Synthesis and anti-inflammatory activity evaluation of novel AZT and adenosine derivatives

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ABSTRACT

A five-step procedure was used to synthesize four novel conjugates of AZT and adenosine with quinazolinone scaffold. In the last step, alkynes-1 of quinazolinone were coupled to adenosine azide and AZT by Click chemistry to yield the designed conjugates. Their structures were characterized by full-length data of spectra including ^{1}H-, ^{13}C-NMR and MS. Screening for their in vitro anti-inflammatory activity was performed using Murine macrophage RAW 264.7 cells. The relationship between structure and biological activity was also discussed.

Keywords: Adenosine; AZT; Quinazolinone; Click chemistry; Anti-inflammatory.

1. INTRODUCTION

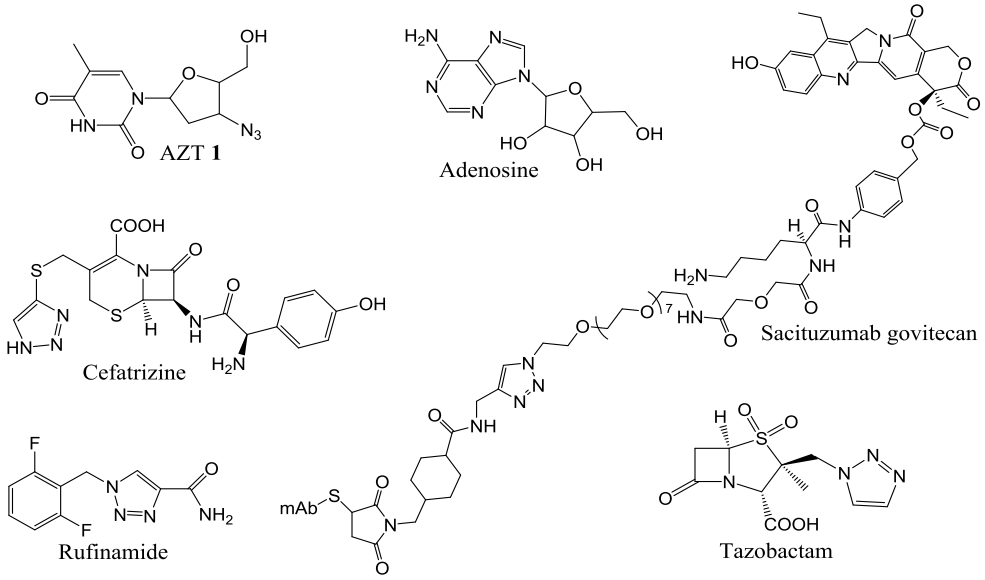


Figure 1. *Structure of AZT, adenosine and four approved drugs based Click chemsitry.*

Nowadays, the Click chemistry is one of the most important reactions to couple an alkyne with an azide in the presence of cooper (I) as a catalyst. This year, this is the second time, the Click chemistry has been officially announced to win the Nobel prize 2022 in chemistry since it was first introduced by K. B. Sharpless. At this time, triazole cyclisation of alkynes and azides has become a powerful tool for chemists in drug discovery thanks to impressive advantages

including excellent yield, mild and green conditions, high selectivity, no by-product, short-time reaction [1]. In Click chemistry, 1,2,3-triazole moiety was considered as an excellent linker unit of many pharmacophores [2]. Such conjugates have been also reported to have striking pharmacological activities such as antiviral [3], anti-oxidant [4], anti-inflammatory [5], anti-cancer [6, 7]. Despite of its tremendous potential, its commercial applications have still been limited, surprisingly. Presently, only four drugs based Click chemistry were approved and released (figure 1) [8].

Nucleosides are important components of living organism. Although nucleosides and their analogues have been used in clinical studies for more than 50 years [9], these studies mainly focus on antiviral and anticancer effects. A. A. Zenchenko and co-workers reported that half of nucleoside-based drugs are antiviral and a quarter anticancer [10]. Clearly, investigation on nucleoside analogues toward anti-inflammatory activity was neglected. On the other hand, structure of nucleosides is greatly suitable for Click chemistry, therefore, in this contribution, four conjugates of adenosine and AZT with 4(3H)-quinazolinones were designed, synthesized, and evaluated for their anti-inflammatory activity.

