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Anticancer Activity of New 1,2,3-Triazole-Amino Acid Conjugates

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Abstract: A multistep synthesis was developed to prepare new 1,2,3-triazole-amino acid conjugates (**6** and **7**). These compounds contain the diaryl ether moiety and were synthesized via S_NAr reaction under mild condition and in good yield. Their structures were confirmed by spectroscopic analyses (HR-MS, NMR, IR). These compounds showed significant antiproliferative activity (>30%) toward the breast MCF7 and liver HepG2 cancer cells lines at <10 μM concentration.

Keywords: amino acid conjugate; amide coupling; anticancer; diaryl ether; S_NAr reaction; triazole compound

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1. Introduction

1,2,3-triazoles can mimic different functional groups and are used as bioisosteres in a wide range of bioactive compounds in medicinal chemistry [1]. In particular, compounds containing both 1,2,3-triazole and diarylether have shown good anticancer activities [2,3] by inhibiting Bax/Bcl-xL protein–protein interaction in cancer cells [4]. Moreover, amino acid conjugates are known to modulate activities toward challenging targets such as protein–protein interaction [5] or peptide-binding G-protein coupled receptors [6,7]. In a continuation of our study toward 1,2,3-triazole containing diaryl ethers [8], we report an efficient protocol for preparing 1,2,3-triazole-amino acid conjugates as potential new bioactive compounds (Figure 1).

