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# Semi-synthesis of β-keto-1,2,3-triazole derivatives from ethinylestradiol and evaluation of the cytotoxic activity

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ABSTRACT

In this study, we report our contribution to the application of the copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction for the synthesis of β-keto-1,2,3-triazole derivatives **3a-f** from ethinylestradiol and their application in the inhibition of two human cancer cells lines: human breast adenocarcinoma (MCF-7) and human hepatocellular carcinoma (HepG2). The β-keto-1,2,3-triazole derivates **3a-f** exhibited moderate cytotoxic activity for the HepG2 cells with $IC_{50}$ values of 29.7 μM (**3a**), 16.4 μM (**3b**), 17.8 μM (**3c**), 20.4 μM (**3d**), 28.1 μM (**3e**) and 28.2 μM (**3f**). The semi-synthetic β-keto-1,2,3-triazoles derivatives **3a-f** were all characterized by FT-IR, NMR, HRMS and $[\alpha]_D$.

## 1. Introduction

Steroids are organic compounds that contain a tetracyclic ring system. They are present in a wide variety of plants, animals and fungi [1, 2]. Members of this class of compounds differ in their oxidation state, chains and functional groups that are attached to the tetracyclic core [3, 4].

Steroidal compounds are widely used as anti-inflammatory, immunosuppressive, anabolic and contraceptive agents [1, 2, 5, 6]. They have also been used against leishmaniasis and to treat breast and prostate cancer [2, 6]. Due to their wide variety of biological activities, many natural steroidal compounds, together with synthetic and semi-synthetic steroids, are routinely prepared and evaluated as drug candidates [1, 7].

Addition or replacement of one or more carbon atoms in a steroidal compound by nitrogen atoms changes its chemical and biological properties [8]. Thus, 1,2,3-triazole scaffolds are of interest for drug development because they do not readily undergo metabolic degradation [4, 7]. These compounds have a wide variety of biological activity including antimicrobial, antiviral, antiepileptic and anti-HIV activity, and they are also active against leishmaniasis [8, 9].

Among the methodologies used in the synthesis of 1,2,3-triazole compounds is the Huisgen 1,3-dipolar cycloaddition. This reaction occurs between azides and terminal alkynes through a concerted mechanism. However, the Huisgen reaction has some drawbacks, such as long reaction time and high temperatures, as well as the formation of 1,4-disubstituted and 1,5-disubstituted regioisomers [10].

In 2002, Sharpless's and Meldal's groups independently discovered the copper-catalyzed azide-alkyne cycloaddition (CuAAC) for the synthesis of 1,2,3-triazole compounds. The reaction can be performed under mild conditions with high regioselectivity and yield. This reaction became also known as the *click reaction* [11, 12, 13, 14]. Deobald's group reported the application of the copper-catalyzed azide-alkyne cycloaddition reaction in the synthesis of 1,2,3-triazole compounds containing steroids, saponins and digitalis analogues [7]. Conner and co-workers reported the application of the *click reaction* in the synthesis of 17α-(2*H*-2,3,4-triazolyl)-estradiol from ethinylestradiol and their interactions in Cytochrome P450 [15].

Recently, Liu's group synthesized a new estradiol derivate, $^{18}$F-17-(1-(2-(dimethyl((trifluoro-14-boranyl)methyl)-14-azanyl)ethyl)-1*H*-1,2,3-triazol-4-yl)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[a]phenanthrene-3,17-diol as a potential Positron Emission Tomography (PET) imaging agent for estrogen receptor-positive breast cancer [16]. Additionally, the synthesis of 1,2,3-triazole derivates possessing a carbonyl group at β-position is also known. For example, Mareddy and co-workers synthesized a new class of 1,2,3-triazole derivates

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from nimesulide as potential inhibitors of phosphodiesterases 4 (PDE 4B). Synthesis of these compounds was carried out via of the copper-catalyzed azide-alkyne cycloaddition reaction [17].

In this study, we report our contribution to the application of the copper-catalyzed azide-alkyne cycloaddition reaction for the semi-synthesis of β-keto-1,2,3-triazole derivatives **3a-f** from ethinylestradiol **2** and their application in the inhibition of growth of two human cancer cells lines: human breast adenocarcinoma (MCF-7) and human hepatocellular carcinoma (HepG2).

## 2. Experimental

### 2.1. General

Deuterated chloroform ($CDCl_3$, 99.8% with 0.5% tetramethylsilane-TMS), deuterated methanol ($CD_3OD$, 99.9%), deuterated dimethylsulfoxide (DMSO-$d_6$, 99.9%) and deuterated acetone (acetone-$d_6$, 99.9%) were purchased from Sigma-Aldrich. Ethyl acetate and hexane were purchased from Synth. Sodium azide was purchased from Merck. The reagents 2-bromo-1-phenylethanone (98%), 2-bromo-1-(4-methoxyphenyl)ethanone (97%), 2-bromo-1-(4-chlorophenyl)ethanone (98%), 2-bromo-1-(4-bromophenyl)ethanone (98%), 2-bromo-1-(4-fluorinephenyl)ethanone (98%), 2-bromo-1-(3-fluorinephenyl)ethanone (98%), (+)-sodium L-ascorbate (98%) and ethinylestradiol (≥98%) were purchased from Sigma-Aldrich.