2. MATERIALS AND METHODS

2.1. Chemistry

2.1.1. Chemicals and instruments

All chemicals were purchased from Sigma Aldrich and used without further purification. ^{1}H-NMR and ^{13}C-NMR spectra were recorded at ambient temperature on a Bruker Avance 600 MHz spectrometer in DMSO-d_6. Chemical shifts δ are quoted in parts per million (ppm) referenced to the residual solvent peak, (DMSO-d_6 at 2.49 ppm and 39.5 ppm) relative to TMS. Mass spectra were recorded by using an Agilent LC/MSD Trap SL. Thin layer chromatography (TLC) was performed on a pre-coated aluminum sheet of Silica Gel 60 F254 (Merck), and products were visualized by UV lamp at 254 nm. Column chromatography was carried out on silica gel (40-230 mesh).

2.1.2. Synthetic procedure

Synthesis of 3-substituted-6-hydroxy-2-methyl-4(3H)-quinazolinone derivatives ***7a-b***. quinazolin derivatives **7a-b** were prepared from 5-hydroxyanthranilic acid **4** and amines: 4-methoxybenzylamine **6a**, 4-methybenzylamine **6b** according to the pathway reported by T. D. Thinh and co-workers [11].

Synthesis of 3-substituted-6-hydroxy-2-methyl-4(3H)-quinazolinone derivatives containing propargyl group ***9a-b***. Each compound **7a** or **7b** (1 mmol, 1.0 equiv.) was dissolved in dry DMF (5 mL), then potassium carbonate (1.5 equiv.) and propargyl bromide **8** (1.0 equiv.) were added. Mixture of reactions was stirred at room temperature for 10h, then quenched in cool water and extracted with ethyl acetate (3×15 mL). The combined organic layer was dried over anhydrous Na_2SO_4, then filtered and evaporated under reduced pressure. Crudes of **9a-b** were purified by column chromatography on silica gel eluting with *n*-hexane/acetone to obtain the pure **9a** and **9b**.

3-(4-methoxybenzyl)-2-methyl-6-(prop-2-yn-1-yloxy)quinazolin-4(3H)-one **9a.** Yield 89%, grey solid, m.p. 110-112^{o}C; ESI-MS $[M+H]^+$ *m/z*: 335.1466; ^{1}H-NMR (600 MHz, DMSO-d_6, δ (ppm)): 7.64 (d, J = 2.1 Hz, 1H, H-5), 7.57 (d, J = 8.7 Hz, 1H, H-8), 7.45 (dd, J_1 = 2.1 Hz, J_2 = 8.7 Hz, 1H, H-7), 7.15 (dd, J_1 = 2.1 Hz, J_2 = 8.7 Hz, 2H, H-2', H-6'); 6.90 (dd, J_1 = 2.1 Hz, J_2 = 8.7 Hz, 2H, H-3', H-5'), 5.30 (s, 2H, 1'-CH_2-)), 4.94 (d, J = 2.4 Hz, 2H, H-11), 3.72 (s, 3H, 4'-OCH_3)), 3.61 (t, J = 2.4 Hz, 1H, H-13), 2.48 (s, 3H, 2-CH3); ^{13}C-NMR (600 MHz, DMSO-d_6, δ (ppm)): 161.2 (C-4), 158.5 (C-4'), 156.3 (C-6), 152.8 (C-2), 141,8 (C-10), 128.38 (C-8), 128.36 (C-1'), 127.9 (C-2', C-6'), 124.4 (C-7), 120.6 (C-9), 114.1 (C-3', C-5'), 107.6 (C-5), 78.8 (C-12), 78.6 (C-13), 55.9 (C-11), 55.0 (4'-OCH_3), 45.9 (1'-CH2-), 22.7 (2-CH_3).

