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Synthesis and Evaluation of (1,4-Disubstituted)-1,2,3-triazoles as Estrogen Receptor Beta Agonists

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Abstract: Estrogen receptors (ER) are nuclear hormone receptors which are responsible for sex hormone signaling in women. A series of (1,4-disubstituted)-1,2,3-triazoles 5-21 were prepared by reaction of azidophenols with terminal alkynes under Fokin reaction conditions. The products were purified by column chromatography or recrystallization and characterized by NMR and HRMS. The compounds were tested for binding to ERβ via a ligand displacement assay, and 1-(4-hydroxyphenyl)-α-phenyl-1,2,3-triazole-4-ethanol (21) was found to be the most potent analog (EC50 = 1.59 μM). Molecular docking of 5-21 within the ligand binding pocket of ERβ (pdb 2jj3) was performed and the docking scores exhibited a general qualitative trend consistent with the measured EC50 values.

Keywords: estrogen receptor ligand; alkyne–azide cycloaddition; molecular docking

1. Introduction

The estrogen receptors α and β (ERα and ERβ) belong to the nuclear hormone family of intracellular receptors for which 17β-estradiol (E2, Figure 1) is the predominant endogenous ligand. The two receptors exhibit overlapping but distinct patterns of tissue distributions as well as different types of transcriptional regulation [1]. ERα is highly expressed in the breast, liver and uterus and contributes to malignant growth in these tissues [2]. ERβ is more highly expressed in the lungs, prostate, colon, brain and gastrointestinal tract, and binding of E2 exerts beneficial effects in these organs/tissues without the risk of breast cancer [3].

![Figure 1. Structures of 17β-estradiol (E2), ERα-selective agonist 1,3,5-tris(4-hydroxyphenyl)-4-propyl-1H-pyrazole (PPT), and ERβ-selective agonist 2,3-bis(4-hydroxyphenyl)propionitrile (DPN).](https://www.mdpi.com/journal/scipharm)

Numerous non-steroidal molecules are known [4,5] to bind to ERα and/or ERβ, including the ERα-selective agonist PPT [6] and the ERβ-selective agonist DPN [7] (Figure 1).
The majority of these molecules contain a phenol moiety which is responsible for hydrogen-bonding interactions between the phenolic hydroxyl and residues Glu353/Arg394 of ERα, (Glu305/Arg346 of ERβ), and generally between an active site histidine and a second aliphatic or aromatic hydroxyl group located ~11Å distant from the phenolic hydroxyl group [8].

The Cu(I)-catalyzed alkyne–azide cycloaddition reaction, pioneered by the groups of Tornoe [9] and Sharpless [10], has revolutionized the preparation of 1,2,3-triazoles and thrust this moiety into prominence as a linkage. A recent search of SciFinder revealed >12,000 journal references containing the topic “azide alkyne cycloaddition”. A number of review articles [11–16] have covered 1,2,3-triazole as a pharmacological scaffold of importance. This may be attributed to the fact that the triazole is stable to metabolic degradation [17] and imparts improved solubility due to the possibility of hydrogen bonding.

Tron’s group synthesized a family of 1,4-di(hydroxyphenyl)-1,2,3-triazoles [18]. All of these were highly toxic to MCF-7 and MDA-MB-231 cells after 5 days of incubation at a concentration of 100 μM; however, only one compound (1, Figure 2) had high affinity (IC50~45 pM) in a radiolabel displacement assay for the estrogen receptor isolated from cytosolic extracts of porcine uterus. Subsequently, the group of de Pascual-Teresa and Ramos reported the synthesis of 1,4-diaryl-1,2,3-triazoles, their affinity for ERα and ERβ, and their effect on MCF-7 proliferation [19]. Their results revealed that compounds 2a and 2b are full agonists of ERβ at 20 μM, though only 2b exhibited no proliferation effect on MCF-7 cells.

![Figure 2](image-url)

**Figure 2.** Structures of 1,4-di(hydroxyphenyl)-1,2,3-triazoles with reported estrogen receptor binding activity.

Given our interest in the discovery and application of ERβ-selective agonists [20–24], we undertook to prepare and assess a series of (1,4-disubstituted)-1,2,3-triazoles. The results are reported herein.