### 2.2. General procedure for the synthesis of 2-azido-1-phenylethanone derivatives (*1a-f*)

In a round-bottomed flask (100 mL) was added 2-bromo-1-phenylethanone **1a** (5.02 mmol), sodium azide (15.38 mmol) and acetone (30 mL). The reaction mixture was kept under magnetic stirring (450 rpm) at room temperature for 5 h and monitored by TLC. After the reaction finished, the acetone was evaporated under reduced pressure and water was added to the crude reaction. Then, the mixture was extracted with EtOAc (2 × 30 mL), dried over anhydrous $Na_2SO_4$, filtered and evaporated under reduced pressure. The same reaction conditions were applied in the synthesis of the 2-azido-1-phenylethanones **1b-f**. These compounds were purified by column chromatography on silica gel eluted with solution of hexane and EtOAc (8:2). The 2-azido-1-phenylethanones **1a-f** were characterized by $^1$H NMR and $^{13}$C NMR, FTIR and mp (see Supplementary material). The spectroscopy data of the compounds **1a-f** are in accordance to the literature. [18, 19].

*2-azido-1-phenylethanone* (**1a**): Molecular formula: $C_8H_7N_3O$; MM: 161.16 g mol$^{-1}$; yield: 87%, yellow liquid; IR (silicon plate) ν (cm$^{-1}$): 3062, 2900, 2096, 1692, 1597, 1449, 1214, 752, 686; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.56 (s, 2H), 7.50 (t, 2H, $J$ = 7.7 Hz), 7.63 (tt, 1H, $J$ = 7.6 and 1.2 Hz), 7.91 (dd, 2H, $J$ = 8.6 and 1.2 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$) δ (ppm): 55.0, 128.1, 129.1, 134.3, 134.5, 193.3.

*2-azido-1-(4-methoxyphenyl)ethanone* (**1b**): Molecular formula: $C_9H_9N_3O_2$; MM: 191.19 g mol$^{-1}$; yield: 89%, cream solid; mp 61–64 °C; IR (KBr) ν (cm$^{-1}$): 3032, 2905, 2124, 1686, 1599, 1516, 1466, 1271, 1238, 1179, 826; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm): 3.88 (s, 3H), 4.50 (s, 2H), 6.95 (d, 2H, $J$ = 8.9 Hz), 7.88 (d, 2H, $J$ = 9.1 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$) δ (ppm): 54.7, 55.7, 114.3, 127.5, 130.4, 164.4, 191.8.

*2-azido-1-(4-chlorophenyl)ethanone* (**1c**): Molecular formula: $C_8H_6ClN_3O$; MM: 195.61 g mol$^{-1}$; yield: 84%, yellow solid; mp 59–62 °C; IR (silicon plate) ν (cm$^{-1}$): 3089, 2907, 2098, 1690, 1592, 1571, 1489, 1216, 814; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.53 (s, 2H), 7.48 (d, 2H, $J$ = 8.6 Hz), 7.85 (d, 2H, $J$ = 8.6 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$) δ (ppm): 55.0, 129.5, 129.6, 132.8, 140.8, 192.2.

*2-azido-1-(4-bromophenyl)ethanone* (**1d**): Molecular formula: $C_8H_6BrN_3O$; MM: 240.06 g mol$^{-1}$; yield: 78%, orange solid; mp 65–68 °C; IR (KBr) ν (cm$^{-1}$): 3086, 2903, 2114, 1694, 1585, 1485, 1219, 814; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.52 (s, 2H), 7.64 (d, 2H, $J$ = 8.7 Hz), 7.77 (d, 2H, $J$ = 8.7 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$) δ (ppm): 54.9, 129.5, 129.6, 132.5, 133.2, 192.4.

*2-azido-1-(4-fluorophenyl)ethanone* (**1e**): Molecular formula: $C_8H_6FN_3O$; MM: 179.15 g mol$^{-1}$; yield: 80%, yellow solid; mp 57–60 °C; IR (KBr) ν (cm$^{-1}$): 3062, 2900, 2096, 1692, 1597, 1449, 1214, 907, 752; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.53 (s, 2H), 7.17 (ddd, 2H, $J$ = 2.3, 8.7 and 9.1 Hz), 7.94 (ddd, 2H, $J$ = 4.6, 5.3 and 9.1 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$) δ (ppm): 54.9, 116.5 (d, $^2J_{CF}$ = 22.1 Hz), 130.8 (d, $^3J_{CF}$ = 9.5 Hz), 131 ($^4J_{CF}$ = 3.1 Hz), 166.4 ($^1J_{CF}$ = 256.8 Hz), 191.8.

*2-azido-1-(3-fluorophenyl)ethanone* (**1f**): Molecular formula: $C_8H_6FN_3O$; MM: 179.15 g mol$^{-1}$; yield: 70%, orange solid; mp 59–62 °C; IR (KBr) ν (cm$^{-1}$): 2978, 2908, 2096, 1692, 1593, 1488, 1342, 1216, 1090, 730; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.54 (s, 2H), 7.33 (tdd, 1H, $J$ = 1.0, 2.6 and 8.2 Hz), 7.46–7.52 (m, 1H), 7.61 (ddd, 1H, $J$ = 1.6, 2.6 and 9.1 Hz), 7.67 (ddd, 1H, $J$ = 1.0, 1.6 and 7.7 Hz)); $^{13}$C NMR (100 MHz, $CDCl_3$) δ (ppm): 55.1, 115.0 (d, $^2J_{CF}$ = 22.5 Hz), 121.4 (d, $^3J_{CF}$ = 21.5 Hz), 123.8 (d, $^6J_{CF}$ = 3.3 Hz), 130.9 (d, $^4J_{CF}$ = 7.8 Hz), 136.5 (d, $^5J_{CF}$ = 6.4 Hz), 164.0 (d, $^1J_{CF}$ = 249.4 Hz), 192.2.