2-methyl-3-(4-methylbenzyl)-6-(prop-2-yn-1-yloxy)quinazolin-4(3H)-one **9b**. Yield 91%, brown solid, m.p. 101-103^{o}C; ESI-MS $[M+H]^+$ *m/z*: 318.1559; ^{1}H-NMR (600 MHz, DMSO-d_6, δ (ppm)): 7.64 (d, J = 3.0 Hz, 1H, H-5), 7.58 (d, J = 8.4 Hz, 1H, H-8), 7.46 (dd, J_1 = 3.0 Hz, J_2 = 8.4 Hz, 1H, H-7), 7.15 (d, J = 8.4 Hz, 2H, H-3', H-5'); 7.08 (d, J = 8.4 Hz, 2H, H-2', H-6'), 5.33 (s, 2H, 1'-CH_2-), 4.94 (d, J = 2.4 Hz, 2H, H-11), 3.62 (t, J = 2.4 Hz, 1H, H-13), 2.46 (s, 3H, 2-CH_3), 2.27 (s, 3H, 4'-CH_3); ^{13}C-NMR (600 MHz, DMSO-d_6, δ (ppm)): 161.2 (C-4), 155.3 (C-6), 153.0 (C-2), 142.0 (C-10), 136.4 (C-4'), 133.4 (C-1'), 129.3 (C-2', C-6'), 128.4 (C-8), 126.3 (C-3', C-5'), 124.5 (C-7), 120.5 (C-9), 107.9 (C-5), 78.8 (C-12), 78.6 (C-13), 55.9 (C-11), 22.6 (2-CH_3), 20.6 (4'-CH_3).

*Synthesis of 5'-azidoadenosine **3**.* 5'-tosyladenosine **2** (2 mmol, 1.0 equiv.) was dissolved in dry DMF (8 mL), then sodium azide (2.0 equiv.) and K_2CO_3 (2 mmol) were added. Mixture of reactions was stirred at room temperature 12h. Then water (30ml) was added, then extracted with dichloromethane (3×25 mL). The combined organic layer was dried over anhydrous Na_2SO_4, then evaporated under reduced pressure to give crude of 5'-azidoadenosine **3** which were purified by column chromatography on silica gel eluting with dichloromethane/methanol to obtain the pure product.

Yield 82%, white solid, m.p. 190-192^{o}C; ESI-MS $[M+H]^+$ *m/z*: 293.1039; ^{1}H-NMR (600 MHz, DMSO-d_6, δ (ppm)): 8.36 (s, 1H, H-8), 8.17 (s, 1H, H-2), 7.28 (s, 2H, 6-NH_2), 5.94 (d, J = 5.4 Hz, 1H, H-1'), 5.57 (d, J = 6.0 Hz, 1H, 3-OH), 5.37 (d, J = 5.4 Hz, 1H, 2'OH), 4.76 (dd, J_1 = 5.4 Hz, J_2 = 6.0 Hz, 1H, H-2'), 4.21 (dd, J_1 = J_2 = 5.4 Hz, 1H, H-3), 4.06 (ddd, J_1 = 3.6 Hz, J_2 = J_3 = 4.2 Hz, 1H, H-4'), 3.69 (dd, J_1 = 7.2 Hz, J_2 = 13.2 Hz, 1H, H-5a), 3.57 (dd, J_1 = 3.6 Hz, J_2 = 13.2 Hz, 1H, H-5b); ^{13}C-NMR (600 MHz, DMSO-d_6, δ (ppm)): 165.7 (C-2), 162.1 (C-6). 149.4 (C-4), 139.3 (C-8), 128.7 (C-5), 97.3 (C-1'), 92.4 (C-4'), 82.2 (C-2'), 80.4 (C-3'), 61.2 (C-5').

*Synthesis of 1,2,3-triazole products **10a-b.*** To a mixture of 1 mmol of quinazolinone derivative containing propargyl group **9a** or **9b** and AZT **1** in 4 ml of mixture solvent tetrahydrofurane/water (1:2, v/v) 10% $CuSO_4$ solution (1,2 mL) and hydrazine hydrate (0,1 mL) were added and contents were further stirred for 5h at the same conditions. The mixture was then diluted with cool water (30 mL) to produce crudes of products **11a-b** as a precipitate that were then filtered, dried over desiccator and purified by column chromatography with dichloromethane:methanol as eluent.

