Cytotoxicity Screening and Molecular Docking Studies of Newly Synthesized 1,4-Disubstituted 1,2,3-Triazoles as Potential anticancer agents.

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Abstract

Ten new 2,6-dimethyl quinoline triazole derivatives with different substituents in the triazole moiety were synthesized via copper-catalyzed cycloaddition (CuAAC) click chemistry between 4-Azido-2,6-dimethylquinoline and ten different terminal alkynes. All the synthesized compounds were characterized via different spectroscopic tools such as IR, MS, ^{1}H NMR, and ^{13}C NMR, techniques to elucidate their structures and the spectral analysis of the compounds was in agreement with the proposed structures. Then all the synthetic compounds were evaluated for their cytotoxic activity against breast cancer (MCF-7) and prostate cancer (PC-3) cell lines using MTT colorimetric assay. Among all the synthesized ten compounds, 24 was the most active compound in the cytotoxic activity. The molecular docking study of the synthesized compounds was performed against 5EF5 (Chaetomium thermophilum Raptor) and 3HB5 (a novel inhibitor of 17 beta-HSD type 1: a lead compound for breast cancer therapy) to understand the binding interactions and the relationship between structure and activity.

Key Words: 1,4-Disubstituted 1,2,3-Triazoles, Anticancer activity, Molecular docking

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1. Introduction

Globally, there is a serious public health issue with cancer as it is a primary cause of death besides cardiovascular disease. Incidence of cancer is predicted to rise globally due to demographic factors over the coming decades, with more than 20 million new cases of cancer annually anticipated by 2025. GLOBOCAN data show that in 2012 there were 14.1 million new cancer cases and 8.2 million deaths because of cancer [1], while in 2020, 19.3 million cancer cases were recorded, and

around 10 million deaths primarily due to cancer [6] which meaning the problem is exacerbating and spreading year by year. Cancer is a distinct collection of diseases marked by the uncontrolled growth and spread of abnormal cells in the body. Cancer can start almost anywhere in the body and can spread to other parts of the body through the bloodstream or lymphatic system [2]. There are so many factors can cause cancer such as genetic factors, environmental elements, the habits of human beings like smoking tobacco, consuming alcohol and eating red meat, etc., A few kinds of cancer are also caused by microbial infections such as *H. pylori*, HPV and HBV infections [6]. Although there is a much progress in chemotherapy, the problem of drug resistance has led to the search for newer leads with superior efficacy [5], also it is an extended process for developing a drug without any side effects like stasis of the lower bowel, mouth sores, diarrhea, hair loss, neuropathy, bone marrow suppression, and other life-threatening problems, that are difficult to identify even with the anticancer drugs that have been authorized by the FDA [6].

The term "triazole" was initially introduced by Bladin in 1885 to designate a specific type of heterocyclic aromatic ring system. This ring system consists of five members and contains three nitrogen atoms, with a chemical formula of $C_2H_3N_3$.The chemistry of triazole underwent sluggish development and was subsequently accelerated with the implementation of several synthetic processes that were both convenient and efficient. Additionally, the diverse interaction between triazole and biological systems further contributed to its advancement [4]. Triazole scaffold is a major pharmacophore between nitrogen containing heterocyclic compounds, it can be synthesized easily using “click” chemistry with copper- or ruthenium-catalyzed azide-alkyne cycloaddition reactions and it can also act as a linker between different pharmacophores [3]. According to structure, there are two types of five-membered triazoles: 1,2,3-triazole and 1,2,4-triazole. Because of its unique structure and having many positions for binding, both 1,2,3- and 1,2,4-triazoles are able to react with different substituents (electrophiles and nucleophiles) around the core structures and form diverse new bioactive molecules [4]. Triazole could form different non-covalent interactions, such as hydrogen bonding, van der Waals forces, and dipole–dipole interactions with various enzymes, proteins, and receptors. Therefore, triazoles show a variety of potential biological properties, such as antibacterial, antimalarial, antitubercular, antiviral, and anticancer effects [3], anticonvulsant, analgesic, antioxidant, anti-inflammatory, and antidepressant activities, also have important in organo-catalysis, agrochemicals, and materials science [4]. The antifungal activity of triazoles has been extensively studied and is known to entail the suppression of

ergosterol synthesis and the blockage of the P450-dependent enzyme (CYP 51). The heme iron of the CYP enzyme is capable of coordinating with ring structures of the triazole type [4]. It is found that 1,2,3-triazoles could act as anticancer agents by inducing the cell cycle arrest and apoptosis of cancer cells. It is found that 1,2,3-triazole derivatives with other pharmacophores increase the anticancer activity of 1,2,3-triazole such as triazoles-containing chalcone derivatives, chalcones are an important structural component in many natural products and have some useful biological properties as they are potential inhibitors of aromatase, P-glycoprotein (P-gp), histone deacetylase (HDAC), matrix metalloproteinase (MMP), NFκB, tubulin, vascular endothelial growth factor, and vascular endothelial growth factor receptor 2 (VEGFR-2) kinase; therefor, chalcones have broad-spectrum antiproliferative activity against drug-susceptible and drug-resistant cancers and even MDR (multidrug resistance) cancers. Also 1,2,3-triazole-containing quinoline or quinolone derivatives, quinoline and quinolone derivatives are potential inhibitors of hepatocyte growth factor receptor, proto-oncogene receptor tyrosine kinase/KIT, platelet-derived growth factor receptorβ/PDGFR-β and VEGFR2, and some quinoline-or quinolone-based agents, such as anlotinib and lenvatinib, have already been approved for lung cancer therapy [3].

2. Materials and methods

All the solvents and reagents were purchased from commercial suppliers and were used without further purification. Melting points were determined on Electrothermal apparatus. Flash chromatography was carried out on silica gel (Baker, 30–60 μm). TLC Monitoring tests were carried out using plastic sheets precoated with silica gel 60 F245 (layer thickness 0.2 mm) purchased from Merck. Spots were visualized by their fluorescence under UV-lamp (λ 245 and 366 nm) or staining with iodine vapor, 15% H_2SO_4, $KMnO_4$, Hanessian's stain; Cerium ammonium molybdate stain (Mostain). NMR spectra were recorded on Bruker 400 MHz spectrometer, NMR unit, Faculty of Pharmacy, El Mansoura University. IR-spectra were recorded on Thermo Fisher FT-IR Spectrophotometer from 500 to 4000 cm^{-1} at the microanalytical unit, Faculty of Science, El Mansoura University.

2.1. Synthesis of *4-Azido-2,6-dimethylquinoline (6)*

A mixture of 4-chloro-2,6-dimethylquinoline (2.0 g, 10.4 mmol) and NaN_3 (2.5 g, 38.4 mmol) in DMF (4.0 ml) was heated in a sand bath at 95-100 °C overnight. The Mixture was evaporated *in vacuo*, and the residue was taken in acetone then co-evaporated with silica gel in vacuo. Flash chromatography (petroleum ether/ethyl acetate, 4:1) afforded compound **6** (1.19 g, 57%) as creamy crystals. R_f 0.28 (petroleum ether/ethyl acetate, 4:1), Mp 70-72 °C. IR ($\acute{\upsilon}$, cm^{-1}): 3043, 3015 (C–$H_{str.Ar}$), 2914 (C–$H_{asy.str.\ Me}$), 2856 (C–$H_{sym.str.Me}$), 2111 ($N_{3str.}$), 1383 ($CH_{3Rock.}$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.86 (d, 1H, $J_{7,8}$ 8.0 Hz, H–8), 7.73 (s, 1H, H–3), 7.52 (dd, 1H, $J_{5,7}$ 4.0, $J_{7,8}$ 8.0 Hz, H–7), 6.94 (d, 1H, $J_{5,7}$ 4.0 Hz, H–5), 2.70 (s, 3H, CH_3–2), 2.50 (4, 3H, CH_3–6); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.93 (C=N), 147.10, 147.60, 135.73, 132.79, 127.92, 120.62, 119.90, 109.17 (8 C–Ar), 25.18 (CH_3–2), 21.63 (CH_3–6). EI-MS (*m/z*, %) for $C_{11}H_{10}N_4$ (198.23): 198.34 (M+), 197.56 (M-1,100), 184.09 (46.27), 160.29 (71.70), 156.30 (80), 125.99 (51.41), 121.12 (66.66), 119.10 (82.33), 115.14 (49.88), 10.32 (67.79), 94.29 (59.63), 81.13 (73.83).

2.2. General procedure for Claisen Schmitt reaction (10, 11)

A mixture of *p*-hydroxyacetophenone (2.13 mmol), benzaldehyde derivative (2.13 mmol) and KOH (5.32 mmol, 2.5 eq.) in EtOH (3.0 ml) was stirred overnight. The product is neutralized with AcOH, then crystals are formed with standing, the crystals are filtered and washed with MeOH then purified by flash chromatography.

2.3.General procedure for the synthesis of propargyl derivatives (13b, e)

A mixture of hydroxy chalcones **10b, e** (1.0 mmol), propargyl bromide (4.98 mmol, 5.0 eq.), K_2CO_3 (1.19 mmol, 1.2 eq.) and KOH (0.98 mmol, 1.0 eq.) in Acetone (5.0 ml) was stirred overnight at ambient temperature. Then purified by flash chromatography.