### 2.3. General procedure for the semi-synthesis of β-keto-1,2,3-triazole derivatives *3a-f* from ethinylestradiol *2*

In a round-bottomed flask (10 mL) was added 2-azido-1-phenylethanone (0.3 mmol) **1a**, ethinylestradiol **2** (0.33 mmol) and acetone (1 mL). Then, a solution of sodium ascorbate (20 mol%) and copper sulfate (10 mol%) in distilled water (1 mL) was added to the mixture. The reaction mixtures was kept under magnetic stirring (450 rpm) at room temperature for 24 h and monitored by TLC. After the reaction went to completion, water was added to the crude reaction. Then, the mixture was extracted with EtOAc (2 × 30 mL), dried over anhydrous $Na_2SO_4$, filtered and evaporated under reduced pressure. The same reaction conditions were applied in the synthesis of the β-keto-1,2,3-triazoles **3a-f**. These compounds were purified by column chromatography on silica gel eluted with solution of hexane and EtOAc (3:7). The β-keto-1,2,3-triazole derivatives **3a-f** were characterized by $^1$H NMR and $^{13}$C NMR, FTIR, HRMS, mp and $[\alpha]_D$ (see Supplementary Material).

*2-(4-((8R,9S,13S,14S,17S)-3,17-dihydroxy-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a] phenanthren*-17-yl)*-1H-1,2,3-triazol*-1-yl)*-1-phenylethan-1-one* (**3a**): Molecular formula: $C_{28}H_{31}N_3O_3$; MM: 457.57 g mol$^{-1}$; yield: 92%, white solid; mp 201–204 °C; $[\alpha]_D$ [23] +34 (*c* 0.05, $CH_3OH$); IR (silicon plate) ν (cm$^{-1}$): 3346, 2924, 2855, 1692, 1596, 1498, 1449, 1225, 1180, 1059, 817; $^1$H NMR (500 MHz, $CD_3OD$) δ (ppm): 0.77–0.85 (m, 1H), 1.06 (s, 3H), 1.31–1.48 (m, 4H), 1.53–1.60 (m, 1H), 1.64–1.67 (m, 1H), 1.82–1.96 (m, 3H), 2.10–2.22 (m, 2H), 2.48–2.55 (m, 1H), 2.74–2.81 (m, 2H), 6.08 (d, 2H, $J$ = 2.9 Hz), 6.47 (d, 1H, $J$ = 2.6 Hz), 6.51 (dd, 1H, $J$ = 8.4 and 2.7 Hz), 7.01 (d, 1H, $J$ = 8.5 Hz), 7.57 (t, 2H, $J$ = 7.8 Hz), 7.69 (tt, 1H, $J$ = 7.6 and 1.2 Hz), 7.84 (s, 1H), 8.08 (dd, 2H, $J$ = 8.4 and 1.2 Hz); $^{13}$C NMR (125 MHz, $CD_3OD$) δ (ppm): 14.9. 24.7, 27.6, 28.7, 30.7, 34.2, 38.4, 41.1, 44.9, 48.5, 49.6, 57.0, 83.2, 113.7, 116.1, 126.0, 127.2, 129.4, 130.1, 132.6, 135.3, 135.7, 138.9, 155.4, 155.9, 193.1. HRMS calcd for $C_{28}H_{31}N_3O_3$ $[M + H]^+$ 458.2438, found 458.2439.

*2-(4-((8R,9S,13S,14S,17S)-3,17-dihydroxy-13-methyl-7,8,7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren*-17-yl)*-1H-1,2,3-triazol*-1-yl)*-1-(4-methoxyphenyl)ethan-1-one* (**3b**): Molecular formula: $C_{29}H_{33}N_3O_4$; MM: 486.60 g mol$^{-1}$; yield: 83%, white solid; mp 250–254 °C; $[\alpha]_D$ [23] +32 (*c* 0.05, $CH_3OH$); IR (KBr) ν (cm$^{-1}$): 3194, 2926, 2855, 1688, 1603, 1508, 1460, 1240, 1180, 1055, 826; $^1$H NMR (500 MHz, DMSO-$d_6$) δ (ppm): 0.64–0.72 (m, 1H), 0.94 (s, 3H), 1.24–1.52 (m, 5H), 1.63–1.72 (m, 1H), 1.74–1.88 (m, 3H), 1.92–1.98 (m, 1H), 2.09–2.15 (m, 1H), 2.35–2.43 (m, 3H), 2.64–2.77 (m, 2H), 3.87 (s, 3H), 5.14 (s, 1H), 6.07 (d, 2H, $J$ = 2.9 Hz), 6.42 (d, 1H, $J$ = 2.5 Hz), 6.48 (dd, 2H, $J$ = 8.4 and 2.6 Hz), 6.98 (d, 1H, $J$ = 8.5 Hz), 7.11 (d, 2H, $J$ = 9 Hz), 7.8 (s, 1H), 8.04 (d, 2H, $J$ = 9 Hz), 8.96 (s, 1H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ (ppm): 14.4, 23.6, 26.1, 27.2, 29.3, 32.6, 37.2, 39.8, 43.2, 46.8, 47.5, 55.3, 55.7, 81.1, 112.7, 114.2, 114.9, 124.4, 126.0, 127.1, 130.4, 130.5, 137.2, 154.0, 154.9, 163.8, 190.6. HRMS calcd for

$C_{29}H_{33}N_3O_4$ $[M + H]^+$ 488.2549, found 488.2541.