*1-(5-(hydroxymethyl)-4-(4-(((3-(4-methoxybenzyl)-2-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)ox -y)methyl)-1H-1,2,3-triazol-1-yl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione **10a.*** Yield 70%, white solid, m.p. 246-247^{o}C; ESI-MS $[M+H]^+$ *m/z*: 602.2561; ^{1}H-NMR (600 MHz, DMSO-d_6, δ (ppm)): ^{1}H-NMR (600 MHz, DMSO-d_6, δ (ppm)): 11.34 (s, 1H, H-3''''); 8.45 (s, 1H, H-5''); 7.82 (d, J = 1.2 Hz, 1H, H-6''''); 7.72 (d, J = 3 Hz, 1H, H-5); 7.57 (d, J = 9 Hz, 1H, H-8); 7.48 (dd, J_1 = 3 Hz, J_2 = 9 Hz, 1H, H-7); 7.16 (d, J = 8.4 Hz, 2H, H-2', H-6'); 6.91 (dd, J_1 = 1.8 Hz, J_2 = 6.6 Hz, 2H, H-3', H-5'); 6.42 (t, J = 6.6 Hz, 1H, H-1'''); 5.41 (m, 1H, H-3'''); 5.30 (d, J = 4.2 Hz, 4H, 4''-CH_2-, 1'-CH_2-); 5.27 (t, J = 5.4 Hz, 1H, 5'''-OH); 4.25 (m, 1H, H-4'''); 3.71 (m, 4H, H-5'''a, 4'-OCH_3); 3,64 (m, 1H, H-5'''b); 2.76 (m, 1H, H-2'''a); 2.67 (m, 1H, H-2'''b); 2.50 (s, 3H, 2-CH_3); 1.82 (s, 3H, 5''''-CH_3). ^{13}C-NMR (150 MHz, DMSO-d_6, δ (ppm)): 163.7 (C-4''''); 161.3 (C-4); 158.5 (C-4'); 156.3 (C-6); 152.8 (C-3); 150.4 (C-2''''); 142.6 (C-4''); 141.8 (C-10); 136.2 (C-6''''); 128.4 (C-8); 127.9 (C-2', C-6'); 124.4 (C-5''); 120.6 (C-9); 114.1 (C-3', C-5'); 109.6 (C-5''''); 107.6 (C-5); 84.4 (C-4'''); 83.9 (C-1'''); 61.6 (4''-CH_2-); 60.7 (C-5'''); 55.0 (4'-OCH_3); 45.9 (1'-CH_2-); 37.1 (C-2'''); 22.7 (2-CH_3); 12.2 (5''''-CH_3).

*1-(5-(hydroxymethyl)-4-(4-(((3-(4-methylbenzyl)-2-methyl-4-oxo-3,4-dihydroquinazolin-6yl)-oxy)methyl)-1H-1,2,3-triazol-1-yl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4-(1H,3H)-dione **10b***. Yield 68%, white solid, m.p. 240-241^{o}C; ESI-MS $[M+H]^+$ *m/z*: 586.2470; ^{1}H-NMR (600 MHz, DMSO-d_6, δ (ppm)): ^{1}H-NMR (600 MHz, DMSO-d_6, δ (ppm)): 11.34 (s, 1H, H-3''''); 8.45 (s, 1H,