### 2. Materials and Methods

#### 2.1. Chemistry

Purifications by chromatography were carried out using flash silica gel (32–63 μm). Melting points were measured in open capillary tubes on a MelTemp melting point apparatus and are uncorrected. NMR spectra were recorded on either a Varian Mercury+ 300 Hz or a Varian UnityInova 400 MHz instrument. CHCl₃, CD₂OD and DMSO-d₅ were purchased from Cambridge Isotope Laboratories. Hydrogen-1 NMR spectra were calibrated to 7.27 ppm for residual CHCl₃, 3.95 ppm for CD₂HOD or 2.49 ppm for d₅-DMSO. Carbon-13 NMR spectra were calibrated from the central peak at 77.23 ppm for CDCl₃, 49.3 ppm for CD₃OD or 39.7 ppm for d₅-DMSO. Fluorine-19 NMR spectra were calibrated from internal CF₃CO₂H (~76.55 ppm). Coupling constants are reported in Hz. High-resolution mass spectra were obtained from the COSMIC lab at Old Dominion University.

#### 2.1.2. General Procedure for the Preparation of Azidophenols

To a solution of aminophenol (9.163 mmol) in ethyl acetate (20 mL) and water (2 mL) at 0 °C was added 5 mL of concentrated HCl. The reaction mixture was stirred for 10 min and then sodium nitrate (1.309 g, 15.40 mmol) in water (3 mL) was added to the solution over 3 min and stirred for an additional 30 min. A solution of sodium azide (1.000 g, 15.38 mmol) in water (5 mL) was added dropwise and the mixture was stirred for 30 min.
The mixture was then diluted with water (20 mL) and extracted several times with ethyl acetate. The combined organic layers were washed with dilute NaOH (20 mL) followed by water (20 mL). The organic layer was dried (MgSO₄) and concentrated to afford the desired azide. The crude azidophenols were used without further purification.

4-Azidophenol (3a). The reaction of 4-aminophenol (1.009 g, 9.246 mmol) following the general procedure gave the product as a dark-brown liquid (1.164 g, 94%). IR: 3318, 2106 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.16 (t, J = 8.1 Hz, 1H), 6.71–6.51 (m, 2H), 6.52 (t, J = 2.2 Hz, 1H) ppm.

3-Azidophenol (3b). The reaction of 3-aminophenol (1.000 g, 9.163 mmol) following the general procedure gave the product as a dark-brown liquid (1.139 g, 92%). IR: 3322, 2109 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.16 (t, J = 8.1 Hz, 1H), 6.71–6.51 (m, 2H), 6.52 (t, J = 2.2 Hz, 1H) ppm.

4-Azido-2-methylphenol (3c). The reaction of 4-aminophenol (1.010 g, 8.300 mmol) following the general procedure gave the product as a black liquid (783 mg, 65%). IR: 3320, 2117 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 6.86 (t, J = 9.1 Hz, 1H), 6.65–6.56 (m, 2H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 155.1 (J_fc = 248.5 Hz), 153.6 (J_fc = 10.5 Hz), 121.5 (J_fc = 2.8 Hz), 120.2 (J_fc = 11.6 Hz), 111.8 (J_fc = 3.1 Hz), 104.7 (J_fc = 22.1 Hz) ppm.

4-Azido-2-chlorophenol (3f). The reaction of 4-amino-2-chlorophenol (1.004 g, 6.993 mmol) following the general procedure gave the product as a dark-red liquid (1.090 g, 92%). IR: 3318, 2106 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.71–7.34 (m, 1H), 7.04–6.75 (m, 2H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 158.7, 148.4, 131.9, 130.8, 119.0, 118.0, 113.8, 17.6 ppm.

2.1.3. General Procedure for Triazole Synthesis

To a solution of alkyne (1.00 mmol), sodium ascorbate (0.40 mmol), and copper (II) sulfate (0.20 mmol) in water/tBuOH (1:1, 10 mL) was added 1.00 mmol of the desired azide. The solution was stirred for 30 h. The reaction was extracted with ethyl acetate (2 × 10 mL). The combined organic layers were washed with brine (15 mL), dried (MgSO₄) and concentrated. The resulting triazole compound was purified by column chromatography.