*2-(4-((8R,9S,13S,14S,17S)-3,17-dihydroxy-13-methyl-7,8,7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-*17-yl*)-1H-1,2,3-triazol*-1-yl*)-1-(4-chlorophenyl)ethan-1-one* (**3c**): Molecular formula: $C_{28}H_{30}ClN_3O_3$; MM: 492.02 g $mol^{-1}$; yield: 89%, white solid; mp 225–228 °C; $[\alpha]_D$ [23] +104 (*c* 0.05, $CH_3OH$); IR (silicon plate) $\nu$ ($cm^{-1}$): 3365, 2925, 2855, 1665, 1591, 1490, 1452, 1227, 1173, 1090, 1013, 847; $^1H$ NMR (500 MHz, $CD_3OD$) δ (ppm): 0.76–0.84 (m, 1H), 1.06 (s, 3H), 1.28–1.48 (m, 4H), 1.51–1.61 (m, 1H), 1.63–1.68 (m, 1H), 1.82–1.99 (m, 3H), 2.10–2.21 (m, 2H), 2.47–2.55 (m, 1H), 2.71–2.81 (m, 2H), 6.06 (d, 2H, $J = 1.7$ Hz), 6.46 (d, 1H, $J = 2.6$ Hz), 6.51 (dd, 1H, $J =$ 8.4 and 2.7 Hz), 7.01 (d, 1H, $J = 8.4$ Hz), 7.60 (d, 2H, $J = 8.8$ Hz), 7.83 (s, 1H), 8.06 (d, 2H, $J = 8.7$ Hz); $^{13}C$ NMR (125 MHz, $CD_3OD$) δ (ppm): 14.9, 24.7, 27.6, 28.8, 30.7, 34.3, 38.4, 41.1, 44.9, 48.5, 49.6, 56.9, 83.2, 113.7, 116.0, 126.0, 127.2, 130.3, 131.0, 132.5, 134.3, 138.8, 141.6, 155.5, 155.9, 192.0. HRMS calcd for $C_{28}H_{30}ClN_3O_3$ $[M + H]^+$ 492.2054, found 492.2044.

*2-(4-((8R,9S,13S,14S,17S)-3,17-dihydroxy-13-methyl-7,8,7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-*17-yl*)-1H-1,2,3-triazol*-1-yl*)-1-(4-bromophenyl)ethan-1-one* (**3d**): Molecular formula: $C_{28}H_{30}BrN_3O_3$; MM: 536.47 g $mol^{-1}$; yield: 90%, white solid; mp 230–234 °C; $[\alpha]_D$ [23] +42 (*c* 0.05, $CH_3OH$); IR (KBr) $\nu$ ($cm^{-1}$): 3136, 2928, 2864, 1688, 1584, 1499, 1454, 1288, 1229, 1069, 993, 816; $^1H$ NMR (500 MHz, acetone-$d_6$) δ (ppm): 0.76–0.85 (m, 1H), 1.06 (s, 3H), 1.27–1.47 (m, 4H), 1.51–1.61 (m, 2H), 1.84–1.95 (m, 3H), 2.08–2.21 (m, 2H), 2.44–2.54 (m, 1H), 2.66–2.87 (m, 2H), 6.11 (s, 2H), 6.51 (d, 1H, $J =$ 2.6 Hz), 6.56 (dd, 1H, $J = 8.4$ and 2.7 Hz), 7.03 (d, 1H, $J = 8.1$ Hz), 7.79 (d, 2H, $J = 8.8$ Hz), 7.85 (s, 1H), 8.04 (d, 2H, $J = 8.6$ Hz); $^{13}C$ NMR (125 MHz, acetone-$d_6$) δ (ppm): 14.9, 24.4, 27.3, 28.4, 30.1, 33.8, 38.5, 40.7, 44.5, 48.1, 49.0, 56.4, 82.7, 113.6, 115.9, 124.8, 127.0, 129.3, 130.9, 132.0, 133.0, 134.5, 138.4, 155.2, 156.0, 191.9. HRMS calcd for $C_{28}H_{30}BrN_3O_3$ $[M + H]^+$ 536.1549, found 536.1543.

*2-(4-((8R,9S,13S,14S,17S)-3,17-dihydroxy-13-methyl-7,8,7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-*17-yl*)-1H-1,2,3-triazol*-1-yl*)-1-(4-fluorinephenyl)ethan-1-one* (**3e**): Molecular formula: $C_{28}H_{30}FN_3O_3$; MM: 475.56 g $mol^{-1}$; yield: 65%, white solid; mp 232–235 °C; $[\alpha]_D$ [23] +52 (*c* 0.05, $CH_3OH$); IR (KBr) $\nu$ ($cm^{-1}$): 3279, 2926, 2857, 1695, 1599, 1506, 1456, 1288, 1234, 1055, 1001, 828; $^1H$ NMR (500 MHz, $CD_3OD$) δ (ppm): 0.77–0.85 (m, 1H), 1.06 (s, 3H), 1.28–1.49 (m, 4H), 1.51–1.60 (m, 1H), 1.63–1.68 (m, 1H), 1.85–2.00 (m, 3H), 2.09–2.20 (m, 2H), 2.47–2.56 (m, 1H), 2.70–2.81 (m, 2H), 6.06 (d, 2H, $J = 1.7$ Hz), 6.46 (d, 1H, $J = 2.6$ Hz), 6.51 (dd, 1H, $J =$ 2.7 and 8.4 Hz), 7.01 (d, 1H, $J = 8.4$ Hz), 7.30 (ddd, 2H, $J = 2.3$, 8.7 and 9.1 Hz); $^{13}C$ NMR (125 MHz, $CD_3OD$) δ (ppm): 14.9, 24.7, 27.6, 28.7 30.8, 34.3, 38.4, 41.1, 44.9, 48.5, 49.6, 56.8, 83.2, 113.7, 116.0, 117.1 (d, $^2J_{CF} = 22.4$ Hz), 126.0, 127.1, 132.2 (d, $^3J_{CF} = 9.5$ Hz), 132.3, 132.5, 138.8, 155.4, 155.9, 167.7 (d, $^1J_{CF} = 254.6$ Hz), 191.6. HRMS calcd for $C_{28}H_{30}FN_3O_3$ $[M + H]^+$ 476.2344, found 476.2343.