H-5''); 7.82 (d, *J* = 1.2 Hz, 1H, H-6''''); 7.71 (d, *J* = 3 Hz, 1H, H-5); 7.57 (d, *J* = 8.4 Hz, 1H, H-8); 7.48 (dd, J_1 = 2.7 Hz, J_2 = 8.4 Hz, 1H, H-7); 7.15 (d, *J* = 8,4 Hz, 2H, H-2', H-6'); 7.08 (d, J_1 = 8.4 Hz, 2H, H-3', H-5'); 6,43 (t, *J* = 6,6 Hz, 1H, H-1'''); 5,40 (m, 1H, H-3'''); 5.33 (s, 2H, 1'-CH_2-); 5.30 (s, 2H, 4''-CH_2-); 5,29 (t, J = 5.1 Hz, 1H, 5'''-OH); 4,24 (m, 1H, H-4'''); 3,71 (m, 1H, H-5'''a); 3,64 (m, 1H, H-5'''b); 2,75 (m, 1H, H-2'''a); 2.66 (m, 1H, H-2'''b); 2,45 (s, 3H, 4'-CH_3); 2.26 (s, 2-CH_3), 1.82 (s, 3H, 5''''-CH_3); ^{13}C-NMR (150 MHz, DMSO-d_6, δ (ppm)): 163.8 (C-4''''); 161.3 (C-4); 156,3 (C-6); 152.9 (C-2); 150.5 (C-2''''); 142.6 (C-4''); 141.9 (C-10); 136.5 (C-4'); 136,3 (C-6''''); 133.5 (C-1'); 129.3 (C-3', C-5'); 128.4 (C-8); 126.3 (C-2', C-6'); 124.5 (C-7); 124,4 (C-5''); 120.6 (C-9); 109.7 (C-5''''); 107.7 (C-5); 84.5 (C-4'''); 83.9 (C-1'''); 61.6 (4''-CH_2-), 60.8 (C-5'''); 59.4 (C-3'''); 46.2 (1'-CH_2); 37.2 (C-2'''); 22.7 (2-CH_3); 20.6 (4'-CH_3), 12.2 (5''''-CH_3).

Synthesis of 1,2,3-triazole products ***11a-b.*** These products were prepared according to the above recipe for **10a-b** in which 5'-azidoadenosine **3** was used instead of AZT **1**.

6-((1-((5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-(4-methoxybenzyl)-2-methylquinazolin-4(3H)-one **11a.** Yield 65%, white solid, m.p. 295-297^{o}C; ESI-MS $[M+H]^+$ *m/z*: 627.2466; ^{1}H-NMR (600 MHz, DMSO-d_6, δ (ppm)): 8.27 (s, 1H, H-8''''), 8.17 (s, 1H, H-2''''), 8.12 (s, 1H, H-5''), 7.70 (d, *J* = 3.0 Hz, 1H, H-5), 7.56 (d, *J* = 8.7 Hz, 1H, H-8), 7.45 (dd, J_1 = 3.0 Hz, J_2 = 8.7 Hz, 1H, H-7), 7.30 (s, 2H, 6''''-NH_2), 7.15 (d, *J* = 8.7 Hz, 2H, H-2', H-6'), 6.91 (d, *J* = 8.7 Hz, 2H, H-3', H-5'), 5.93 (d, *J* = 5.4 Hz, 1H, H-1'''), 5.61 (d, *J* = 6.0 Hz, 1H, 2'''-OH), 5.50 (d, *J* = 4.8 Hz, 1H, 3'''-OH), 5.30 (s, 2H, N3-CH_2-), 5.23 (d, *J* = 12.0 Hz, 1H, 4''-CH_{2a}-), 5.20 (d, *J* = 12.0 Hz, 1H, 4''-CH_{2b}-), 4.81 (m, 2H, H-5'''), 4.66 (dd, J_1 = 5.4 Hz, J_2 = 6.0 Hz, 1H, H-2'''), 4.30 (m, 2H, H-3''', H-4'''), 3.72 (s, 3H, 4'-OCH_3), 2.48 (s, 3H, 2-CH_3); ^{13}C-NMR (600 MHz, DMSO-d_6, δ (ppm)): 161.3 (C-4), 158.5(C-4'), 156.3 (C-6), 156.1 (C-2), 152.8 (C-2''''), 152.7 (C-6''''), 149.3 (C-4''''), 142.3 (C-10), 141.8 (C-4''), 139.9 (C-8''''), 128.4 (C-8), 128.3 (C-1'), 127.9 (C-2', C-6'), 125.4 (C-5''), 124.4 (C-7), 120.6 (C-9), 119.2 (C-5''''), 114.2 (C-3', C-5'), 107.6 (C-5), 87.8 (C-1'''), 82.4 (C-4'''), 72.6 (C-2'''), 71.0 (C-3'''), 61.5 (4''-CH_2-), 55.1 (4'-OCH_3-), 51.5 (C-5'''), 45.9 (N3-CH_2-), 22.7 (2-CH_3).