1-(4-Hydroxyphenyl)-1-phenyl-1,2,3-triazole (5). The reaction of ethynylbenzene (98 mg, 0.96 mmol) with 3a (132 mg, 0.977 mmol) was carried out by the general procedure. Purification of the residue by column chromatography (SiO₂, hexane-ethyl acetate = 1:1 to 100% ethyl acetate gradient) gave 5 (173 mg, 70%) as a pale-tan solid. mp 204–205 °C (lit. [25] mp 206–208 °C). ¹H NMR (400 MHz, CD₂OD) δ 8.74 (1H, s, H-5), 7.90 (2H, d, J = 7.6 Hz), 7.68 (2H, J = 8.8 Hz), 7.46 (2H, t, J = 7.6 Hz), 7.37 (1H, t, J = 7.4 Hz), 6.97 (d, 2H, J = 8.8 Hz); ¹³C NMR (400 MHz, CD₂OD) δ 158.7, 148.4, 131.9, 130.8, 129.7, 128.8, 126.3, 122.9, 119.6, 117.0 ppm. The 1H NMR spectral data for this compound (in CD₂OD) were consistent with the literature values [25].

1-(3-Hydroxyphenyl)-1-phenyl-1,2,3-triazole (6). The reaction of ethynylbenzene (97 mg, 0.95 mmol) with 3b (130 mg, 0.962 mmol) was carried out by the general procedure. Purification of the residue by column chromatography (SiO₂, hexane-ethyl acetate = 1:1 to 100% ethyl acetate gradient) gave 6 (166 mg, 68%) as a dark-brown solid. mp 235–237 °C; ¹H NMR (400 MHz, CD₂OD) δ 8.85 (1H, s, H-5), 7.92 (2H, d, J = 7.7 Hz), 7.47 (2H, t, J = 7.3 Hz, 7.41–7.32 (4H, m), 6.92 (1H, d, J = 7.7 Hz) ppm; ¹³C NMR (75 MHz, d₆-DMSO)
δ 158.6, 147.3, 137.7, 130.7, 128.6, 128.5, 126.3, 120.7, 115.4, 110.3, 106.8, 31.2 ppm. HRMS m/z 274.0951.

4-Benzyl-1-(3-hydroxyphenyl)-1,2,3-triazole (8). The reaction of 3-phenyl-1-propyne (0.13 mL, 1.0 mmol) with 3a (129 mg, 0.960 mmol) was carried out by the general procedure. Purification of the residue by column chromatography (SiO₂, hexane–ethyl acetate = 1:1 to 100% ethyl acetate gradient) gave 8 (121 mg, 48%) as a light-brown solid. mp 193–194 °C; 1H NMR (300 MHz, CD₂OD) δ 8.22 (1H, s, H-5), 7.38–7.19 (8H, m), 6.90–6.84 (1H, m), 4.12 (2H, s, CH₂Ph); 13C NMR (100 MHz, CD₂OD) δ 158.7, 149.5, 130.3, 129.1, 128.3, 119.6, 115.8, 110.5, 107.0 ppm. HRMS m/z 274.0950 (calcd. for C₁₃H₁₂N₂O⁺ 274.0951). The NMR spectral data for this compound were consistent with the literature values [27].