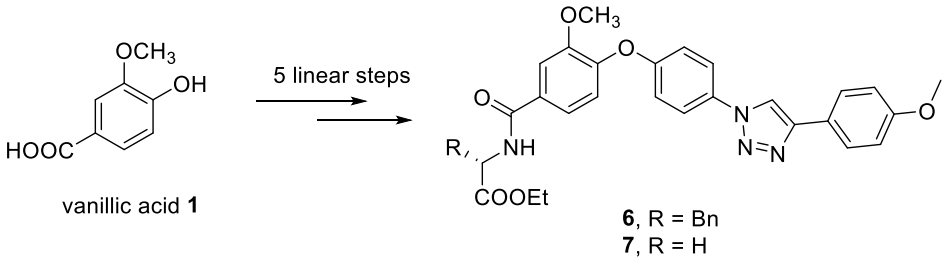


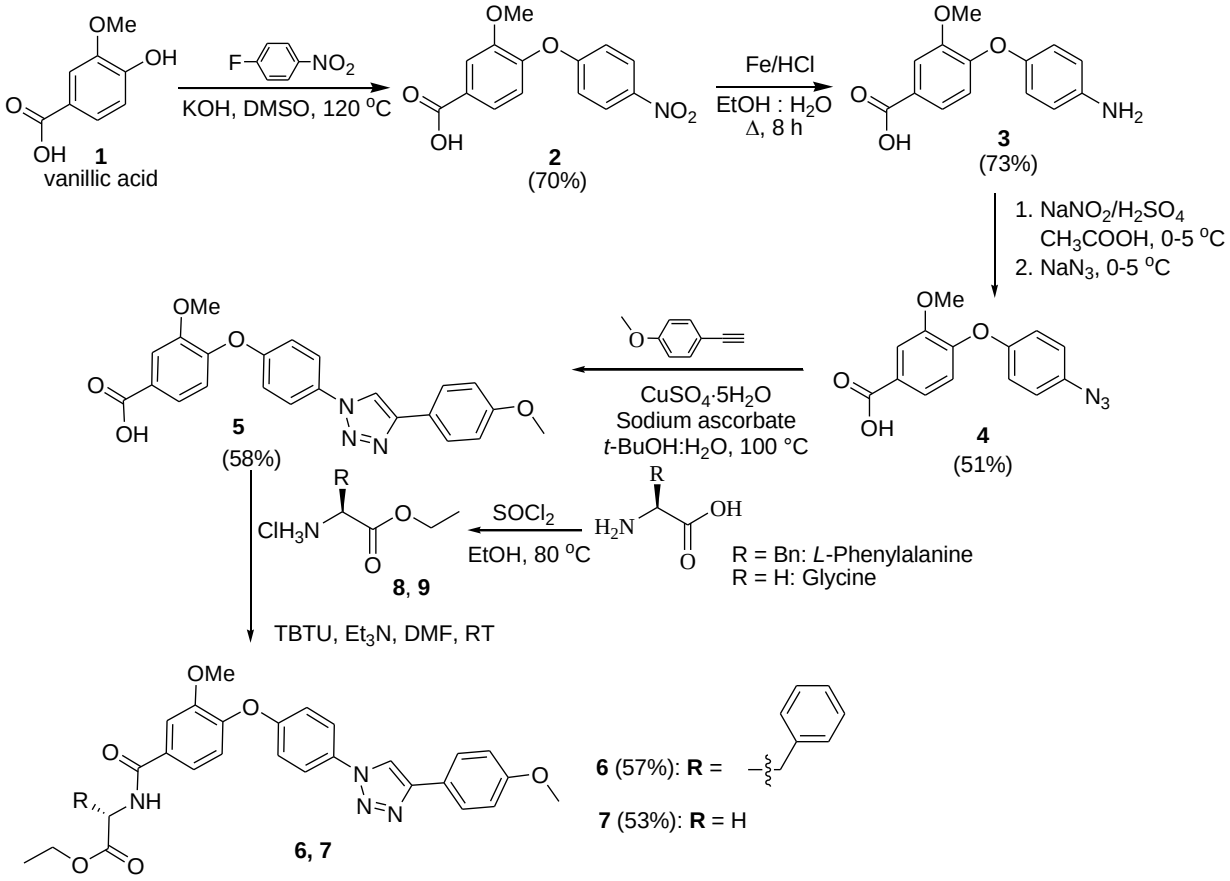
Figure 1. Synthesis of 1,2,3-triazole-aminoacid conjugates **6** and **7** from vanillic acid.

2. Results and Discussion

2.1. Synthesis

The synthesis strategy of the triazole-amino acid conjugates **6** and **7** is depicted in Scheme 1. The synthesis started with an S_NAr reaction between vanillic acid **1** and 1-fluoro-4-nitrobenzene in DMSO using KOH as base. The ratio between vanillic acid **1** and KOH is important, and was optimized to improve the yield of compound **2** (Table 1). Two

equivalents of KOH was not enough, giving only 59% yield. The best yield (70%) was obtained when 3 to 5 equivalents of KOH were used.



Scheme 1. Synthesis of 1,2,3-triazole-amino acid conjugates **6** and **7**.

Table 1. Effect of KOH equivalent in the synthesis of diaryl ether **2**.

Entry	KOH Equivalents	Isolated Yield (%)
1	2	59
2	3	70
3	5	70

Reduction of nitro group of compound **2** using Fe/HCl in EtOH/H_2O produced the aniline **3** in 73% yield. This aniline was then converted to azide using a two-step procedure (diazotization and azidation) providing compound **4** in 51% yield. A click reaction was employed for the synthesis of triazole **5** using Sharpless conditions [9]. Compound **5** was obtained in 58% yield. The ^{1}H-NMR spectrum of compound **5** showed a new singlet at 9.12 ppm (1H) which is the proton of the newly formed triazole ring. In parallel, ethyl ester of natural amino acid *L*-phenylalanine **8** and glycine **9** were prepared by reacting the corresponding amino acids with $SOCl_2$ in EtOH at 80 °C. Amide coupling between **5** and **8** (or **9**) was successful using 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) as coupling reagent. The target triazole-amino acid conjugates **6** and **7** were obtained in good yield (57% and 53%, respectively). The structures of compounds **6** and **7** were confirmed by IR, NMR and HR-MS spectroscopies (see Supplementary Materials).