2.3.1. (E)-3-(4-Methylphenyl)-1-(4-(prop-2-ynyloxy)phenyl)prop-2-en-1-one (13b)

Creamy crystals (0.828 g, 71%) from (petroleum ether/ethyl acetate, 7:3). R_f 0.59 (petroleum ether/ethyl acetate, 7:3), Mp 92–94 °C; IR ($\acute{\upsilon}$, cm^{-1}): 3284 (≡C–H*str*), 3027 (=C–H*str.*), 2916 (–C–H*str.*), 2119 (C≡C*str.*), 1654 (C=O*str.*), 1598 (C=C*str.*), 1225 (C_{Ar}–O*str*), 1017 ($C_{Al.}$–O*str.*); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 (d, 2H, J_{AB} 12.0 Hz, Ar), 7.80 (d, 1H, $J_{\alpha,\beta}$ 16.0 Hz, C*H*=CHCO), 7.54 (d, 2H, J_{AB} 8.0 Hz, Ar), 7.51 (d, 1H, $J_{\alpha,\beta}$ 16.0 Hz, CH=C*H*CO), 7.22 (d, 2H, J_{AB} 8.0 Hz, Ar), 7.06 (d, 2H, J_{AB} 8.0 Hz, Ar), 4.76 (d, 2H, $J_{gem.}$, $J_{1,3}$ < 1.0 Hz, OCH_2), 2.60 (dd, 1H, $J_{1,3}$, $J_{1,3`}$ <1, ≡C–H), 2.38 (s, 3H, CH_3–4). ^{13}C NMR (100 MHz, $CDCl_3$): δ 188.73 (C=O), 161.15, 144.13, 140.95, 132.25, 131.92, 130.73, 130.36, 129.73, 129.43, 128.69, 127.38, 125.91, 120.70, 114.69 (12 C_{Ar}, *C*H=*C*HCO), 77.92–76.33 (≡C_{ipso}, H–C≡), 55.87 (OCH_2), 21.58 (CH_3). EI-MS (*m/z*, %) for $C_{19}H_{16}O_2$ (276.34): 276.45 (M+, 22.00), 238.34 (32.24), 211.88 (29.36), 186.74 (86.18), 137.30 (77.44)100.84 960.42), 77.96 (100), 65.00 (70.96), 44.70 (32.82).

2.3.2. (E)-3-(4-Chlorophenyl)-1-(4-(prop-2-ynyloxy)phenyl)prop-2-en-1-one (13e)

Yellow crystals (0.34 g, 74%) from (petroleum ether/ethyl acetate, 4:1). R_f 0.33 (petroleum ether/ethyl acetate, 7:3), Mp 98–100 °C; IR ($\acute{\upsilon}$, cm^{-1}): 3298 (≡C–H*str.*), 3069 (=C–H*str.*), 2924 (–C–H*str.*), 2123 (C≡C*str.*), 1657 (C=O*str.*), 1600 (C=C*str.*), 1224 (C_{Ar}–O*str.*), 1009 ($C_{Al.}$–O*str.*); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 (d, 2H, J_{AB} 8.0 Hz, H–2`$_{Ar}$, H–6`$_{Ar}$), 7.76 (d, 1H, $J_{\alpha,\beta}$ 16.0 Hz, C*H*=CHCO), 7.58 (d, 2H, J_{AB} 8.0 Hz, H–2_{Ar}, H–6_{Ar}), 7.52 (d, 1H, $J_{\alpha,\beta}$ 16.0 Hz, CH=C*H*CO), 7.40 (d, 2H, J_{AB} 8.0 Hz, H–3_{Ar}, H–5_{Ar}), 7.08 (d, 2H, J_{AB} 8.0 Hz, H–3`$_{Ar}$,H–5`$_{Ar}$), 4.78 (d, 2H, $J_{gem.}$, $J_{1,3}$ 4.0 Hz, OCH_2), 2.59 (dd,1H, $J_{1,3}$, $J_{1,3`}$ 4.0 Hz, ≡C–H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 188.83 (C=O), 161.30 (*C*H=CHCO), 142.71, 136.26, 133.50, 131.01, 130.79, 130.35, 129.57, 129.24, 128.76, 128.30, 127.78, 122.18, 114.78 (12 C_{Ar}, CH=*C*HCO), 77.77–76.24 (≡C_{ipso}, ≡C–H), 55.92 (OCH_2); EI-MS (*m/z*, %) for $C_{18}H_{13}ClO_2$ (296.75): 296.72 (M^+,40.00), 295.47 (M-1, 10.78), 294.60 (M^{-2}, 17.13), 267.26 (80.81), 242.21 (80.73), 201.72 (73.15), 159.11 (36.49), 125.23 (100.00), 91.10 (32.91), 66.22 (91.79).

The propargylated compounds 1-(4-(prop-2-ynyloxy)phenyl)ethenone **12**, (E)-3-phenyl-1-(4-(prop-2-ynyloxy)phenyl)prop-2-en-1-one **13a**, (E)-3-(4-methoxyphenyl)-1-(4-prop-2-ynyloxy) phenyl)prop-2-ene-1-one **13c**, (E)-3-(4-(dimethylamino)phenyl)-1-(4-(prop-2-ynyloxy)phenyl) prop-2-en-1-one **13d**, (E)-3-(4-(furan-2-yl) -1-(4-(prop-2-ynyloxy)phenyl) prop-2-en-1-one **14a**, (E)-1-(4-(prop-2-ynyloxy)phenyl)-3-(thiophen-2-yl)prop-2-en-1-one **14b**, 4-propenyloxy benzaldehyde **18**, 1,3-dimethyl-7-(prop-2-ynyl)-1H-purine-2,6(3H,7H)-dione **19**, (3β)Propargyloxycholesterol **20** and (2E)-1-(Ferrocen-3-yl)-3-[4-(prop-2-yn-1-ylox-y)phenyl]prop-2-en-1-one **21** were compared with authentic samples and they were correct [7].

2.4. General procedure for the synthesis of clicked derivatives

A mixture of the terminal alkynes **13, 14, 19-21** (0.65 mmol), 4-azido-2,6-dimethylquinoline **6** (0.5 mmol), $CuSO_4$. $5H_2O$ (0.24 mmol) and L-ascorbic acid (1.4 mmol) in THF-H_2O (4:1, 5 ml) was gently refluxed with stirring for 4 h. The mixture was diluted with acetone then co-evaporated with silica gel in *vacuo* then purified by flash chromatography.

2.4.1. (E)-1-(4-((1-(2,6-dimethylquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-3phenylprop-2-en-1-one (22a)

yellow crystals (0.11 g, 73%) from MPLC (petroleum ether/ethyl acetate, 2:1); R_f 0.32 (petroleum ether/acetone, 2:1); Mp 120–125 °C; IR ($\acute{\upsilon}$, cm^{-1}): 3055 (=C–$H_{str.}$), 2919 (–C–$H_{Asy.str.}$), 1658 (C=O_{str}), 1603 (C=$N_{str.}$, C=$C_{str.}$), 1338 (C_{Ar}–$N_{str.}$), 1223 (C_{Ar}–O_{str}), 1020 (C_{Al}–$O_{str.}$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05–8.00 (m, 4H, J_{AB} 8.0 Hz, H–$5_{Triaz.}$, H–$8_{Quin.}$, 2Ar), 7.98–7.96 (d, 2H, J_{AB} 8.0 Hz, Ar), 7.93–7.90 (dd, 1H, J_{AB} 4.0, 4.0 Hz, H–$7_{Quin.}$), 7.76–7.72 (d, 1H, $J_{\alpha,\beta}$ 16.0, C*H*=CHCO), 7.58–7.56 (d, 2H, J_{AB} 8.0 Hz, Ar), 7.54 (s, 1H, H–$3_{Quin.}$), 7.50–7.49 (d,1H, J_{AB} 4.0 Hz, H–$5_{Quin.}$), 7.49–7.46 (d, 1H, $J_{\alpha,\beta}$ 12.0, CH=C*H*CO), 7.09–7.07 (d, 2H, J_{AB} 8.0 Hz, Ar), 7.04–7.02 (d, 1H, J_{AB} 8.0 Hz, Ar), 5.40 (s, 2H, OCH_2), 2.73 (s, 3H, C_2–CH_3), 2.42 (s, 3H, C_6–CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 188.68 (C=O), 161.85, 161.77, 157.93, 144.33, 144.23, 140.99, 138.49, 134.96, 133.67, 131.84, 130.93, 130.73, 130.49, 129.35, 128.98, 128.42, 128.26, 124.93, 121.68, 121.27, 120.62, 117.38, 114.68, 114.56, 114.52 (23 C_{Ar}, *C*H=*C*HCO), 61.95 (OCH_2),

26.42 (C_2–CH_3), 21.94 (C_6–CH_3). EI-MS (*m/z*, %) for $C_{29}H_{24}N_4O_2$ (460.54): 459.99 (M+, 33.71), 457.72 (M-3, 21.87), 422.23 (64.13), 393.54 (62.15), 345.34 (55.32), 296.24 (68.79), 255.27 (70.18), 212.55 (100.00), 159.82 (70.29), 120.02 (80.55).