4-Hydroxymethyl-1-(3-hydroxyphenyl)-1,2,3-triazole (9). The reaction of 3-propargyl alcohol (0.10 mL, 1.5 mmol) with 3a (200 mg, 1.48 mmol) was carried out by the general procedure. Purification of the residue by column chromatography (SiO₂, hexane-ethyl acetate = 1:1 to 100% ethyl acetate gradient) gave 9 (255 mg, 90%) as a colorless solid. mp 181–185 °C; 1H NMR (400 MHz, CDCl₃) δ 8.13 (1H, s, H-5), 7.35 (1H, t, J = 8.0 Hz), 7.29–7.22 (2H, m), 6.90 (1H, dd, J = 1.4, 8.0 Hz), 4.75 (2H, br s, CHO₂H); 13C NMR (100 MHz, CDCl₃) δ 158.5, 147.0, 139.3, 137.7, 130.7, 128.6, 128.5, 126.3, 120.7, 115.4, 110.3, 106.8, 31.2 ppm. HRMS m/z 274.0950 (calcd. for C₁₃H₁₂N₂O⁺ 274.0957). The NMR spectral data for this compound were consistent with the literature values [27].
4-(1-Hydroxy-1-methylethyl)-1-(3-hydroxyphenyl)-1,2,3-triazole (13). The reaction of 2-methyl-3-butyne-2-ol (0.20 mL, 2.1 mmol) with 3b (300 mg, 2.22 mmol) was carried out by the general procedure. Purification of the residue by column chromatography (SiO₂, hexane-ethyl acetate = 1:1 to 100% ethyl acetate gradient) gave 13 (310 mg, 69%) as a colorless solid. mp 201–202 °C; 1H NMR (300 MHz, CD₂OD) δ 8.25 (1H, s, H-5), 7.71 (1H, d, J = 2.4 Hz), 7.56 (1H, dd, J = 2.4, 8.7 Hz), 7.05 (1H, d, J = 8.7 Hz), 1.66 (6H, s, C(OH)Me₂) ppm; 13C NMR (75 MHz, CD₂OD) δ 157.7, 155.1, 131.0, 123.5, 122.5, 121.4, 120.2, 118.0, 69.0 (C(OH)Me₂), 30.6 (C(OH)Me₂) ppm. HRMS m/z 242.0900 (calcd. for C₁₁H₁₃N₃O₂Na⁺ 242.0900).

4-(1-Hydroxy-1-methylethyl)-1-(4-hydroxy-3-methylphenyl)-1,2,3-triazole (14). The reaction of 2-methyl-3-butyne-2-ol (0.20 mL, 2.1 mmol) with 3c (300 mg, 2.07 mmol) was carried out by the general procedure. Purification of the residue by column chromatography (SiO₂, hexane-ethyl acetate = 1:1 to 100% ethyl acetate gradient) gave 14 (387 mg, 80%) as a reddish-brown solid. mp 144-144 °C; 1H NMR (CD₂OD, 400 MHz) δ 9.48 (1H, br s, 7.14 (1H, d, J = 8.4 Hz, H-6'), 6.80 (d, J = 2.3 Hz, H3'), 6.74 (dd, J = 2.3, 8.4 Hz, H5'), 2.03 (3H, s, Me-2'). 1.63 (6H, s, Me) ppm; 13C NMR (CD₂OD, 100 MHz) δ 157.5, 131.0, 127.3, 124.3, 120.5, 116.0, 30.7, 16.2 ppm; HRMS m/z 256.1058 (calcd. for C₁₂H₁₃N₃O₂Na⁺ 256.1056).

4-(1-Hydroxy-1-methylethyl)-1-(4-hydroxy-2-methylphenyl)-1,2,3-triazole (15). The reaction of 2-methyl-3-butyne-2-ol (0.20 mL, 2.1 mmol) with 3d (300 mg, 2.07 mmol) was carried out by the general procedure. Purification of the residue by column chromatography (SiO₂, hexane-ethyl acetate = 1:1 to 100% ethyl acetate gradient) gave 15 (387 mg, 91%) as a light-yellow solid. mp 144-144 °C; 1H NMR (CD₂OD, 400 MHz) δ 7.95 (1H, br s, 7.14 (1H, d, J = 8.4 Hz, H-6'), 6.80 (d, J = 2.3 Hz, H3'), 6.74 (dd, J = 2.3, 8.4 Hz, H5'), 2.03 (3H, s, Me-2'). 1.63 (6H, s, Me) ppm; 13C NMR (CD₂OD, 100 MHz) δ 161.5 (d, JCF = 11.1 Hz), 157.2, 156.8 (d, JCF = 248 Hz), 127.8, 123.3 (d, JCF = 4.0 Hz), 118.3 (d, JCF = 11.4 Hz), 113.1, 104.6 (d, JCF = 22.0 Hz), 69.1, 30.6; 19F NMR (376 MHz, CD₂OD) δ -125.3 ppm; HRMS m/z 260.0805 (calcd. for C₁₁H₁₂F₃N₃O₅Na⁺ 260.0806).