*2-(4-((8R,9S,13S,14S,17S)-3,17-dihydroxy-13-methyl-7,8,7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-*17-yl*)-1H-1,2,3-triazol*-1-yl*)-1-(3-fluorinephenyl)ethan-1-one* (**3f**): Molecular formula: $C_{28}H_{30}FN_3O_3$; MM: 475.56 g $mol^{-1}$; yield: 63%, white solid; mp 225–228 °C; $[\alpha]_D$ [23] +26 (*c* 0.05, $CH_3OH$); IR (KBr) $\nu$ ($cm^{-1}$): 2926, 2856, 1704, 1589, 1498, 1447, 1286 1255, 1057, 1004, 872; $^1H$ NMR (500 MHz, $CD_3OD$) δ (ppm): 0.78–0.84 (m, 1H), 1.06 (s, 3H), 1.32–1.49 (m, 4H), 1.54–1.60 (m, 1H), 1.63–1.70 (m, 1H), 1.86–2.00 (m, 3H), 2.09–2.20 (m, 2H), 2.48–2.54 (m, 1H), 2.72–2.80 (m, 2H), 6.08 (d, 2H, $J = 2.0$ Hz), 6.46 (d, 1H, $J = 2.6$ Hz), 6.51 (dd, 1H, $J = 2.7$ and 8.4 Hz), 7.02 (d, 1H, $J = 8.4$ Hz), 7.45 (tdd, 1H, $J = 0.8$, 2.6 and 8.4 Hz), 7.59–7.63 (m, 1H), 7.79 (ddd, 1H, $J = 1.6$, 2.5 and 9.4 Hz), 7.84 (s, 1H), 7.92 (ddd, 1H, $J = 1.0$, 1.4 and 7.8 Hz); $^{13}C$ NMR (125 MHz, $CD_3OD$) δ (ppm): 13.5, 23.3, 26.2, 27.3, 29.3, 32.8, 37.0, 39.7, 43.5, 47.0, 48.1, 55.7, 81.8, 112.3, 114.4 (d, $^2J_{CF} = 23$ Hz), 114.6, 120.8 (d, $^3J_{CF} = 21.7$ Hz), 123.9 (d, $^6J_{CF} = 3.0$ Hz), 124.6, 125.7, 130.8 (d, $^4J_{CF} = 7.7$ Hz), 131.1, 136.5 (d, $^5J_{CF} = 6.6$ Hz), 137.4, 154.1, 163.9 (d, $^1J_{CF} = 244.1$ Hz), 190.6. HRMS calcd for $C_{28}H_{30}FN_3O_3$ $[M + H]^+$ 476.2344, found 476.2343.

### 2.4. Fourier transform infrared analysis (FTIR)

The FTIR spectra of the purified compounds were recorded on a Shimadzu IRAffinity-1 spectrometer model. Analyses were performed using KBr for solid samples and silicon plates for liquid samples. The transmittance was measured in $cm^{-1}$ in the 4000-600 $cm^{-1}$ region.

### 2.5. Nuclear magnetic resonance (NMR)

The $^1H$ and $^{13}C$ nuclear magnetic resonance (NMR) spectra of the purified compounds were recorded on an Agilent Technologies 400/54 Premium Shielded ($^1H$ NMR and $^{13}C$ NMR at 400 and 100 MHz) or Agilent Technologies 500/54 Premium Shielded ($^1H$ NMR and $^{13}C$ at 500 and 125 MHz) spectrometer. The samples were solubilized in acetone-$d_6$, $CDCl_3$, $CD_3OD$ or DMSO-$d_6$, and the chemical shifts were reported in ppm relative to an internal standard, TMS. Coupling constants (*J*) were expressed in hertz (Hz).

### 2.6. High resolution mass spectrometry (HRMS)

The HRMS spectra were recorded on a micro Tof-QII hybrid quadrupole/time-of-flight (QqToF) mass spectrometer, from Daltonics (Bremen, Germany), equipped with an electrospray ionization (ESI) source. ESI source conditions used in the positive ionization mode included a capillary voltage of 4.0 kV, drying gas flow rate of 8.0 L/min, nebulizing gas pressure set at 4 bar and source temperature set at 200 °C. Data acquisition was performed using full MS mode (quadrupole $m/z$ range was set from 50 to 3000 Da) at 1.0 Hz rate. Data processing was performed with software (version 4.2), also from Bruker Daltonics.

### 2.7. Absolute configuration

The optical rotations ($[\alpha]_D^T$) of the β-keto-1,2,3-triazoles **3a-f** were measured at 23 °C with a JASCO P2000 polarimeter equipped with a 589 nm-lamp Na in 1 dm cuvette. The samples were prepared with 1 mg of the purified compound diluted in 2.0 mL of $CH_3OH$ (0.05 g/100 mL).