*2.2. Anticancer Activity of **6** and **7***

The two triazole-amino acid conjugates **6** and **7** were tested for their cancer cell antiproliferative activity against the breast and liver cancer cells lines (MCF7 and HepG2) using the natural alkaloid ellipticine, a topoisomerase II inhibitor, as positive control. The results are shown in Table 2. Compound **6** showed similar IC_{50} for both cancer cells lines, while compound **7** was more selective toward HepG2 than MCF7. Interestingly, the two compounds significantly inhibited both MCF7 and liver HepG2 cancer cell proliferation by >30% at low concentration (<10 μM).

Table 2. Anticancer activity of compounds **6** and **7**. MTT cancer cells antiproliferation assay was done using reported protocols from literature [10,11]. The values are means of triplicate experiments. Incubation time was 72 h.

Compound.	MCF7		HepG2	
	IC_{50} (μM)	% Inhibition at <10 μM	IC_{50} (μM)	% Inhibition at <10 μM
6	129.6 ± 3.7	30–40 (1.7–6.7)	115.5 ± 2.5	23–32 (1.7–6.7)
7	199.6 ± 6.0	28–35 (2–8)	30.3 ± 1.6	29–35 (2–8)
Ellipticine	2.5 ± 0.2	-	1.3 ± 0.2	-

3. Materials and Methods

3.1. Materials

Reagents and solvents were purchased from commercial suppliers and used without further purification. Column chromatography was carried out using Merck Kieselgel 60 silica gel (particle size: 32–63 Å). Analytical TLC was performed using Merck precoated silica gel 60 F-254 sheets.

NMR spectroscopic data were acquired on Bruker Avance III at 500 MHz for ^{1}H–NMR and 125 MHz for ^{13}C–NMR. HR–MS spectra were recorded on a Bruker MICROTOF-Q 10,187 and a LC-MS Thermo, model: UltiMate 3000/ISO EC. Infrared spectra were taken on a SHIMADZU FTIR 8400S (KBr).

3.2. Synthesis Procedure

3-methoxy-4-(4-nitrophenoxy)benzoic acid **2**: To a stirred mixture of vanillic acid **1** (504 mg, 3 mmol, 1 equiv.) and KOH (504 mg, 9 mmol, 3 equiv.) in DMSO (1.5 mL) at room temperature was added 1-fluoro-4-nitrobenzene (0.3 mL, 3.3 mmol, 1.1 equiv.). The reaction was heated at 120 °C for 3 h. After cooling down, the water was added and the mixture was washed with ethyl acetate (30 mL × 3). The aqueous layer was acidified with 1N HCl to pH = 1. The resulting solid was filtered and dried. The solid was recrystallized from ethanol to afford the desired product. Yield: 607 mg (70%), white solid. Mp 216–217 °C. ^{1}H-NMR δ_H (500 MHz, $CDCl_3$, δ ppm): 13.14 (1H, br), 8.22 (2H, d, *J* = 9.0 Hz), 7.70 (1H, d, *J* = 1.5 Hz), 7.65 (1H, dd, *J* = 1.5 Hz, *J* = 8.5 Hz), 7.31 (1H, d, *J* = 8.5 Hz), 7.06 (2H, d, *J* = 9.0 Hz), 3.80 (3H, s). ^{13}C-NMR δ_C (125 MHz, $CDCl_3$, δ ppm): 167.1, 162.9, 151.5, 146.0, 142.7, 130.0, 126.5, 123.4, 122.8, 116.8, 114.5, 56.4. HR-MS calcd $C_{14}H_{11}NO_6Na$ ($[M + Na]^+$): 312.0484, found: 312.0466.