2.4.2. (E)-1-(4-((1-(2,6-dimethylquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-3-(p-tolyl)prop-2-en-1-one (22b)

Faint brown crystals (0.22 g, 86%) from (Petroleum ether/Acetone, 2:1); R_f 0.26 (petroleum ether/acetone, 2:1); Mp 156–158 °C; IR ($\acute{\upsilon}$, cm^{-1}): 3021 (=C–$H_{str.}$), 2918 (–C–$H_{Asy.str.}$), 2868 (–C–$H_{Sym.str.}$), 1657 (C=O_{str}),1601 (C=$N_{str.}$, C=$C_{str.}$), 1337 ($CH_{3Rock.}$), 1229 (C_{Ar}–O_{str}), 1030 (C_{Al}–$O_{str.}$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14 (s, 1H, H–$5_{Triaz.}$), 8.10–8.05 (2d, 3H, J_{AB} 8.0, 12.0 Hz, H–$8_{Quin.}$, 2Ar), 7.83–7.79 (d, 1H, $J_{\alpha,\beta}$ 16.0 Hz, C*H*=CHCO), 7.64 (d, 1H, J_{AB} 8.0, H–$7_{Quin.}$), 7.57–7.51 (2d, 3H, J_{AB} 8.0, $J_{\alpha\beta}$ 16.0, CH=C*H*CO, 2Ar), 7.41 (s, 1H, H–$5_{Quin.}$), 7.28 (s, 1H, H–$3_{Quin.}$), 7.25–7.23 (d, 2H, J_{AB} 8.0, Ar), 7.16 (d, 2H, J_{AB} 8.0, Ar), 5.49 (s, 2H, OCH_2), 2.82 (s, 3H, C_2–CH_3), 2.51 (s, 3H, C_6-CH_3), 2.41 (s, 3H, Tol-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 188.79 (C=O), 161.69, 157.93, 146.81, 144.43, 144.25, 141.03, 138.49, 133.69, 132.21, 131.97, 131.46, 130.88, 129.72, 129.49, 128.98, 128.45, 128.01, 124.89, 121.29, 120.66, 120.62, 117.36, 114.64 (23 C–Ar, *C*H=*C*HCO), 61.95 (OCH_2), 24.54 (C_2–CH_3), 21.93(C_6–CH_3), 21.56 (Tol–CH_3); EI-MS (*m/z*,%) for $C_{30}H_{26}N_4O_2$ (474.56): 472.73 (M-2, 20.31), 461.40 (80.63), 368.40 (62.16), 327.95 (29.92), 255.33 (100), 159.35 (36.05), 79.08 (50.06).

2.4.3. ((E)-1-(4-((1-(2,6-dimethylquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-3-(4methoxyphenyl)prop-2-en-1-one (22c)

Yellow crystals (0.3 g, 63%) from (petroleum ether/acetone, 2:1); Mp 124–126 °C; IR ($\acute{\upsilon}$, cm^{-1}): 3139 (=C–H_{str}), 2921 (–C–$H_{str.Asy.}$), 1656 (C=O_{str}), 1601 (C=$N_{str.}$, C=$C_{Str.}$), 1223 (C_{Ar}–$O_{str.}$), 1033 (C_{Al}–$O_{str.}$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14 (s, 1H, H–$5_{Triaz.}$), 8.08 (d, 1H, J_{AB} 12.0 Hz, H–$8_{Quin.}$), 8.06 (d, 2H, J_{AB} 12.0 Hz, Ar), 7.82, 7.79 (d, 1H, $J_{\alpha,\beta}$ 12.0 Hz, C*H*=CHCO), 7.65–7.58 (2d, 4H, J_{AB} 8.0, 12.0 Hz, H–$3_{Quin.}$, H–$7_{Quin.}$, 2Ar), 7.47, 7.43 (d, 1H, $J_{\alpha,\beta}$ 16.0, CH=C*H*CO), 7.41 (s, 1H, H–$5_{Quin.}$), 7.16, 6.95 (2d, 4H, J_{AB} 8.0, 8.0 Hz, H–3, H–3`, H–5, H–5`), 5.49 (s, 2H, OCH_2), 3.86 (s, 3H, OCH_3), 2.82 (s, 3H, C_2–CH_3), 2.51 (s, 3H, C_6–CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 188.733 (C=O), 161.64, 161.61, 158.10, 147.70, 144.16, 144.12, 140.49, 138.05, 133.23, 132.07, 131.52, 131.36, 130.81, 130.18, 128.69, 127.68, 124.90, 121.09, 120.59, 119.33, 117.35, 114.62,

114.42, 113.61, 101.38 (23 C–Ar, *C*H=*C*HCO), 61.97 (OCH_2), 55.44 (OCH_3), 25.01 (C_2–CH_3), 21.80 (C_6–CH_3); EI-MS (*m/z*, %) for $C_{30}H_{26}N_4O_3$ (490.56): 490.08 (M+,30), 479.58 (51.85), 338.83 (41.61), 283.47 (76.48), 255.47(63.57), 202.97(51.44), 185.28(100), 132.50(38.70), 97.84(44.68), 74.33(70.06), 41.29(84.01).

2.4.4. (E)-1-(4-((1-(2,6-dimethylquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-3-(4(dimethylamino)phenyl)prop-2-en-1-one (22d)

Orange crystals (0.11 g, 98%) from (petroleum ether/ethyl acetate, 1:1); R_f 0.17 (petroleum ether/ethyl acetate, 1:1); Mp 124–126 °C; IR ($\acute{\upsilon}$, cm^{-1}): 3147 (≡C–H_{str}), 2921 (–C–$H_{Asy.str.}$), 1651 (C=O_{str}), 1601 (C=$N_{str.}$, C=C), 1228 (C_{Ar}–$O_{str.}$), 1170 (C_{Al}–$N_{str.}$), 1009 (C_{Al}–$O_{str.}$) ; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 (4, 1H, H–$5_{Triaz.}$), 8.09–8.05 (m, 3H, J_{AB} 8.0 Hz, Ar), 7.83–7.80 (d, 1H, $J_{\alpha,\beta}$ 12.0 Hz, C*H*=CHCO), 7.65–7.63 (dd, 1H, H–$7_{Quin.}$), 7.57 (d, 3H, *J* 8.0 Hz, Ar), 7.41 (s, 1H, Ar), 7.37 (d, 1H, $J_{\alpha,\beta}$ 16.0 Hz CH=C*H*CO), 7.15 (d, 2H, J_{AB} 8.0 Hz, Ar), 6.71 (d, 2H, J_{AB} 8.0 Hz, Ar), 5.49 (s, 2H, OCH_2), 3.06 (s, 6H, NMe_2), 2.83 (s, 3H, C_2–CH_3), 2.51 (s, 3H, C_6–CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 188.83 (C=O), 161.27, 157.78, 151.91, 145.32, 144.50, 141.46, 138.85, 134.05, 132.64, 130.64, 130.37, 127.43, 124.85, 122.83, 121.47, 120.65, 117.37, 116.46, 114.50, 111.94 (23 C–Ar, *C*H=*C*HCO), 61.91 (OCH_2), 40.23 (NMe_2), 21.97 (CH_3); EI-MS (*m/z*, %) for $C_{31}H_{29}N_5O_2$ (503.61): 503.45 (M+), 477.10 (50.32), 458.59 (18.07), 442.02 (26.16), 406.15 (39.15), 375.00 (52.13), 333.75 (57.36), 294.85 (47.79), 255.35 (73.84), 238.30 (95.41), 195.16 (60.58), 182.23 (100).

2.4.5. *(E)-3-(4-chlorophenyl)-1-(4-((1-(2,6-dimethylquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)prop-2-en-1-one (22e)*

Yellow crystals (0.06 g, 20%) from (petroleum ether/acetone, 2:1); R_f 0.25(petroleum ether/acetone, 2:1); Mp 152–154 °C; IR ($\acute{\upsilon}$, cm^{-1}): 3144 (=C–$H_{str.}$), 2920 (–C–$H_{Asy.str.}$), 2852 (–C–$H_{Sym.str.}$), 1657 (C=O_{str}), 1604 (C=$N_{str.}$, C=$C_{str.}$), 1227 (C_{Ar}–$O_{str.}$), 1014 (C_{Al}–$O_{str.}$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.22– 8.20 (d, 2H, H–$5_{Triaz.}$, H–$8_{Quin.}$), 8.09–8.07 (d, 2H, J_{AB} 8.4 Hz, Ar), 8.00–7.98 (d, 2H, J_{AB} 8.8 Hz, Ar), 7.78–7.74 (d, 1H, $J_{\alpha\beta}$ 15.6 Hz, C*H*=CHCO), 7.67 (s, 1H, H–$3_{Quin.}$), 7.59–7.57 (d, 1H, $J_{\alpha,\beta}$ 8.0 Hz, CH=C*H*CO), 7.48 (s, 1H, H–$5_{Quin.}$), 7.41– 7.39 (d, 1H, J_{AB} 8.0 Hz, H–$7_{Quin.}$), 7.17– 7.15 (d, 2H, J_{AB} 8.4 Hz, Ar), 7.12–7.10 (d, 2H, J_{AB} 8.4 Hz, Ar), 5.48–5.46 (d, 2H, $J_{gem.}$, $J_{1,3}$ 9.2 Hz, OCH_2), 2.893 (s, 3H, C_2–CH_3), 2.523 (s, 3H, C_6–CH_3); ^{13}C NMR