### 2.8. Cytotoxic assay

Cancer cell lines, MCF-7 (human breast adenocarcinoma) and HepG2 (human hepatocellular carcinoma) and non-cancer cell line, MRC-5 (human lung fibroblast), were obtained from the American Type Culture Collection (ATCC) [20]. The cells were cultured in cell culture bottles (75 $cm^3$, 250 mL volume) in RPMI 1640 medium and supplemented with 10% fetal bovine serum. The cells were maintained in incubators under a 5% $CO_2$ atmosphere at 37 °C. Cellular growth was monitored daily using an inverted microscope. The medium was changed whenever the cell growth reached the necessary confluence for nutrient renewal. For the maintenance of adherent cells, trypsin (0.25%) was used detach the cells from the surface of the bottles. Cell cultures were mycoplasma negative, as assessed by incubation with Hoechst (Mycoplasma Stain Kit, Cat, MYC1, Sigma-Aldrich, St. Louis, MO, USA).

Cell viability was quantified using alamar blue assay, as previously described [21]. Initially, the cells were plated in 96-well plates (100 μL per well of a solution of $0.3 \times 10^6$ cells per mL for cells in suspension and $0.7 \times 10^5$ cells per mL for adhered cells). After 24 h of incubation, the compounds **3a-f** solubilized in DMSO were added to the cells and incubated for 72 h. Doxorubicin (purity >95%, Laboratórios IMA S.A.I.C., Buenos Aires, Argentina) was used as a positive control and the negative control received the same amount of DMSO. Then, 4 h before the end of the incubation period, 20 μL of stock solution (0.312 mg $mL^{-1}$) of alamar blue (resazurin) was added to each well. Absorbance was measured at wavelengths of 570 nm (reduced) and 595 nm (oxidized) using a plate reader. $IC_{50}$ values were determined from the non-linear regression of the

percentage of inhibition × log of the concentration, using the program Prisma version 5.0 (GraphPad Software).

## 3. Results and discussion

### 3.1. Semi-synthesis of β-keto-1,2,3-triazole derivatives 3a-f from ethinylestradiol 2

To obtain the optimal reaction conditions for 1,3-dipolar cycloaddition of 2-azido-1-phenylethanone **1a** with ethinylestradiol **2**, the reaction was performed in a mixture of organic solvent and $H_2O$ (1:1) in the presence of $CuSO_4.5H_2O$ (10 mol%) and sodium ascorbate (20 mol%) (Scheme 1).

The first reaction condition tested was the organic solvent. The reaction was performed at room temperature for 14 h (Table 1, entries 1–4). Using the $H_2O$ and isopropanol (1:1) system, compound **3a** was obtained with a 38% yield (Table 1, entry 1). A mixture of ethanol and $H_2O$ (1:1) produced the desired product **3a** with a 56% yield (Table 1, entry 2). However, when the reaction was performed in THF and $H_2O$ (1:1) or acetone and $H_2O$ (1:1), product **3a** was obtained with 72% and 83% yields, respectively (Table 1, entries 3 and 4). Therefore, the acetone and $H_2O$ solution was chosen as the solvent for the reaction because it afforded a higher reaction yield and good solubility of 2-azido-1-phenylethanone **1a** and ethinylestradiol **2** among the reaction conditions studied.

To evaluate the influence of temperature on this reaction, a study was performed using three temperature conditions (26 °C, 40 °C, and 50 °C) (Table 1, entries 5–7). When the reaction was performed in a mixture of acetone and $H_2O$ (1:1) at room temperature for 6 h, the β-keto-1,2,3-triazole derivative **3a** was obtained with a 70% yield (Table 1, entry 5). When the reaction was performed at 40 °C or 50 °C for 6 h, compound **3a** was obtained with 66% and 63% yields, respectively (Table 1, entries 6 and 7). Investigation of the temperature effects on the reaction revealed that the increase in temperature has no significant influence on the yield of product **3a**.

Another condition tested was the reaction time (Table 1, entries 4, 5, and 8). When the reaction time was decreased from 14 to 6 h, the chemical yield decreased from 83% to 70% (Table 1, entries 4 and 5). The reaction was performed for 24 h and its chemical yield increased to 92% (Table 1, entries 4 and 8).

The optimized reaction conditions were used to synthesize the five different β-keto-1,2,3-triazole derivatives **3b-f** (Scheme 2). High isolated yields were obtained for compounds **3a** (92%), **3b** (83%), **3c** (89%), and **3d** (90%). Good isolated yields were obtained for compounds **3e** (65%) and **3f** (63%), as reported in Table 2. Compounds **3a-3f** were isolated using column chromatography containing silica gel as a stationary phase and characterized using Fourier transform infrared (FTIR), nuclear magnetic resonance (NMR), high resolution mass spectrometry (HRMS), optical rotation $[\alpha]_D$, and melting point (mp).

Compound **3a** was purified as a white solid (mp 201–204 °C). The molecular formula $C_{28}H_{31}N_3O_3$ was established using HRESIMS data (*m/z* for 458.2438 $[M + H]^+$, establishing an index of hydrogen deficiency (IDH) of 15. The IR spectrum of compound **3a** showed an absorption band at 3200 $cm^{-1}$ for a hydroxyl group and an absorption band at 1691 $cm^{-1}$ for a carbonyl group. It was also showed a C–H stretching band at 2926 $cm^{-1}$ and a C–H asymmetrical band at 2840 $cm^{-1}$.