4-(4-aminophenoxy)-3-methoxybenzoic acid **3**: To a mixture of **2** (650 mg, 2.25 mmol) in 1:1 mixture of EtOH:H_2O (33 mL) at room temperature was added Fe (2.8 g) and concentrated HCl (3 mL). The reaction was stirred at reflux for 8 h. After cooling down, the mixture was acidified using 5% HCl to pH = 1 and diluted with ethyl acetate. The mixture was extracted with H_2O (30 mL × 3). The combined water layer was basicified using 1N NaOH to pH = 6 and extracted with ethyl acetate (30 mL × 3). The combined organic layer was dried over Na_2SO_4, filtered and concentrated to give the desired product which was used as such for the next step without purification. Yield: 426 mg (73%), beige solid. Mp 190–191 °C. ^{1}H-NMR δ_H (500 MHz, DMSO-d_6, δ ppm): *δ* 7.55 (1H, d, *J* = 1.5 Hz),

7.47 (1H, dd, *J* = 7.0, 1.5 Hz), 6.74 (2H, d, *J* = 7.0 Hz), 6.69 (1H, d, *J* = 7.0 Hz), 6.59 (2H, d, *J* = 7.0 Hz), 3.85 (3H, s). ^{13}C-NMR δ_C (125 MHz, $CDCl_3$, δ ppm): *δ* 166.9, 151.7, 149.0, 145.6, 145.1, 124.6, 122.8, 120.4, 115.5, 114.9, 113.0, 55.6. HR-MS calcd $C_{14}H_{12}NO_4$ ($[M - H]^-$): 258.0766, found: 258.0772.

4-(4-azidophenoxy)-3-methoxybenzoic acid **4**: To a mixture of **3** (259 mg, 1 mmol) in AcOH (4 mL) at room temperature was added concentrated H_2SO_4 (1 mL). The mixture was cooled down to 0–5 °C, then added a 2M solution of $NaNO_2$ (1 mL, 2 equiv.). The mixture was stirred at 0–5 °C for 60 min before adding NaN_3 (65 mg, 1 equiv.). The reaction was stirred at room temperature for 30 min before extracting with ethyl acetate (10 mL × 3). The combined organic layer was dried over Na_2SO_4, filtered and concentrated to give a residue which was purified by silica gel column chromatography using *n*-hexane:ethyl acetate (2:3) to afford the desired product. Yield: 145 mg (51%), beige solid. Mp 152–153 °C. ^{1}H-NMR δ_H (500 MHz, DMSO-d_6, δ ppm): 12.95 (1H, br), 7.63 (1H, d, *J* = 1.5 Hz), 7.56 (1H, dd, *J* = 1.5 Hz, *J* = 8.5), 7.12 (2H, d, *J* = 8.5 Hz), 6.99–7.01 (3H, m), 3.82 (3H, s). ^{13}C-NMR δ_C (125 MHz, DMSO-d_6, δ ppm): 167.2, 154.3, 150.9, 148.9, 134.8, 127.8, 123.3, 121.1, 120.0, 119.6, 114.1, 56.2. IR (KBr, ν (cm^{-1})): 3080.3, 2918.3, 2852.7, 1685.8, 1587.4, 1496.8. HR-MS calcd $C_{14}H_{10}N_3O_4$ ($[M - H]^-$): 284.0671, found: 284.0678.

3-methoxy-4-(4-(4-(4-methoxyphenyl)-1H-1,2,3-triazol-1-yl)phenoxy)benzoic acid **5**: The mixture of *p*-methoxyphenylacetylene (53 mg, 0.4 mmol, 4 equiv.), $CuSO_4 \cdot 5H_2O$ (25 mg, 0.1 mmol, 1 equiv.), sodium ascorbate (20 mg, 0.1 mmol, 1 equiv.) in 1:1 mixture *t*-BuOH: H_2O (4 mL) was stirred at room temperature for 5 min. **4** (114 mg, 0.4 mmol, 1 equiv.) was added and the mixture was stirred at 100 °C for 25 min. After cooling down and filtering, the mixture was extracted with ethyl acetate (20 mL × 3). The combined organic layer was dried over Na_2SO_4, filtered and concentrated to give the crude product which was purified by silica gel column chromatography using *n*-hexane:ethyl acetate (2:3) to afford the desired product. Yield: 96 mg (58%), white solid. Mp 266–267 °C. ^{1}H-NMR δ_H (500 MHz, DMSO-d_6, δ ppm): 13.01 (1H, br), 9.12 (1H, s), 7.90 (2H, d, *J* = 8.5 Hz), 7.86 (2H, d, *J* = 8.5 Hz), 7.68 (1H, s), 7.62 (1H, d, *J* = 8.0 Hz), 7.15–7.19 (3H, m), 7.06 (2H, d, *J* = 8.5 Hz), 3.84 (3H, s), 3.81 (3H, s). ^{13}C-NMR δ_C (125 MHz, DMSO-d_6, δ ppm): *δ* 167.2, 159.8, 157.2, 151.2, 147.9, 147.7, 132.5, 128.5, 127.2, 123.4, 123.2, 122.4 121.2, 119.2, 118.5, 114.9, 114.3, 56.3, 55.7. HR-MS calcd $C_{23}H_{20}N_3O_5$ ($[M + H]^+$): 418.1403, found: 418.1399.