(100 MHz, $CDCl_3$): δ 188.42 (C=O), 161.85, 157.87, 146.27, 144.24, 142.85, 141.31, 138.76, 136.36, 133.97, 133.44, 131.65, 130.96, 130.74, 129.57, 129.25, 128.75, 128.47, 127.55, 124.95, 124.92, 122.07, 121.40, 120.63, 117.40, 114.51 (23 C–Ar, *C*H=*C*HCO), 61.88 (OCH_2), 24.24 (C_2–CH_3), 21.94 (C_6–CH_3) ; EI-MS (*m/z*, %) for $C_{29}H_{23}ClN_4O_2$ (494.98): 495.60 (M+,18.1), 468.61 (59.90), 381.47 (85.42), 363.57 (100.00), 272.28 (45.94), 205.66 (70.17), 137.75 (36.99), 76.23 (52.20).

2.4.6. (E)-1-(4-((1-(2,6-dimethylquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-3-(furan-2yl)prop-2-en-1-one (23a)

Creamy crystals (0.28 g, 11%) from (petroleum ether/acetone, 1.5:1); R_f 0.22 (Petroleum ether/Acetone, 1.5:1); Mp 158–160 °C; IR ($\acute{\upsilon}$, cm^{-1}): 3921 (–C–$H_{Asy.str.}$), 1658 (C=$O_{str.}$), 1604 (C=$N_{str.}$, C=$C_{str.}$), 1229 (C_{Ar}–O_{str}), 1015 (C_{Al}–$O_{str.}$); ^{1}H NMR (400 MHz,): δ 8.11 (s, 1H, H–$5_{Triaz.}$), 8.01–7.99 (d, 3H, J_{AB} 6.4 Hz, H–$8_{Quin.}$, 2Ar), 7.58 (s, 1H, H–$3_{Quin.}$), 7.53–7.39 (3d, 4H, $J_{\alpha,\beta}$ 15.6, 15.2 Hz, CH=CHCO, $1H_{Fur.}$, H–$7_{Quin.}$), 7.20 (s, 1H, H–$5_{Quin.}$), 7.07–7.05 (d, 2H, J_{AB} 6.4 Hz, Ar), 6.64 ($1H_{Fur.}$), 6.45 ($1H_{Fur.}$), 5.39 (s, 2H, OCH_2), 2.79 (s, 3H, C_2–CH_3), 2.435 (s, 3H, C_6–CH_3); ^{13}C NMR (100 MHz, DMSO): δ 188.10 (C=O), 161.74, 157.88, 151.72, 146.44, 144.86, 144.28, 141.21, 138.67, 133.87, 131.81, 130.85, 130.30, 129.58, 127.68, 124.91, 121.36, 120.62, 118.95, 117.38, 116.15, 114.64, 112.70 (21 C–Ar, *C*H=*C*HCO), 61.91 (OCH_2), 24.36 (C_2–CH_3), 21.94 (C_6–CH_3); EI-MS (*m/z*, %) for $C_{27}H_{22}N_4O_3$ (450.50): 450.93 (M+, 22.51), 375.29 (40.72), 255.44 (100), 209.42 (35.26), 144.44 (54.19), 82.36 (49.61), 44.29 (22.93).

2.4.7. (E)-1-(4-((1-(2,6-dimethylquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-3(thiophen-2-yl)prop-2-en-1-one (23b)

Faint brown crystals (0.46 g, 98%) from (petroleum ether/acetone, 2:1); R_f 0.27 (petroleum ether/acetone, 2:1); Mp 200–202 °C; IR ($\acute{\upsilon}$, cm^{-1}): 3063 (=C–$H_{str.}$), 2922 (–C–$H_{Asy.str.}$), 1650 (C=O_{str}),1598 (C=$N_{str.}$, C=$C_{str.}$), 1221 (C_{Ar}–O_{str}), 1019 (C_{Al}–$O_{str.}$); ^{1}H NMR (400 MHz, DMSO): δ 8.98 (s, 1H, H–$5_{Triaz.}$), 8.17 (d, 2H, J_{AB} 8.0 Hz, Ar), 8.02 (d, 1H, J_{AB} 8.0 Hz, H–$8_{Quin.}$), 7.91 (d, 1H, $J_{\alpha,\beta}$ 16.0 Hz, C*H*=CHCO), 7.80 (d, 1H, J_{AB} 8.0 Hz, H–$7_{Quin.}$), 7.73 (s, 1H, H–$3_{Quin.}$), 7.71–7.70 (d, 1H, J_{AB} 4.0 Hz, Ar), 7.64–7.60 (d, 1H, $J_{\alpha,\beta}$ 16.0 Hz, CH=C*H*CO), 7.55 (s, 1H, H–$5_{Quin.}$), 7.31–7.28 (d, 2H, J_{AB} 12.0 Hz, Ar), 7.22–7.20 (t, 1H, J_{AB} 4.0 Hz, Ar), 5.49 (s, 2H, OCH_2), 2.75 (s, 3H, C_2–CH_3), 2.47 (s, 3H, C_6–CH_3); ^{13}C NMR (100 MHz, DMSO): δ 187.34 (C=O), 162.33,

158.86, 147.73, 143.39, 140.32, 140.26, 138.49, 137.68, 136.50, 133.22, 133.07, 131.30, 131.21, 131.08, 130.70, 129.18, 129.08, 127.58, 121.55, 120.77, 120.69, 118.31, 115.37 (21 C–Ar, *C*H=*C*HCO), 61.66 (OCH_2), 25.09 (C_2–CH_3), 21.82 (C_6–CH_3); EI-MS (*m/z*, %) for $C_{27}H_{22}N_4O_2S$ (466.56): 466.05 (M+, 11.71), 429.70 (39.95), 378.07 (39.78), 375.29 (40.72), 331.58 (37.88), 255.44 (100), 221.00 (13.11).

2.4.8. 7-((1-(2,6-dimethylquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methyl)-1,3-dimethyl-1H-purine2,6(3H,7H)-dione (24)

Creamy crystals (0.3 g, 71%) from (petroleum ether/acetone, 1:1); R_f 0.19 (petroleum ether/acetone, 1:1); Mp 85–90 °C; IR ($\acute{\upsilon}$, cm^{-1}): 1705 ($C=O_{str}$– 2), 1663 ($C=O_{str}$– 6); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.42 (s, 1H, H–$5_{Triaz.}$), 8.21 (s, 1H, H–$8_{Theoph.}$), 7.97 (s, 1H, Ar), 7.71-7.65 (m, 2H, Ar), 7.44 (s, 1H, Ar), 5.77 (s, 2H, NCH_2), 3.62 (s, 3H, N3–$CH_{3Theoph.}$), 3.43 (s, 3H, N1–$CH_{3Theoph.}$), 2.89 (s, 3H, CH_3–$2_{Quin.}$), 2.54 (s, 3H, CH_3–$6_{Quin.}$); ^{13}C NMR (100 MHz, DMSO): δ 158.83, 154.96, 151.55, 148.94, 147.66, 143.67, 143.16, 140.16, 137.65, 133.17, 129.00, 126.55, 121.58, 120.53, 118.11, 106.54 (2 C=O, 14 C–Ar), 41.65 (NCH_2), 29.95, 28.07 (2 $CH_{3Theoph.}$), 25.04 (C2-$CH_{3Quin.}$), 21.77 (C6-$CH_{3Quin.}$) ppm; EI-MS (*m/z*, %) for $C_{21}H_{20}N_8O_2$ (416.45): 416.54 (M+, 28.85), 413.84 (M-3 , 23.60), 395.20 (36.99), 362.36 (32.08), 240.84 (52.31), 197.40 (43.34), 158.36 (100), 111.83 (57.18), 59.95 (26.28).

