxin **5** under click chemistry reaction conditions to get a series of target compounds **6a-6j**. The podophyllotoxin was converted to 4β-azidopodophyllotoxin by the known literature method[17].

All the products were characterized by ^{1}H NMR, ^{13}C NMR, IR, ESI-MS and elemental analysis and were assayed for *in vitro* cytotoxicity against two human cancer cell lines using sulforhodamine B assay with etoposide as a reference compound. The cells were allowed to proliferate in presence of test material for 48 hours and the results are reported in terms of IC_{50} values. It was seen that all compounds showed good cytotoxic



Scheme 1 Click chemistry approach for the synthesis of podophyllotoxin-1,2,3-triazole-thiazolidinedione hybrids.

For 6a, R = H; 6b, R = 2-methyl; 6c, R = 3-methyl; 6d, R = 4-methyl; 6e, R = 2-chloro; 6f, R = 3-chloro; 6g, R = 3-nitro; 6h, R = 4-nitro; 6i, R = 3-floro; 6j, R = 4-nitro;

activity but compound 6h and 6i showed potent activity against DU-145 and HCT-15 respectively.

4. References

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