L-phenylalanine ethyl ester hydrochloride **8**: To the mixture of *L*-phenylalanine (495 mg, 3 mmol) in EtOH (15 mL) at 0 °C was added dropwise $SOCl_2$ (0.4 mL). The reaction was stirred at reflux for 3 h. After cooling down, the mixture was concentrated and dried to give desired product which was used as such for the next step without purification.

(3-methoxy-4-(4-(4-(4-methoxyphenyl)-1H-1,2,3-triazol-1-yl)phenoxy)benzoyl)-L-phenylalanin ethyl ester **6**: To the mixture of *L*-phenylalanine ethyl ester hydrochloride **8** (33 mg, 0.13 mmol) in DMF (0.4 mL) at room temperature was added **5** (49 mg, 0.12 mmol), TBTU (45 mg, 0.14 mmol), Et_3N (0.1 mL). The reaction mixture was stirred at room temperature for 30 min. The mixture was diluted with 5% $NaHCO_3$ to pH = 9 and extracted with ethyl acetate (30 mL × 3). The combined organic layer was dried over Na_2SO_4, filtered and concentrated to give a residue which was purified by silica gel column chromatography using *n*-hexane:ethyl acetate (1:2) to afford the desired product. Yield: 40 mg (57%), beige solid. Mp 129–130 °C. ^{1}H-NMR δ_H (500 MHz, DMSO-d_6, δ ppm): 9.11 (1H, s), 8.87 (1H, d, *J* = 8.0 Hz), 7.85–7.88 (4H, m), 7.59 (1H, d, *J* = 1.5 Hz), 7.51 (1H, dd, *J* = 1.5 Hz, *J* = 8.5 Hz), 7.28–7.33 (4H, m), 7.19–7.23 (2H, m), 7.11 (2H, d, *J* = 9.0 Hz), 7.06 (2H, d, *J* = 9.0 Hz), 4.65–4.69 (1H, m), 4.10 (2H, q, *J* = 7.0 Hz), 3.82 (3H, s), 3.81 (3H, s), 3.09–3.20 (2H, m), 1.15 (3H, *t*, *J* = 7.0 Hz). ^{13}C-NMR δ_C (125 MHz, DMSO-d_6, δ ppm): *δ* 172.2, 166.1, 159.8, 157.6, 151.3, 147.6, 146.4, 138.1, 132.2, 131.7, 129.6, 128.7, 127.1, 127.0, 123.3, 122.3, 121.5, 121.2, 119.2, 118.0, 114.9, 113.1, 61.1, 56.4, 55.7, 54.9, 36.9, 14.5. IR (KBr, ν (cm^{-1})): 3352.3, 2927.9, 2852.7, 1737.9, 1639.5, 1494.8. HR-MS calcd $C_{34}H_{33}N_4O_6$ ($[M + H]^+$) 593.240, found: 593.324.

Glycine ethyl ester hydrochloride **9**: To the mixture of glycine (150 mg, 2 mmol) in EtOH (10 mL) at 0 °C was added dropwise $SOCl_2$ (0.6 mL). The reaction was stirred at

reflux for 3 h. After cooling down, the mixture was concentrated and dried to give the desired product, which was used as such for the next step without purification.