2.4.9. 4-(4-((3β-cholesteroyloxy)methyl)-1H-1,2,3-triazol-1-yl)-2,6-dimethylquinoline (25)

Yellow crystals (0.15 g, 88%) from (petroleum ether/ethyl acetate, 6:1 then 4:1); R_f 0.26 (petroleum ether/ethyl acetate, 4:1); Mp 86–90 °C; IR ($\acute{\upsilon}$, cm^{-1}): 3141 (≡C–H_{str}), 2935 (–C–$H_{Asy.str.}$), 2865(–C–$H_{Sym.str.}$), 1605 (C=$N_{str.}$), 1228 (C_{Ar}–$O_{str.}$), 1107 (C_{Al}–$O_{str.}$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (d, 1H, *J 8.0* Hz, H–$8_{Quin.}$), 7.94 (s, 1H, H–$5_{Triaz.}$), 7.55–7.54 (m, 2H, H–$3_{Quin.}$, H–$7_{Quin.}$), 7.20 (s, 1H, H–$5_{Quin.}$), 5.32 (d, 1H, *J* 8.0 Hz, H-$6_{Chol.}$), 4.79 (s, 2H, OCH_2), 3.36 (m, 1H, H–$3_{Chol.}$), 2.73 (s, 3H, CH_3–$2_{Quin.}$), 2.43 (s, 3H, CH_3-$6_{Quin.}$), 2.25 (t, $1H_{Chol.}$), 1.96-1.75 (m, $6H_{Chol.}$), 1.51- 1.37 (m, $7H_{Chol.}$), 1.33- 0.98 (m, $14H_{Chol.}$), 0.95 (s, 3H, CH_3–$19_{Chol.}$), 0.85 (d, 3H, *J* 4.0 Hz, CH_3–$21_{Chol.}$), 0.80, 0.79 (2d, 6H, *J* 4.0 Hz, CH_3–$26_{Chol.}$, CH_3–$27_{Chol.}$), 0.61 (s, 3H, CH_3–$18_{Chol.}$); ^{13}C NMR (100 MHz, $CDCL_3$): δ 157.85 (C=$N_{Quin.}$), 140.53, 138.39, 133.67, 127.86, 124.25, 122.05, 121.55, 120.70,117.23 (8$C_{Quin.}$, C–$4_{Triaz.}$, C–$5_{Triaz.}$, C–$5_{Chol.}$, C–$6_{Chol.}$), 79.43

(C–3$_{Chol.}$), 61.60 (OCH_2), 56.76, 56.15 (C–14$_{Chol.}$, C–17$_{Chol.}$), 50.17 (C–9$_{Chol.}$), 42.33, 39.77, 39.52, 39.08 (C–4$_{Chol.}$, C–13$_{Chol.}$, C–12$_{Chol.}$, C–24$_{Chol.}$), 37.18, 36.88, 36.19, 35.79 (C–1$_{Chol.}$, C–10$_{Chol.}$, C–20$_{Chol.}$, C–22$_{Chol.}$), 31.96, 31.89 (C–2$_{Chol.}$, C–7$_{Chol.}$, C–8$_{Chol.}$), 28.38, 28.24, 28.03 (C–16$_{Chol.}$, C–25$_{Chol.}$, CH_3-2$_{Quin.}$), 24.49 (CH_3–6$_{Quin.}$), 24.30 (C–15$_{Chol.}$), 23.83 (C–23$_{Chol.}$), 22.84, 22.58 (C–26$_{Chol.}$, C–27$_{Chol.}$), 21.09 (C–11$_{Chol.}$), 19.40 (C–19$_{Chol.}$), 18.73 (C–21$_{Chol.}$), 11.88 (C–18$_{Chol.}$); EI-MS (*m/z*, %) for $C_{41}H_{58}N_4O$ (622.94): 623.49 (M+1, 20.00), 570.18 (14.89), 488.92 (25.42), 368.55 (33.63), 255.33 (79.04), 193.34 (53.95), 153.26 (57.46), 95.29 (40.20), 57.23 (100.00).

2.4.10. (2E)-3-[4-[[1-(2,6-dimethylquinolin-4-yl)-1H-1,2,3triazol-4-yl]methoxy]phenyl]-1-(ferrocen-3-yl)prop-2-en1-one (26)

Red crystals (0.33 g, 97%) from (petroleum ether/acetone, 2:1); R_f 0.26 (petroleum ether/acetone, 2:1); Mp 172-174 °C; IR ($\acute{\upsilon}$, cm^{-1}): 3130 (=C–H), 2923 (–C–H$_{Asy.str.}$), 1645 (C=O$_{str.}$), 1588 (C=N$_{str.}$), 1239 (C$_{Ar}$-O$_{str.}$), 1021 (C$_{Al}$–O$_{str.}$); ^{1}H NMR (400 MHz, DMSO): δ 8.97 (s, 1H, H–5$_{Triaz.}$), 8.03–8.01 (d, 2H, J_{AB} 8.0 Hz, Ar), 7.90–7.88 (d, 1H, J_{AB} 8.0 Hz, Ar), 7.73–7.71 (m, 2H, J_{AB} 8.0 Hz, Ar), 7.65–7.62 (d, 1H, $J_{\alpha,\beta}$ 12.0 Hz, C*H*=CHCO), 7.55 (s, 1H, H–5$_{Quin.}$), 7.38–7.34 (d, 1H, $J_{\alpha,\beta}$ 16.0 Hz, CH=C*H*CO), 7.24–7.22 (d, 2H, J_{AB} 8.0 Hz, Ar), 5.43, 5.06, 4.67 (s, 7H–Fc), 4.23 (s, 5H, OCH_2, 3H–Fc), 2.74 (s, 3H, CH_3–2$_{Quin.}$), 2.48 (s, 3H, CH_3–6$_{Quin.}$); ^{13}C NMR (100 MHz, DMSO): δ 192.46 (C=O), 160.08, 158.86, 147.72, 143.64, 140.29, 140.04, 137.68, 133.22, 130.92, 129.06, 128.50, 127.46, 122.09, 121.56, 120.70, 118.27, 116.26, 115.74, 81.34, 73.05, 72.61, 70.23, 70.11, 70.07, 69.84 (29 C), 61.54 (OCH_2), 25.09 (C2-CH_3), 21.83 (C6-CH_3) ppm; EI-MS (*m/z*, %) for $C_{33}H_{29}FeN_4O_2$ (568.16): 566.30 (M-2, 32.50), 539.85 (61.49), 488.46 (84.09), 390.61 (83.15), 315.54 (45.00), 249.25 (100), 217.55 (55.89), 173.32 (57.09), 137.74 (37.83), 94.42 (46.94), 48.63 (67.45).

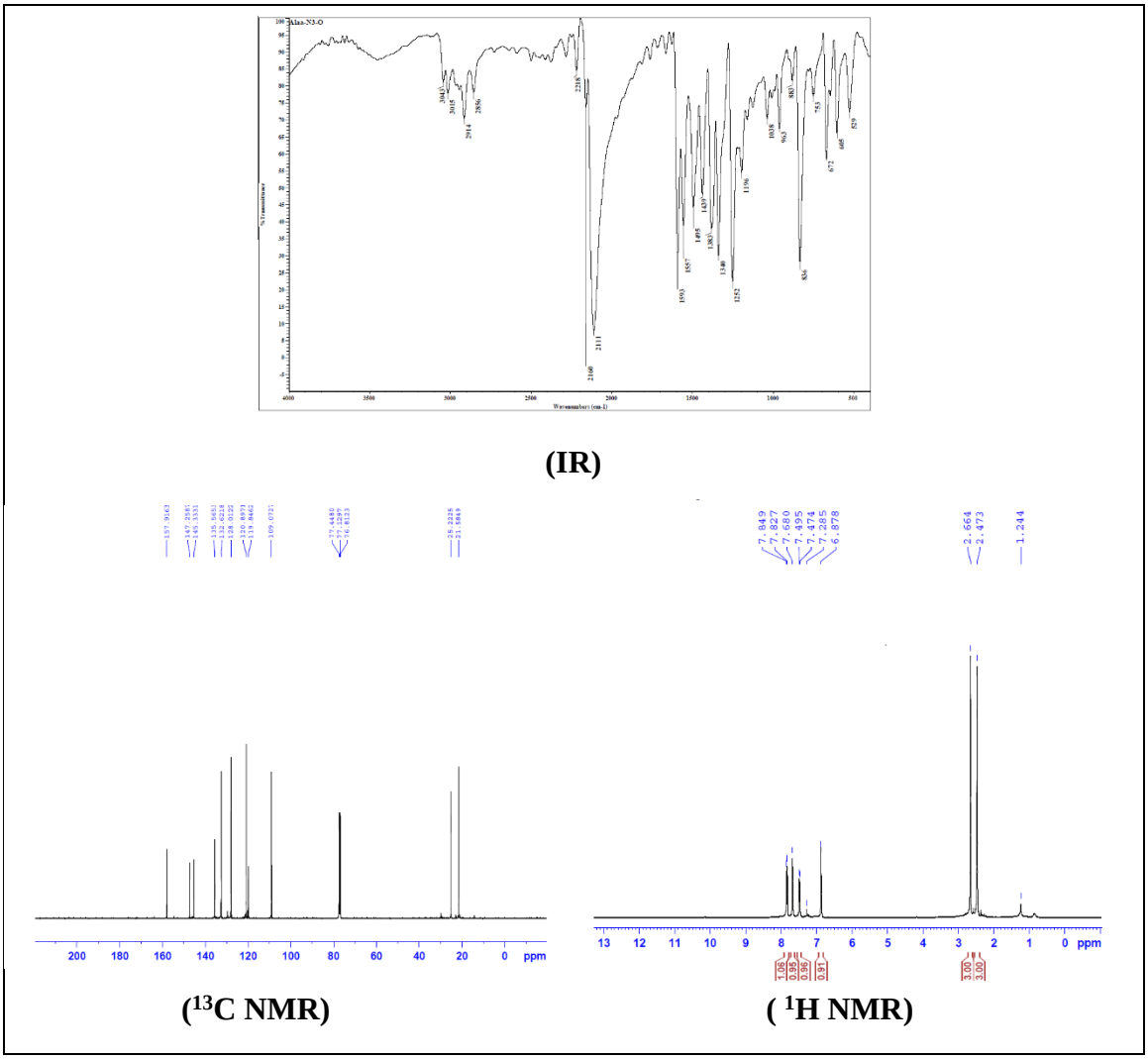


Fig. 1. IR, ^{1}H MR and ^{13}C MR spectra of 4-Azido-2,6-dimethylquinoline (6)