**Table 1**
Optimization of the 1,3-dipolar cycloaddition reaction conditions using 2-azido-1-phenylethanone **1a** and ethinylestradiol **2** in the presence of $CuSO_4.5H_2O$ and sodium ascorbate.

| Entry | Solvente-$H_2O$ (1:1) | Temperature (°C) | Yield[a] (%) | Reaction time (h) |
|---|---|---|---|---|
| 1[b] | Isopropanol | 26 | 38 | 14 |
| 2[b] | Ethanol | 26 | 56 | 14 |
| 3[b] | THF | 26 | 72 | 14 |
| 4[b] | Acetone | 26 | 83 | 14 |
| 5[c] | Acetone | 26 | 70 | 6 |
| 6[c] | Acetone | 40 | 66 | 6 |
| 7[c] | Acetone | 50 | 63 | 6 |
| 8[d] | Acetone | 26 | 92 | 24 |

[a] Isolated yield.
[b] $CuSO_4.5H_2O$ (10 mol%), sodium ascorbate (10 mol%), reaction time of 14 h.
[c] $CuSO_4.5H_2O$ (10 mol%), sodium ascorbate (10 mol%), reaction time of 6 h.
[d] $CuSO_4.5H_2O$ (10 mol%), sodium ascorbate (10 mol%), reaction time of 24 h.

The $^1H$ NMR spectrum of compound **3a** showed a singlet at $\delta_H$ 7.84 for the vinyl hydrogen. Additionally, three aromatic signals were integrated for four protons with chemical shifts at $\delta_H$ 7.57, 7.69, and 8.08, which confirms the aromatic moiety vicinal to carbonyl. The signals at $\delta_H$ 6.47, 6.51, 7.01, and 7.69 were integrated for three protons, which confirms the aromatic moiety from ethinylestradiol.

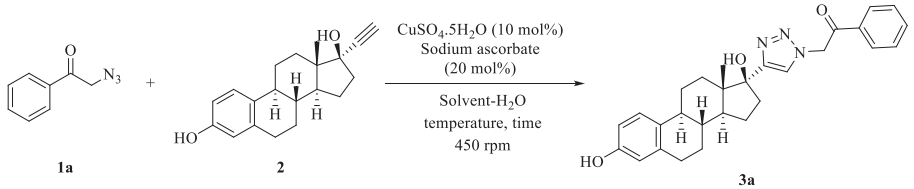
The $^{13}C$ NMR spectrum of compound **3a** showed a signal at $\delta_C$ 57.0 for the methylene carbon vicinal to triazole nucleus. The characteristic signal at $\delta_C$ 83.2 results from the carbinolic carbon vicinal to the triazole nucleus. The presence of ten signals from $\delta_C$ 126.0–155.9 for twelve carbons, with two of these signals having double intensity, confirm the two aromatic moieties. The signals at $\delta_C$ 126.0 and 155.4 are a result of the carbons from triazole nucleus and the typical signal at $\delta_C$ 193.1 is a result of the carbonyl carbon of a ketone. The spectra of all compounds **3a-f** were similar, except for some signals in the aromatic regions that resulted from different 2-azido-1-phenylethanones substitutions on the aromatic ring.

### 3.2. Cytotoxic activity

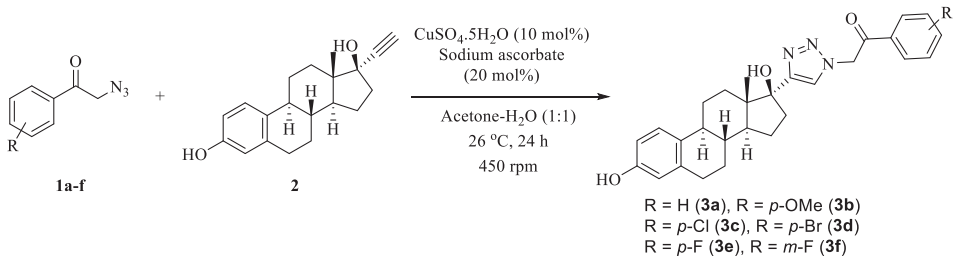
The synthesized compounds **3a-f** were evaluated against two human cancer cell lines, MCF-7 and HepG2, and one non-cancer cell line, MRC-5. Table 3 shows the $IC_{50}$ data and the respective 95% confidence interval obtained by non-linear regression using the GraphPad Prism version 5.0 program. Doxorubicin was used as a positive control for the cytotoxicity assay, with an $IC_{50}$ value of 1.9 μM for MCF-7 cells, 0.2 μM for HepG2 cells and 2.4 μM for MRC-5 cells.

As shown in Table 3, the β-keto-1,2,3-triazole compounds **3a-f** exhibited moderate cytotoxic activity against the cancer cell line HepG2 with $IC_{50}$ values of 29.7, 16.4, 17.8, 20.4, 28.1 and 28.2 μM, respectively. The $IC_{50}$ values found for the cancer cell line MCF-7 were >54.6, 43.6, 44.4, 46.1, 39.3 and >52.5 μM, respectively, while no cytotoxic activity against the non-cancer cell line MRC-5 was found at the experimental concentrations tested.

This is an unpublished study describing the evaluation of cytotoxic activity in semi-synthetic β-keto-1,2,3-triazole derivates **3a-f** from ethinylestradiol **2**. In the literature there are few studies describing the




**Scheme 1.** The 1,3-dipolar cycloaddition reaction using 2-azido-1-phenylethanone 1a and ethinylestradiol 2.




**Scheme 2.** The 1,3-dipolar cycloaddition reaction using 2-azido-1-phenylethanones 1a-f and ethinylestradiol 2.

**Table 2**
Yields for the β-keto-1,2,3-triazole derivatives **3b-f** obtained by click reaction.

| Entry | R | Yield[a] (%) |
|---|---|---|
| 1 | H (**3a**) | 92 |
| 2 | *p*-$OCH_3$ (**3b**) | 83 |
| 3 | *p*-Cl (**3c**) | 89 |
| 4 | *p*-Br (**3d**) | 90 |
| 5 | *p*-F (**3e**) | 65 |
| 6 | *m*-F (**3f**) | 63 |

[a] Isolated yield.