1-(2-Fluoro-4-hydroxyphenyl)-4-(1-methyl-1-hydroxyethyl)-1,2,3-triazole (16). The reaction of 2-methyl-3-butyne-2-ol (84 mg, 1.0 mmol) with 3e (176 mg, 1.15 mmol) was carried out by the general procedure. Purification of the residue by column chromatography (SiO₂, hexane-ethyl acetate = 1:1 to 100% ethyl acetate gradient) gave 16 (130 mg, 55%) as a light-yellow solid. mp 125–126 °C; 1H NMR (CD₂OD, 400 MHz) δ 8.09 (1H, s, H-5), 7.53 (t, J = 8.6 Hz, 1H), 6.78–6.76 (m, 2H), 1.64 (6H, s, Me) ppm; 13C NMR (CD₂OD, 100 MHz) δ 161.5 (d, JCE = 11.1 Hz), 157.2, 156.8 (d, JCF = 248 Hz), 127.8, 123.3 (d, JCF = 4.0 Hz), 118.3 (d, JCF = 11.4 Hz), 113.1, 104.6 (d, JCF = 22.0 Hz), 69.1, 30.6; 19F NMR (376 MHz, CD₂OD) δ -125.3 ppm; HRMS m/z 260.0805 (calcd. for C₁₁H₁₂F₃N₃O₅Na⁺ 260.0806).

1-(3-Chloro-4-hydroxyphenyl)-4-(1-methyl-1-hydroxyethyl)-1,2,3-triazole (17). The reaction of 2-methyl-3-butyne-2-ol (87 mg, 1.0 mmol) with 3f (190 mg, 1.12 mmol) was carried out by the general procedure. Purification of the residue by column chromatography (SiO₂, hexane-ethyl acetate = 1:1 to 100% ethyl acetate gradient) gave 17 (199 mg, 76%) as a dark-brown solid. mp 173–175 °C; 1H NMR (CD₂OD, 400 MHz) δ 8.25 (1H, s, H-5), 7.80–7.77 (1H, m), 7.56 (1H, dd, J = 8.9, 2.4 Hz), 7.05 (1H, d, J = 8.8 Hz), 1.63 (6H, s, C(OH)Me₂) ppm; 13C NMR (CD₂OD, 100 MHz) δ 157.7, 155.1, 131.0, 123.5, 122.5, 121.4, 120.2, 118.0, 69.0 (C(OH)Me₂), 30.5 (C(OH)Me₂) ppm; HRMS m/z 276.0510 (calcd. for C₁₁H₁₂ClN₃O₂Na 276.0510).

4-(2-Hydroxyethyl)-1-(4-hydroxyphenyl)-1,2,3-triazole (18). The reaction of 3-butyne-1-ol (0.14 mL, 1.8 mmol) with 3a (246 mg, 1.82 mmol) was carried out by the general procedure. Purification of the residue by column chromatography (SiO₂, hexane-ethyl acetate = 1:1 to 100% ethyl acetate gradient) gave 18 (291 mg, 78%) as a light-brown solid. mp 153–155 °C; 1H NMR (400 MHz, CD₂OD) δ 8.15 (1H, s, H-5), 7.57 (2H, d, J = 8.6 Hz), 6.92 (2H, d, J = 8.6 Hz), 3.86 (2H, t, J = 4.9 Hz), 2.97 (2H, t, J = 4.9 Hz) ppm; 13C NMR (100 MHz, CD₂OD) δ 159.5, 146.7, 130.9, 123.3, 122.3, 117.0, 62.0, 29.9 ppm. HRMS m/z 228.0743 (calcd. for C₁₀H₁₁N₃O₂Na⁺ 228.0743).