2.5. Experimental protocol for cytotoxic activity

2.5.1. Cytotoxic activity against MCF-7 and PC-3 cell lines using MTT assay.

The inhibitory effects of the compounds **22- 26** on cell growth were evaluated in mammary gland breast cancer (MCF-7) and human prostate cancer (PC-3) cell lines using MTT assay. This colorimetric assay is based on the conversion of the yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. The cell lines were cultured in RPMI-1640 medium with 10% fetal bovine serum. The antibiotics added were 100 units/ml penicillin and 100µg/ml streptomycin at 37˚C in a 5% CO_2 incubator. The cell lines were seeded in a 96 well plate at a density of $1.0x10^4$ cells/well at 37˚C for 48 h under 5% CO_2. After incubation the cells were treated with different concentrations of compounds and incubated for 24 h. After 24 h of drug treatment, 20 µl of MTT solution at 5mg/ml was added and incubated for 4 h. 100 µ of dimethyl sulfoxide (DMSO) was added into each well to dissolve the purple formazan formed [8]. The colorimetric assay is measured and recorded at absorbance of 570 nm using a plate reader (EXL 800, USA). The relative cell viability in percentage was calculated using the formula: % cytotoxicity = (average of control − average of compound)/ (average of control − average of blank) × 100), where control is the culture medium with cells and DMSO while blank is the culture medium without cells. IC50 values were calculated by plotting the percentage survival versus concentrations, using Origin Pro software [9]. The cell lines were obtained from ATCC via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt. The chemical reagents (RPMI-1640 medium, MTT and DMSO) were purchased from sigma co., St. Lous, USA while Fetal Bovine serum from GIBCO, UK.

2.5.2. Molecular docking study.

Molecular docking studies were performed using Molecular Operating Environment (MOE). To study the effect of the synthesized ligands **22-24** and **26** on mTOR (Mammalian target of rapamycin) and on breast cancer, the crystal structure of 5EF5 (Chaetomium thermophilum Raptor) protein and the binary and ternary crystal structures of 3HB5 (a novel inhibitor of 17 beta-HSD type 1: a lead compound for

breast cancer therapy) protein were obtained from the Protein Data Bank and then the proteins were prepared for the docking study. Docking procedure was followed using the standard protocol implemented in MOE software and the compounds were docked against the three-dimensional structure of the 5EF5 and 3HB5 proteins.

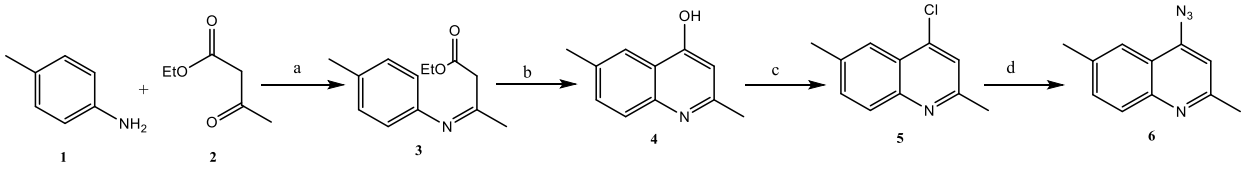
3. Result and discussion

3.1. Chemistry

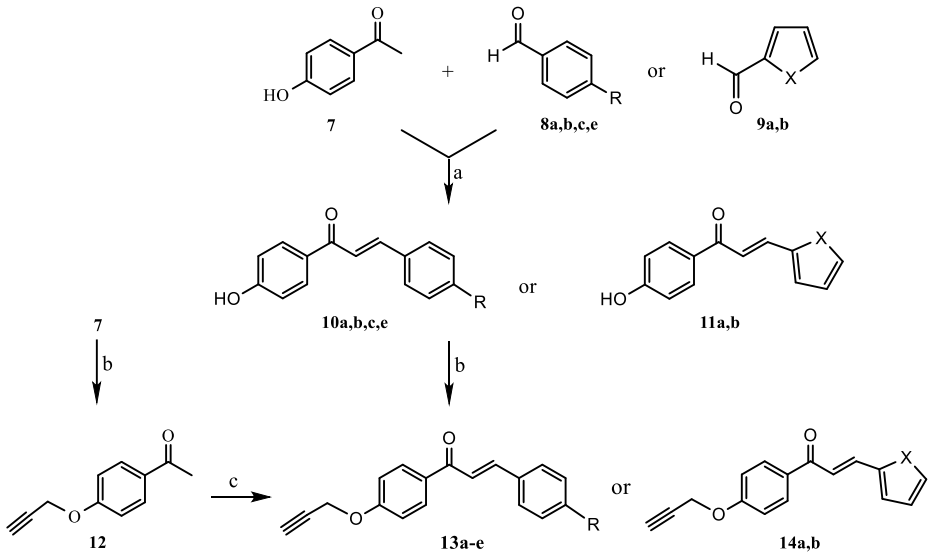
1,4-Disubstituted 1,2,3-Triazole derivatives **22-26** have been obtained by the fusion of the terminal alkynes **13-21** with 4-azido-2,6-dimethylquinoline **6** via click chemistry **Scheme 4**. The synthesis of 4-Azido-2,6-dimethylquinoline **6** has been achieved by heating a mixture of 4-chloro-2,6-dimethylquinoline **5** with NaN_3 in DMF in a sand bath at 95-100 °C overnight **Scheme1**[7,10]. The 1-(4-(prop-2-ynyloxy)benzene derivatives **13a,b,c,e** and **14a,b** have been afforded in good to excellent yield in two steps: first reaction of **8a,b,c,e** and **9a,b** with **7** in the presence of KOH and EtOH to afford **10a,b,c,e** and **11a,b** [11,12] which then have been terminally alkylated with propargyl bromide in the presence of K_2CO_3 as a base in N,N-dimethylformamide (DMF) [7], while **13d** is obtained by the condensation of 1-(4-(prop-2-ynyloxy) phenyl) ethenone **12** with *p*-dimethylaminobenzaldehyde [7] **Scheme2**. The synthesis of the alkylated derivatives **19-21** has been achieved according to a literature procedure as shown in **Scheme 3** [7]. Individual cycloaddition of the azidoquinoline scaffold **6** with these propargylated pharmacophores **13, 14, 19-21** according to the Copper-Catalyzed Azide Alkyne (CuAAC) conditions "Clicking" in the presence of $CuSO_4.5H_2O$, L-ascorbic acid, THF–H_2O 4:1 under reflux afforded the required 1,4-disubstituted-1,2,3-triazole series **22-26** in accepted yields as described in scheme **4** [7]. The structure of these series was elucidated by the integrated IR, ^{1}H NMR and ^{13}C NMR techniques. The spectral analysis of the compounds was in agree with the proposed structures. The structure of the azidoquinoline **6** was confirmed by its ^{1}H NMR that showed the two chemical shifts at δ 2.70 and 2.50 ppm indicated the presence of CH_3-2 and CH_3–6 respectively as well as the chemical shifts of ^{13}C NMR at δ 25.18 and 21.63 ppm also proved the presence of the two methylene groups. In addition, the IR band at 2111 cm^{-1} proved the presence of ($N_{3str.}$). Then the structures of the newly synthesized propargylated derivatives **13b** and **13e** were elucidated by their IR bands at 3284 and 3298 cm^{-1} respectively which indicated (≡C–$H_{str.}$) as well as the bands at 2119 and 2123 cm^{-1} respectively indicated (C≡$C_{str.}$). Their ^{1}H NMR spectra showed doublet peaks with