6-((1-((5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-1H-1,2,3-triaz-ol-4-yl)methoxy)-2-methyl-3-(4-methylbenzyl)quinazolin-4(3H)-one **11b**. Yield 66%, white solid, m.p. 288-289^{o}C; **ESI-MS** $[M+H]^+$ *m/z*: 611.2348; ^{1}H-NMR (600 MHz, DMSO-d_6, δ (ppm)): 8.27 (a, 1H, H-8''''), 8.17 (s, 1H, H-2''''), 8.12 (s, 1H, H-5''), 7.69 (d, *J* = 3.0 Hz, 1H, H-5), 7.56 (d, *J* = 8.4 Hz, 1H, H-8), 7.46 (dd, J_1 = 3.0 Hz, J_2 = 8.4 Hz, 1H, H-7), 7.30 (s, 2H, 6''''-NH_2), 7.15 (d, *J* = 8.4 Hz, 2H, H-2', H-6'), 7.08 (d, *J* = 8.4 Hz, 2H, H-3', H-5'), 5.93 (d, *J* = 5.4 Hz, 2H, H-2', H-6'), 5.61 (d, *J* = 6.0 Hz, 1H, 3'''-OH), 5.50 (d, *J* = 5.4 Hz, 1H, 2'''-OH), 5.33 (s, 2H, N3-CH_2-), 5.23 (d, *J* = 12.0 Hz, 1H, 4''-CH_{2a}-), 5.20 (d, *J* = 12.0 Hz, 1H, 4''-CH_{2b}-), 4.81 (m, 2H, H-5'''), 4.67 (dd, J_1 = J_2 = 5.4 Hz, 1H, H-2'''), 4.30 (m, 2H, H-3''', H-4'''), 2.46 (s, 3H, 4'-CH_3), 2.27 (s, 3H, 2-CH_3); ^{13}C-NMR (600 MHz, DMSO-d_6, δ (ppm)): 161.3 (C-4), 156.3 (C-6), 156.1 (C-2), 152.8 (C-2''''), 152.7 (C-6''''), 149.3 (C-4''''), 142.3 (C-10), 141.8 (C-4''), 139.9 (C-8''''), 136.5 (C-4'), 133.5 (C-1'), 129.3 (C-2', C-6'), 128.4 (C-8), 126.3 (C-3', C-5'), 125.4 (C-5''), 124.4 (C-7), 120.6 (C-9), 119.2 (C-5''''), 107.6 (C-5), 87.8 (C-1'''), 82.4 (C-4'''), 72.6 (C-2'''), 71.0 (C-3'''), 61.5 (4''-CH_2-), 51.5 (C-5'''), 46.2 (N3-CH_2-), 22.6 (2-CH_3), 20.6 (4'-CH_3).

2.2. Biology

The *in vitro* anti-inflammatory activity of tested compounds was determined using nitrite assay in murine macrophages following published method with minor modifications [12, 13].