4-(2-Hydroxyethyl)-1-(3-hydroxyphenyl)-1,2,3-triazole (19). The reaction of 3-butyne-1-ol (0.32 mL, 4.2 mmol) with 3b (571 mg, 4.23 mmol) was carried out by the general
procedure. Purification of the residue by column chromatography (SiO$_2$, hexane-ethyl acetate = 1:1 to 100% ethyl acetate gradient) gave 19 (712 mg, 82%) as a light-yellow solid. mp 129–130 °C; $^1$H NMR (300 MHz, CD$_3$OD) δ 8.07 (1H, s, H-5), 7.15 (1H, t, $J = 8.1$ Hz), 7.10–7.01 (2H, m), 6.70 (1H, d, $J = 7.7$ Hz), 3.70 (2H, t, $J = 6.4$ Hz) ppm; $^{13}$C NMR (75 MHz, CD$_3$OD) δ 160.0, 139.4, 131.7, 122.3, 122.2, 116.8, 112.1, 108.5, 62.0, 23.0 ppm. HRMS m/z 409.1626 (calcd. for [C$_{10}$H$_{11}$N$_3$O$_2$]$^2$–H$^+$, 409.1630).

4-(2-Hydroxypropyl)-1-(4-hydroxyphenyl)-1,2,3-triazole (20). The reaction of 4-pentyn-2-ol (0.13 mL, 1.4 mmol) with 3a (189 mg, 1.40 mmol) was carried out by the general procedure. Purification of the residue by column chromatography (SiO$_2$, hexane-ethyl acetate = 1:1 to 100% ethyl acetate gradient) gave (±)-20 (204 mg, 62%) as a colorless solid. mp 142–144 °C; $^1$H NMR (300 MHz, CD$_3$OD) δ 8.10 (1H, s, H-5), 7.54 and 6.89 (4H, AA$'$$B'B'$, $J_{AB} = 7.7$ Hz), 4.06 (1H, sextet, $J = 6.1$ Hz, C$_2$H(Ph)(OH)Me), 2.84 (2H, t, $J = 6.1$ Hz, C$_2$H$_2$CH(OH)Me), 1.20 (3H, d, $J = 6.1$ Hz, CH(OH)C$_3$H$_7$) ppm; $^{13}$C NMR (100 MHz, CD$_3$OD) δ 159.5, 146.6, 130.7, 123.3, 122.6, 117.1, 68.0, 36.0, 23.1 ppm. HRMS m/z 437.1940 (calcd. for [C$_{11}$H$_{13}$N$_3$O$_2$]$^2$–H$^+$, 437.1943).

1-(4-Hydroxyphenyl)-α-phenyl-1,2,3-triazole-4-ethanol (21). The reaction of 2-methyl-3-butyn-2-ol (0.20 mL, 2.1 mmol) with 3a (300 mg, 2.22 mmol) was carried out by the general procedure. The product was recrystallized from ethyl acetate/hexanes to afford (±)-21 (310 mg, 69%) as a pale-tan solid. mp 142–143 °C; $^1$H NMR (400 MHz, CD$_3$OD) δ 7.97 (1H, s, H-5), 7.54 (2H, d, $J = 8.8$ Hz), 6.92 (2H, d, $J = 8.8$ Hz), 5.02–4.95 (m, 1H, CH$_2$(Ph)OH), 3.23–3.10 (m, 2H, CH$_2$CH(Ph)OH) ppm; $^{13}$C NMR (100 MHz, CD$_3$OD) δ 159.5, 145.5, 130.8, 129.4, 128.6, 127.1, 117.1, 74.4, 36.5 ppm. HRMS m/z 280.1090 (calcd. for C$_{16}$H$_{15}$N$_3$O$_2$–H$^+$, 280.1092).

2.2. ER$\beta$ Assays

2.2.1. TR-FRET Assay

ER$\beta$ ligand displacement measurements were performed using the SelectScreen assay service from Thermo Fisher Scientific. The TR-FRET assay involves human ER$\beta$ ligand-binding domain (LBD) that is tagged with glutathione-S-transferase (GST), a Tb-anti-GST antibody that binds to the tag, and a fluorescently labeled estrogen bound in the active site pocket. The TR-FRET signal obtained decreases when competitor compounds displace the fluorescently labeled tracer. Individual dose–response curves are found in Figure S1.