integration of one H and a coupling constant of $J_{\alpha,\beta}$ 16.0 Hz at δ 7.80 and 7.76 ppm respectively which represented the beta hydrogen of (C*H*=CHCO) group also showed doublet beaks with integration of one H and a coupling constant of $J_{\alpha,\beta}$ 16.0 Hz at δ 7.51 and 7.52 ppm respectively which represented the alpha H of (CH=C*H*CO) group. The structure of the 1,4-disubstituted-1,2,3-trizole derivatives **22a-e** was elucidated by C=N_{str} band appeared in their IR spectrum at 1603, 1601, 1601, 1601 and 1604 cm^{-1} respectively, also their ^{1}H NMR spectrum elucidated their structure through the singlet peaks with integration of one H at δ 8.05, 8.14, 8.14, 8.13 and (8.22-8.20) ppm respectively which referred to the presence of H–5_{Triaz} , the peaks at δ (7.76-7.72), (7.83-7.79), (7.82-7.79), (7.83-7.80) and (7.78-7.74) ppm respectively referred to beta H of (C*H*=CHCO) group while the peaks at (7.49-7.46), (7.57-7.51), (7.47-7.43), (7.39-7.35) and (7.59-7.57) ppm respectively referred to the presence of alpha H of (CH=C*H*CO) group, the singlet peaks at δ 5.40, 5.49, 5.49, 5.49 and 5.46 ppm respectively proved the presence of OCH_2, the singlet peaks at δ 2.73, 2.82, 2.82, 2.83 and 2.89 ppm respectively proved the presence of C_2–CH_3 while the singlet peaks at δ 2.42, 2.51, 2.51, 2.51 and 2.52 ppm respectively proved the presence of C_6–CH_3. Moreover, for 22b the singlet peak at δ 2.41 ppm proved the presence of Tol-CH_3, for **22c** the singlet peak at δ 3.86 ppm proved the presence of OCH_3 and for 22d the singlet peak at 3.06 ppm proved the presence of $N(CH_3)_2$. In their ^{13}C NMR spectrum the peaks at of and the peaks at δ 188.68, 188.79, 188.73, 188.83 and 188.42 ppm respectively of (C=O), the peaks at 61.95, 61.95, 61.97, 61.91 and 61.88 ppm respectively of (OCH_2), the peaks at δ 26.42, 24.54, 25.01, 21.97 and 24.24 ppm respectively of C2-CH_3, the peaks at δ 21.94, 21.93, 21.80, 21.97 and 21.94 ppm respectively for C6-CH_3 also proved their structure. Moreover, for **22b** the peak at δ 21.56 ppm proved the presence of Tol-CH_3, for **22c** the peak at δ 55.44 ppm proved the presence of OCH_3 and for **22d** the peak at δ 40.23 ppm proved the presence of $N(CH_3)_2$. The structure of the 1,4-disubstituted-1,2,3-trizole derivatives **23a,b** was elucidated in a similar manner as mentioned before with **22a-e** derivatives moreover with the chemical shifts δ 6.64, 6.45 ppm in the ^{1}H NMR spectrum of **23a** which indicated the 2H of furan ring. The structure of the derivative **24** was elucidated in a similar manner besides the characteristic ^{1}H NMR peaks of theophylline nucleus at δ 5.77 (s, 2H, NCH_2), 3.62 (s, 3H, N3–$CH_{3Theoph.}$), 3.43 (s, 3H, N1–$CH_{3Theoph.}$) ppm and ^{13}C NMR (100 MHz, DMSO): δ 158.83, 154.96, 151.55, 148.94, 147.66, 143.67, 143.16, 140.16, 137.65, 133.17, 129.00, 126.55, 121.58, 120.53, 118.11, 106.54 (2 C=O, 14 C–Ar), 41.65 (NCH_2), 29.95, 28.07 (2 $CH_{3Theoph.}$), 25.04 (C2-$CH_{3Quin.}$), 21.77 (C6-$CH_{3Quin.}$) ppm [7]. Also the structure of

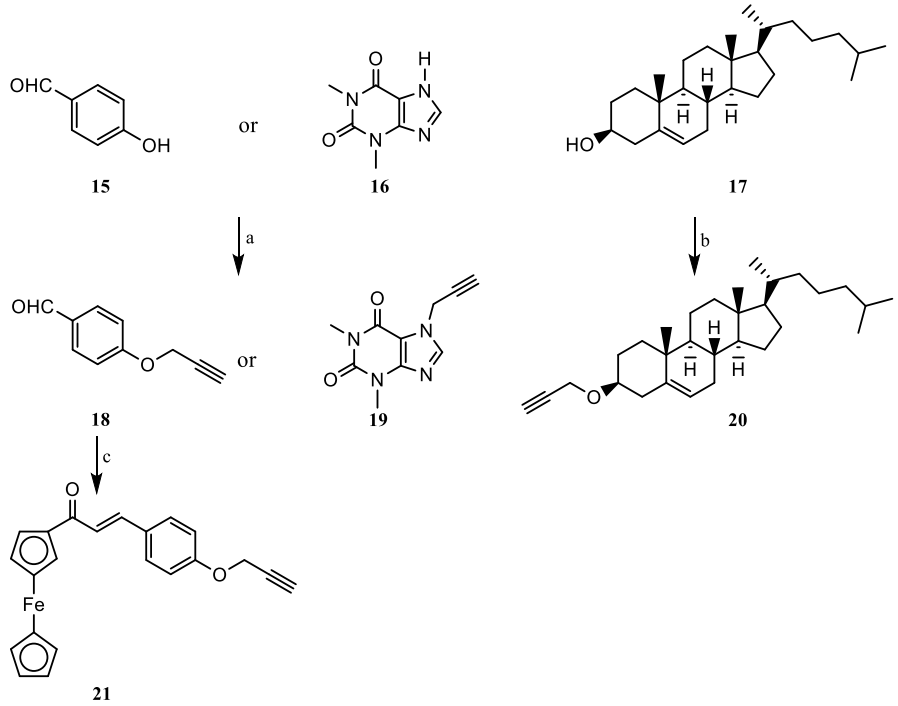
the derivative **25** was elucidated the same way besides the significant peaks that marks the cholesterol nucleus in ^{1}H NMR spectrum at δ 5.32 ppm (d, 1H, *J* 8.0 Hz, H-6$_{Chol.}$), 3.36 ppm (m, 1H, H–3$_{Chol.}$), 2.25 ppm (t, 1H$_{Chol.}$), 1.96-1.75 ppm (m, 6H$_{Chol.}$), 1.51- 1.37 ppm (m, 7H$_{Chol.}$), 1.33-0.98 ppm (m, 14H$_{Chol.}$), 0.95 ppm (s, 3H, CH$_3$–19$_{Chol.}$), 0.85 ppm (d, 3H, *J* 4.0 Hz, CH$_3$–21$_{Chol.}$), 0.80, 0.79 ppm (2d, 6H, *J* 4.0 Hz, CH$_3$–26$_{Chol.}$, CH$_3$–27$_{Chol.}$) and 0.61 ppm (s, 3H, CH$_3$–18$_{Chol.}$) and in ^{13}C NMR spectrum as 124.25-117.23 ppm (C–5$_{Chol.}$, C–6$_{Chol.}$), 79.43 ppm (C–3$_{Chol.}$), 56.76, 56.15 ppm (C–14$_{Chol.}$, C–17$_{Chol.}$), 50.17 ppm (C–9$_{Chol.}$), 42.33, 39.77, 39.52, 39.08 ppm (C–4$_{Chol.}$, C–13$_{Chol.}$, C–12$_{Chol.}$, C–24$_{Chol.}$), 37.18, 36.88, 36.19, 35.79 ppm (C–1$_{Chol.}$, C–10$_{Chol.}$, C–20$_{Chol.}$, C–22$_{Chol.}$), 31.96, 31.89 ppm (C–2$_{Chol.}$, C–7$_{Chol.}$, C–8$_{Chol.}$), 28.38, 28.24, 28.03 ppm (C–16$_{Chol.}$, C–25$_{Chol.}$), 24.30 ppm (C–15$_{Chol.}$), 23.83 ppm (C–23$_{Chol.}$), 22.84, 22.58 ppm (C–26$_{Chol.}$, C–27$_{Chol.}$), 21.09 ppm (C–11$_{Chol.}$), 19.40 ppm (C–19$_{Chol.}$), 18.73 ppm (C–21$_{Chol.}$) and 11.88 ppm (C–18$_{Chol.}$)[7,13,14]. Finally, the structure of the derivative **26** was elucidated by the remarkable ^{1}H NMR peaks of ferrocene nucleus at δ 5.43, 5.06, 4.67 ppm (s, 7H–Fc) and 4.23 ppm (s, 3H–Fc). ^{13}C NMR (100 MHz, DMSO): δ 192.46 (C=O), 160.08, 158.86, 147.72, 143.64, 140.29, 140.04, 137.68, 133.22, 130.92, 129.06, 128.50, 127.46, 122.09, 121.56, 120.70, 118.27, 116.26, 115.74, 81.34, 73.05, 72.61, 70.23, 70.11, 70.07, 69.84 (29 C), 61.54 (OCH_2), 25.09 (C2-CH_3), 21.83 (C6-CH_3) ppm [15].