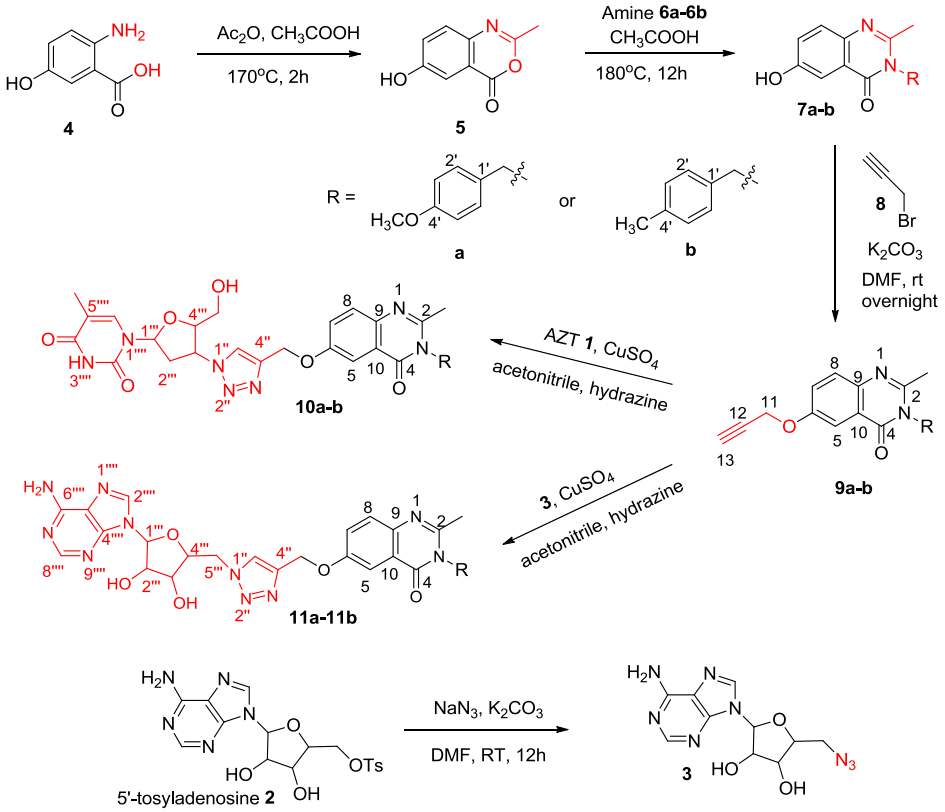
Cell culture: Murine macrophage RAW 264.7 cells (ATCC TIB 71) were maintained and cultured at 37 ^{o}C under humidified air, with 5% CO_2 atmosphere in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 unit/mL penicillin and 100 mg/mL streptomycin.

Nitrite assay: RAW 264.7 macrophages were seeded in 24-well microtiter plates at a density of $2x10^6$ cells per well for exponentially growing. After a cell starvation period in 1% FBS medium for 6 hours, macrophages were then treated or not with test compounds (37 ^oC, 5% CO_2, 24 h) and stimulated with 1 µg/mL LPS in 24 h. Cell-free supernatants were collected to determine the production of nitric oxide (NO) content by reaction with Griess reagent (1% sulfanilamide and 0.1% *N*-1-naphtylethylenediamine dihydrochloride in 2.5% H_3PO_4) (Sigma-Aldrich, Germany) (30 ^oC, 20 min) and detected by absorbance at 550 nm (Tecan F150 microplate reader, Switzerland). The inhibition of NO production by test samples was calculated by interpolation basing on calibration of a standard curve with known sodium nitrite concentrations.

3. RESULTS AND DISCUSSION

3.1. Chemistry

Scheme 1 depicts the route for synthesis of two nucleosides AZT and adenosine conjugates with quinazolinones through a Click chemistry. In the final step, cooper (I) ion was produced *in situ* from $CuSO_4$ using hydrazine hydrate as a reductive agent in a mixture of tetrahydrofurane:water.



Scheme 1. *Pathway for synthesis of four AZT and adenosine derivatives bearing quinazolinone.*

Firstly, the alkynes-1 were prepared from 5-hydroxyanthranilic acid **4** *via* a three-step procedure (Scheme 1). In the first step, the intermediate **5** was achieved by a cyclization at the reflux condition with acetic anhydride which also have the role as medium for the reaction. Next, intermediate **5** was reacted with amines **6a-b** in acetic acid at reflux to afford **7a-b** which are then O-alkylated with propargyl bromide **8** to obtain desired alkynes-1 **9a-b** for the last step. Beside the main products **7a-b**, the acylation of **6a-b** produced by-products that had the same R_f

with **7a-b** under UV lamp at 254 nm. However, mixtures of **7a-b** and by-products were directly used for *O*-propargylation with propargyl bromide **8** in the next step without purification to yield **9a-b** that were easily purified by column chromatography/silica gel eluting with n-hexane:acetone 2:1.

Structures of **9a-b** were confirmed by NMR and MS spectroscopies. In the case of **9a** is an example, ^{1}H-NMR spectrum indicates the signals of 7 protons of quinazolinone moiety appeared in downfield regions with δ: 6.90-7.64 ppm that were in good agreement with given data by T. D. Thinh and co-workers [11]. Next, three singlets at 5.30, 3.72 and 2.48 ppm were assigned to *N3*-CH_2, 4'-OCH_3 and 2-CH_3 protons, respectively. Successful *O*-propargylation was proved by presence of signals including: a doublet at 4.94 (J = 2.4 Hz) of H-11 and a triplet at 3.61 (J = 2.4 Hz) of H-13. The ^{13}C-NMR data of **9a** also agreed well with its structure.