(3-methoxy-4-(4-(4-(4-methoxyphenyl)-1H-1,2,3-triazol-1-yl)phenoxy)benzoyl)glycine ethyl ester **7**: To the mixture of glycine ethyl ester hydrochloride **9** (20 mg, 0.13 mmol) in DMF (0.2 mL) at room temperature was added **5** (49 mg, 0.12 mmol), TBTU (45 mg, 0.14 mmol), Et_3N (0.1 mL). The reaction mixture was stirred at room temperature for 30 min. The mixture was diluted with 5% $NaHCO_3$ to pH = 9 and extracted with ethyl acetate (30 mL × 3). The combined organic layer was dried over Na_2SO_4, filtered and concentrated to give a residue which was purified by silica gel column chromatography using *n*-hexane:ethyl acetate (1:2) to afford the desired product. Yield: 32 mg (53%), beige solid. Mp 123-124 °C. ^{1}H-NMR δ_H (500 MHz, DMSO-d_6, δ ppm): 9.10 (1H, s), 9.00 (1H, s), 7.85–7.89 (4H, m), 7.69 (1H, s), 7.56 (1H, d, *J* = 8.5 Hz), 7.21 (1H, d, *J* = 8.5 Hz), 7.12 (2H, d, *J* = 8.5 Hz), 7.06 (2H, d, *J* = 8.5 Hz), 4.13 (2H, *q*, *J* = 7.0 Hz), 4.02 (2H, d, *J* = 5.5 Hz), 3.83 (3H, s), 3.80 (3H, s), 1.22 (3H, *t*, *J* = 7.0 Hz). ^{13}C-NMR δ_C (125 MHz, DMSO-d_6, δ ppm): 167.2, 159.8, 157.2, 151.2, 147.9, 147.7, 132.5, 128.5, 127.2, 123.4, 123.2, 122.4 121.2, 119.2, 118.5, 114.9, 114.3, 56.3, 55.7, 41.9, 14.6. IR (KBr, ν (cm^{-1})): 3311.8, 2926.0, 2852.7, 1726.3, 1641.4, 1599.01494.8. HR-MS calcd $C_{27}H_{27}N_4O_6$ ($[M + H]^+$): 503.193, found 503.282.

3.3. MTT Assay for Cell Antiproliferative Activity

The anticancer activity of the synthesized compounds **6** and **7** was evaluated on two human cancer cells lines HepG2 (HB-8065^{TM}), and MCF-7 (HTB-22^{TM}) which were obtained from the American Type Culture Collection (USA) ATCC using recent reported protocol [12].

4. Conclusions

An efficient multistep synthetic procedure was reported for preparing new 1,2,3-triazole-amino acid conjugated compounds (**6** and **7**). The structures of these compounds were confirmed by IR, NMR and HR-MS. These conjugates significantly inhibited the breast MCF7 and liver HepG2 cancer cells proliferation of >30% at concentration < 10 μM. Thus, compounds **6** and **7** represent a new class of potential anticancer compounds for further optimization and mechanistic studies. The reported synthetic route can be used to prepare different collections of triazole-amino acid conjugates for screening on diverse pharmacological targets.

Supplementary Materials: The following is available online: supporting information with NMR spectra of compounds **2**–**7**.

Author Contributions: Conceptualization, T.T.L. (Thanh Tin Le), D.D.V.; conducting experiments, P.T.K.L., T.T.L. (Thanh Tin Le); acquisition of data, T.T.L. (Thanh Thanh Le), H.T.T.D.; interpretation of data, T.T.L. (Thanh Tin Le), D.D.V.; writing—original draft preparation, T.T.L. (Thanh Tin Le), D.D.V.; writing—review and editing, T.T.L. (Thanh Tin Le), D.D.V.; supervision, T.T.L. (Thanh Tin Le), D.D.V.; project administration, T.T.L. (Thanh Tin Le); funding acquisition, T.T.L. (Thanh Tin Le). All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: We are pleased to provide additional data on request.

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

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