2.2.2. Cell-Based Functional Assay

Kits from Indigo Biosciences were used to examine the impact of (±)-21 on agonist and antagonist activity for full-length, native ER$\beta$. In this assay, a luciferase reporter gene was downstream from an ER$\beta$-responsive promoter activated by an agonist. Chemiluminescence resulting from ER-induced luciferase expression was measured in a SpectraMax M5 (Molecular Devices). Vehicle and E2 controls were included. E2 had an agonist IC$_{50}$ value of 0.022 ± 0.005 nM. Kit instructions were followed. Data were normalized to controls and EC$_{50}$ values were calculated by performing a nonlinear squares fit using Prism 6 (GraphPad).

2.3. Computational

Computational docking was performed using the on-line 1-Click Docking tool in Mcule (www.mcule.com, accessed on 3 July 2022) into the ER$\beta$ agonist configuration (pdb 2jj3) [28]. The 1-Click Docking tool uses the Vina docking algorithm [29]. As a control experiment, 17$\beta$-estradiol was docked into the structure of ER$\beta$ (pdb2jj3) and found to adopt a binding mode where the phenolic hydroxyl group was hydrogen bound to Glu305 and Arg346 and the 17$\beta$-hydroxyl group was hydrogen bound to His475 (binding score = −9.7).
3. Results

Azidophenols 3a–f were prepared from the corresponding aminophenols by diazotization with sodium nitrite and HCl, followed by reaction with sodium azide (Scheme 1). The crude azidophenols were reacted with terminal alkynes 4a–g under standard Fokin conditions [10] to afford the (1,4-disubstituted)-1,2,3-triazoles 5–21 in 48–90% yields (Table 1).

![Scheme 1. Synthesis of (1,4-disubstituted)-1,2,3-triazoles 5–21.](image)

The structures of 5 [25], 6 [26] and 9 [27] were assigned by comparison of their NMR spectral data with the literature values. All (1,4-disubstituted)-1,2,3-triazoles 5–21 were characterized by $^1$H, $^{13}$C NMR spectroscopy and HRMS. The signals for the 5-H proton of 5–8 and 9–21 appeared at ~8.3–8.9 and ~δ 8.0–8.3 ppm respectively, in their $^1$H NMR spectra.

The ability of (1,4-disubstituted)-1,2,3-triazoles to displace a fluorescent-labelled ligand from the ligand binding domain of ERβ was measured in a TR-FRET assay, with EC50 values ranging from 1.6 to >50 μM (Table 1). The most potent compound, 1-(4-hydroxyphenyl)-α-phenyl-1,2,3-triazole-4-ethanol (21), was assayed for agonism in a cell-based functional assay; the resultant EC50 value (1.80 ± 0.26 μM) was in good agreement with the value determined in the ligand displacement assay. Compound 21 was not found to be an ERβ antagonist.

Molecular volumes for 5–21 were calculated using the online Molinspiration property engine (https://www.molinspiration.com/cgi-bin/properties, accessed on 3 July 2022). O–O distances for 9–21 were generated using the online Biomodel engine using JSmol (https://biomodel.uah.es/en/ DIY/JSME/draw.en.htm, accessed on 3 July 2022). Dockings of 5–21 into the ERβ (pdb 2jj3) were calculated with the Mcule online one-click program which uses the Vina docking algorithm.

**Table 1.** 1,4-Disubstituted-1,2,3-triazole yields, ERβ ligand displacement data and docking scores.

<table>
<thead>
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<th>Compound</th>
<th>Yield</th>
<th>ERβ EC50 (μM)</th>
<th>Volume (calc. Å³)</th>
<th>O–O Distance</th>
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<tr>
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<td>Yield</td>
<td>ERβ EC50 (µM)</td>
<td>Volume (calc. Å³)¹</td>
<td>O–O Distance²</td>
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</tr>
<tr>
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<td>25.8</td>
<td>229</td>
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<td>165</td>
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</tr>
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<td>−7.9</td>
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<tr>
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<tr>
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<td>198</td>
<td>9.3 Å</td>
<td>−6.6</td>
</tr>
<tr>
<td>13</td>
<td>69%</td>
<td>9.15</td>
<td>198</td>
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<td>−7.3</td>
</tr>
<tr>
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<td>215</td>
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<tr>
<td>17</td>
<td>76%</td>
<td>&gt;50</td>
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<tr>
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<tr>
<td>(±)-21</td>
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<td>1.59</td>
<td>254</td>
<td>9.2–10.9 Å</td>
<td>−9.3 (R)</td>
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</table>