In the last step, the alkynes **9a-b** were coupled to AZT and 5'-azidoadenosine by a Click triazole cyclisation using $CuSO_4$ and hydrazine hydrate in a solvent mixture of tetrahydrofurane/water 1:2 (v/v) at room temperature to give novel AZT and adenosine derivatives **10a-b** and **11a-b** (Scheme 1) in yields of 65-70%. The NMR spectra of **10a-b** and **11a-b** exhibit all signals of protons and carbons in quinazolinone, AZT, and adenosine moieties. In addition, the presence of triazole bridge was approved by the singlets at 8.45 ppm (H-5" for AZT derivatives **10a-b**), and 8.12 ppm (H-5" for adenosine derivatives **11a-b**), respectively. The signals of carbons C-4", C-5" of **10a-b** and **11a-b** were found at 142.6, 124.4 for **10a-b** and 142.6 and 125.4 for **11a-b**, respectively.

3.2. Biology

The *in vitro* anti-inflammatory activity of test compounds was evaluated by measuring reduced NO production in cell culture supernatants of LPS-stimulated RAW 264.7 cells. Results showed that two AZT conjugates **10a** and **10b** inhibited NO production with IC_{50} values of 68,23 and 60,04 µg/mL (table 1). Notably, almost no NO reduction was observed for the derivative **11a** (only 2,65%). Obviously, nucleoside AZT might be an advantageous pharmacophore over adenosine in term of anti-inflammatory activity while influence of the methoxy and methyl substituents on the quinazolinone skeleton to the activity is insignificant.

Table 1. *NO inhibitory activity of the synthesized compounds on RAW 264.7 macrophages.*

Sample's name**	**Test concentration (µg/mL)**	**NO inhibition* (%)**	**Cell survival* (%)**	**NO half-maximal inhibitory concentration (IC_{50}, µg/mL)**
(+) control	0.86	86.93±0.96	71.80±0.51	0.61±0.07
10a	100	53.31±0.49	69.49±0.51	68.23±1.38
10b	100	59.83±0,44	91.44±0.43	60.04±0.99
11a	100	2,65±0,09	88,36±0,59	-
11b	100	30.53±0,03	42.76±0.39	-

** Data represent the mean ± standard deviation of three independent wells;*
*** Positive control: Cardamonin (Merck, German)*

4. CONCLUSIONS

An efficient pathway was designed to prepare four novel AZT and adenosine derivatives containing quinazolinone. Their NMR and MS spectral data indicated the good agreement of structures as designed. All synthesized products were evaluated for their *in vitro* anti-inflammatory activity *via* a NO production inhibitory assay. As given results, only two AZT

derivatives **10a** and **10b** expressed a weak activity in reducing the production of NO with IC_{50} values ranging from 59.64-68.23 µg/mL. The results revealed that the presence of AZT in the conjugates with quinazolinone seems to be more beneficial than that of adenosine for their anti-inflammatory activity.

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TÓM TẮT

Tổng hợp và đánh giá hoạt tính kháng viêm các dẫn xuất mới của AZT và adenosine

Bốn tổ hợp mới giữa AZT và adenosine với hợp phần quinazolinone được tổng hợp thông qua một quá trình năm bước phản ứng. Ở bước phản ứng cuối cùng, các ankin-1 của quinazolinone được ghép với các azide adenosine và AZT thông qua phản ứng Click chemistry để tạo thành các sản phẩm như đã thiết kế. Cấu trúc của tất cả các sản phẩm được xác định và chứng minh sử dụng các phương pháp phổ ^{1}H-, ^{13}C-NMR and MS. Hoạt tính kháng viêm in vitro của các sản phẩm thu được được sàng lọc trên các đại thực bào RAW264.7. Từ đó, mối quan hệ hoạt tính sinh học - cấu trúc của các sản phẩm cũng đã được thảo luận.

Từ khóa: Adenosine; AZT; Quinazolinone; Click chemistry; Kháng viêm.