**Table 3**
The $IC_{50}$ values obtained for cytotoxic activity in human cancer cell lines *versus* non-cancer[a] for the β-keto-1,2,3-triazole compounds **3a-f**.

| β-keto-1,2,3-triazoles | $IC_{50}$ (μM) | | |
|---|---|---|---|
| | MCF-7 | HepG2 | MRC-5 |
| **3a** | >54.6 | 29.7<br>24.1–36.5 | >54.6 |
| **3b** | 43.6<br>30.5–62.3 | 16.4<br>12.7–21.2 | >52.5 |
| **3c** | 44.4<br>31.4–62.8 | 17.8<br>13.1–24.3 | >50.8 |
| **3d** | 46.1<br>29.8–71.1 | 20.4<br>15.0–27.7 | >46.6 |
| **3e** | 39.3<br>32.2–47.9 | 28.1<br>20.3–38.7 | >52.5 |
| **3f** | >52.5 | 28.2<br>18.7–42.7 | >52.5 |
| Doxorubicin | 1.9<br>1.4–2.5 | 0.20<br>0.1–0.3 | 2.4<br>1.9–3.0 |

Data are presented as $IC_{50}$ values in μM and 95% confidence interval obtained by nonlinear regression from at the least three independent experiments performed in duplicate, measured by alamar blue assay after 72 h of incubation. Cancer cell lines: MCF-7 (human breast adenocarcinoma) and HepG2 (human hepatocellular carcinoma). Non-cancer cell line: MRC-5 (human lung fibroblast). Doxorubicin was used as a positive control.

synthesis and evaluation of cytotoxic activity of 1,2,3-triazole compounds from steroids. Recently, Ortiz and co-workers reported the synthesis of two pregnane derivates with a triazole (3β-hydroxy-21-(1*H*-1,2,4-triazol-1-yl)pregna-5,16-dien-20-one) or imidazole (3β-hydroxy-21-(1*H*-imidazol-1-yl)pregna-5,16-dien-20-one) ring and their application in inhibiting three human cancer cells lines: prostate cancer (PC-3), breast cancer (MCF7) and lung cancer (SK-LU-1). The results showed that the 3β-hydroxy-21-(1*H*-1,2,4-triazol-1-yl)pregna-5,16-dien-20-one compound exhibited cytotoxic activity for the PC-3, MCF7 and SK-LU-1 cancer cell lines with $IC_{50}$ values of 17, 360 and 230 μM, respectively [4].

Also there are studies in the literature describing the synthesis and evaluation of biological activity of other 1,2,3-triazole derivates [22, 23, 24, 25]. The coumarin-1,2,3-triazole-dithiocarbamate hybrids were designed, synthesized and evaluated for their inhibitory activity towards lysine specific demethylase 1 (LSD1). Several of these compounds presented potent activity against LSD1 [26]. Kuntala's group synthesized novel benzoxepine-1,2,3-triazole hybrids and applied them as antibacterial and anticancer agents. Some of these compounds showed antibacterial activity against gram-positive and gram-negative species. These compounds also showed cytotoxicities against lung and colon cancer cell lines [27].

The 1,2,3-triazole-nimesulide hybrids were designed, synthesized and evaluated as anticancer agents. Several of these compounds showed growth inhibition of A549 (lung cancer), HepG2 (liver cancer), HeLa (cervical cancer) and DU145 (prostate cancer) cancer cell lines [28]. Neeraja and co-workers synthesized 1*H*-1,2,3-triazolyl-substituted 1,3,4-oxadiazole derivates containing structural features of ibuprofen/naproxen as antibacterial agents. Several of these compounds showed good to reasonable antibacterial activities when tested against three gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus subtilis*) and three gram-negative (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*) species.

The 2-(4-((5-(1-(4-isobutylphenyl)ethyl)-1,3,4-oxadiazol-2-ylthio)methyl)-1*H*-1,2,3-triazol-1-yl)-*N*-(2-nitrophenyl)acetamide compound showed promising activities across both the species [29].

Our results demonstrated that compounds **3a-f** were active against HepG2 cell proliferation. Therefore, the β-keto-1,2,3-triazole compounds **3a-f** may be promising for the development of novel therapeutic alternatives to treat cancer. However, new derivatives can be synthesized that may provide better results.

## 4. Conclusions

Synthesis of the six β-keto-1,2,3-triazole derivatives **3a-f** were obtained with good isolated yields (63–92%) were obtained through optimization of the 1,3-dipolar cycloaddition reaction in the presence of $CuSO_4.5H_2O$ and sodium ascorbate using different 2-azido-1-phenylethanones **1a-f** and ethinylestradiol **2.** These compounds were investigated against two human cancer cells lines, MCF-7 and HepG2. Compounds **3a-f** showed moderate cytotoxic activity against the HepG2 cancer cells.

## Declarations

### *Author contribution statement*

Thayane M. Queiroz: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Erika V.M. Orozco: Conceived and designed the experiments; Analyzed and interpreted the data. Valdenizia R. Silva, Luciano S. Santos: Performed the experiments; Analyzed and interpreted the data. Milena B.P. Soares, Daniel P. Bezerra: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data. André L.M. Porto: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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### *Competing interest statement*

The authors declare no conflict of interest.

### *Additional information*

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