¹ Molecular volumes were calculated using the Molinspiration Property Engine. ² O–O distance calculated using Biomodel with JSmol. ³ Docking scores were calculated using Mcule online one-click with the Vina docking algorithm (https://mcule.com, accessed on 3 July 2022). All of the structures of 5–20 docked with modest affinity (−6.6 to −8.0 kcal/mol), while the two enantiomers of 21 docked with slightly higher affinity (−9.3 kcal/mol). Molecular volumes, O–O distances and docking scores appear in Table 1.
4. Discussion

The (1,4-disubstituted)-1,2,3-triazoles exhibited a range of potencies in the TR-FRET ligand displacement assay. The 4-phenyl- and 4-benzyl-substituted 1,2,3-triazoles 5-8 were selected to assess whether the presence of two hydroxyl groups at opposite ends of the ligand were crucial for agonist activity. Similarly, the 4-hydroxyethyl-, 4-(1-methyl-1-hydroxyethyl)-, 4-(2-hydroxyethyl)-, 4-(2-hydroxypropyl), and 4-(2-hydroxy-2-phenylethyl)-substituted 1,2,3-triazoles 9-21 were prepared to assess the effect of a hydroxalkyl substituent on agonist activity. While 1-(4-hydroxyphenyl)-1,2,3-triazoles 10 and 13 were more potent than their corresponding 1-(3-hydroxyphenyl)-1,2,3-triazole counterparts 9 and 12, respectively, the opposite was found to be the case for 7 and 18 compared to 8 and 19. However a general correlation between the observed EC50 values and the calculated docking scores was observed; those (1,4-disubstituted)-1,2,3-triazoles with less negative docking scores were found to be less potent. The most potent ERβ agonist was found to be (±)-21 (EC50 = 1.59 µM). This increased potency might be explained by reference to the computationally generated docking poses (Figure 3). These reveal that the phenolic group is situated in a position similar to the phenolic portion of E2, with hydrogen bonding to both a Glu and Arg residue and a π–π interaction with a Phe residue. Similarly, the aliphatic hydroxyl group of (R)- or (S)-21 is situated in close proximity to the His residue. What perhaps allows the increased binding of (±)-21 is that the aryl group of the α-phenylethanol sidechain is oriented into a cleft between helix 12 and the estradiol binding pocket.

![Docked structures of 1-(4-hydroxyphenyl)-α-phenyl-1,2,3-triazole-4-ethanol (21) in the binding pocket of agonist mode human ERβ (pdb code 2jj3): (a) (R) stereoisomer; (b) (S) stereoisomer.](https://www.mdpi.com/article/10.3390/scipharm90030046/s1)

In summary, a series of (1,4-disubstituted)-1,2,3-triazoles were prepared and assessed as agonists for ERβ. While these exhibited a range of EC50 values, 1-(4-hydroxyphenyl)-α-phenyl-1,2,3-triazole-4-ethanol (21) was found to be a single-digit micromolar ERβ agonist.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/scipharm90030046/s1, Figure S1: ERβ TR-FRET displacement assay data, Pages S5–S42: NMR spectra of (1,4-disubstituted)-1,2,3-triazoles.

Author Contributions: Conceptualization, W.A.D. and D.S.S.; investigation, E.A.W., G.C.C. and S.B.-F.; data curation, W.A.D.; writing—original draft preparation, W.A.D.; writing—review and editing, D.S.S. and W.A.D.; supervision, W.A.D. and D.S.S.; project administration, W.A.D.; funding acquisition, W.A.D. and D.S.S. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in Supplementary Materials.

Conflicts of Interest: Daniel S. Sem and William A. Donaldson are co-founders and shareholders of Estrigenix Therapeutics, Inc., a company which aims to dramatically improve women’s health by developing safe, clinically proven treatments for the mental and physical effects of menopause, to enable and empower women to live happier and healthier lives. Since D.S.S. and W.A.D. have disclosed other more potent and highly selective ERβ agonists, they do not anticipate patenting the compounds described in this manuscript.

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