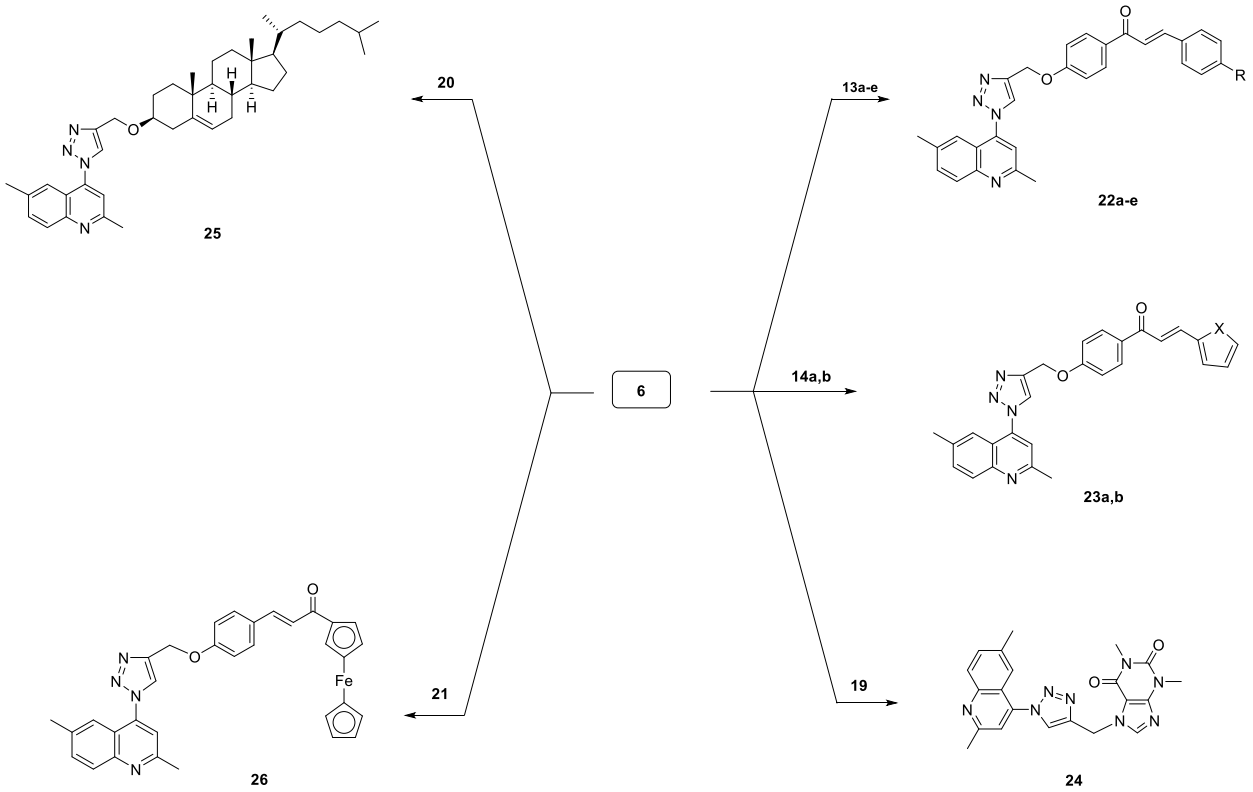
Scheme 1. *Reagents and conditions: (a)* Conc. HCl; *(b)* Paraffin oil 230-240 °C (20 %); *(c)* $POCl_3$, rfx. (66 %); *(d)* NaN_3, DMF, 80-100 °C (57%) [7,10].



Scheme 2. *Reagents and conditions: (a)* KOH, EtOH, rt [**10a** (R = H); **10b** (R = CH_3, 68%), compounds **10c** (R = *p*-OMe); **10d** (R = *p*-NMe_2); **10e** (R = Cl, 98%); **11a** (X= O); **11b** (X= S)[11,12]]; *(b)* propargyl bromide, K_2CO_3, DMF, rt; [**12**, **13a** (R= H, 74%); **13b** (R = CH_3, 71%); **13c** (R = *p*-OMe, 84%); **13e** (R= Cl, 74%); **14a** (X = O, 97%); **14b** (X = S, 20%)]; *(c)* *p*-dimethylaminobenzaldehyde, NaOH, EtOH, rt; → **13d** (R = *p*-NMe_2, 65%)[7].



Scheme 3. *Reagents and conditions: (a)* propargyl bromide, K_2CO_3, DMF, rt [**18** (qual.), **19** (78%)]; *(b)* propargyl bromide, NaH, DMF-Et_2O, 4:1, **20** (93%); *(c)* 3-acetylferrocene, KOH, EtOH, rt, **21** (95%) [7].



Scheme 4. *Reagents and conditions: (a)* ($CuSO_4.5H_2O$, L-ascorbic acid, THF–H_2O 4:1, rfx), [**22a**, R = H (73%); **22b**, R= CH_3 (86%); **22c**, R= *p*-OMe (63%); **22d**, R = *p*-NMe_2 (98%); **22e**, R = Cl, (20%); **23a**, X = O (11%); 23b, X = S (98%); **24** (71%) [7]; **25** (88%) [7,13,14]; **26** (97%) [15]].

3.2. Biological evaluation

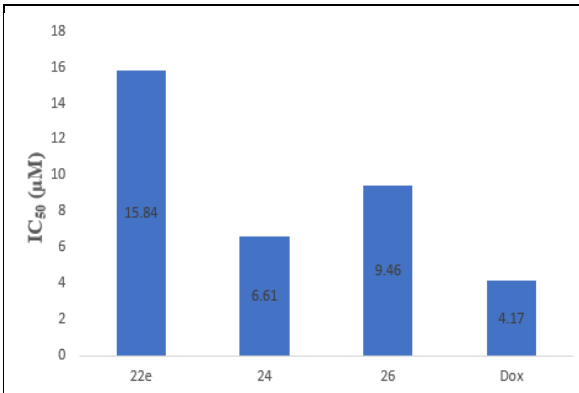
3.2.1. Cytotoxic activity. The synthesized 2,6-dimethylquinolinetriazole derivatives **22-26** were further evaluated for their cytotoxic activity against the two different human cancer cell lines, MCF-7 (human breast cancer cell line) and PC-3 (human prostate cancer cell line), using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) colorimetric assay and doxorubicin (Dox) was used as a reference control in this assay. The cytotoxicity results of the compounds **22-26** against MCF-7 and PC-3 cell lines were represented in **Table 1** and the best results were compared graphically with doxorubicin in **Fig. 1**. The results revealed that the compound **24** showed the potent cytotoxic effect against both MCF-7 and PC-3 cell lines with IC_{50} = 6.61±0.4 and 7.33±0.5 μM respectively, compared to 4.17±0.2 and 8.87±0.6 μM which are the IC_{50} values of doxorubicin against MCF-7 and PC-3 cell lines respectively. The compounds **26** and **22e** also showed a strong cytotoxicity against both MCF-7 and PC-3 cell lines with IC_{50} = 9.46±0.7, 13.89±1.0, 15.84±1.2 and 19.20±1.4 μM respectively. While the compounds **22a**, **22c** and **22d** showed moderate cytotoxicity against both MCF-7 and PC-3 cell lines, the compounds **22b**, **23a** and **23b** showed weak cytotoxicity against both cell lines and the compound **25** showed weak cytotoxicity against MCF-7 cell line and was non-cytotoxic against PC-3 cell line.

Table 1. Cytotoxic activities of the synthesized compounds **22-26** and Doxorubicin against human breast and prostate cancer cell lines (IC_{50} values are expressed in µM ± S.E.)

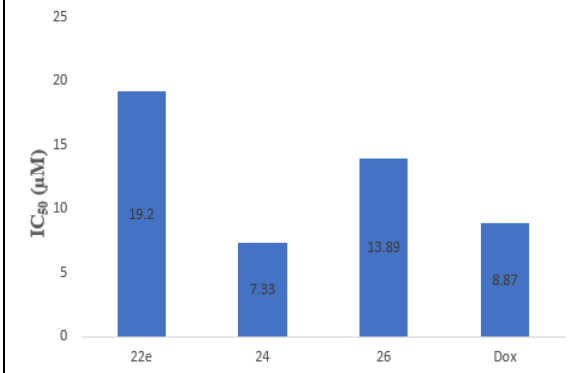
Comp. No.	Molecular weight	*In vitro* Cytotoxicity IC_{50} (µM)	
		MCF-7	PC-3
22a	460.54	37.41±2.3	51.73±2.8
22b	474.56	72.49±3.6	91.43±4.4
22c	490.56	29.48±2.1	35.31±2.2
22d	503.61	21.01±1.6	28.45±2.0
22e	494.98	15.84±1.2	19.20±1.4
23a	450.50	53.96±3.0	76.14±3.5
23b	466.56	45.51±2.6	67.54±3.2
24	416.45	6.61±0.4	7.33±0.5
25	622.94	88.56±4.2	>100
26	568.16	9.46±0.7	13.89±1.0
Dox		4.17±0.2	8.87±0.6

All values represent the average of 3-4 experiments; IC50 values are reported as mean ± SD.

• **IC_{50} (µM)**: 1 – 10 (very strong), 11 – 20 (strong), 21 – 50 (moderate), 51 – 100 (weak) and above 100 (non-cytotoxic)

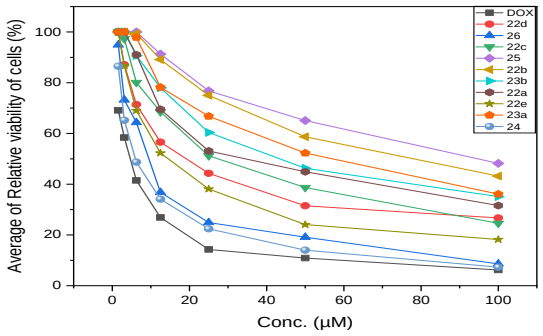


(A)

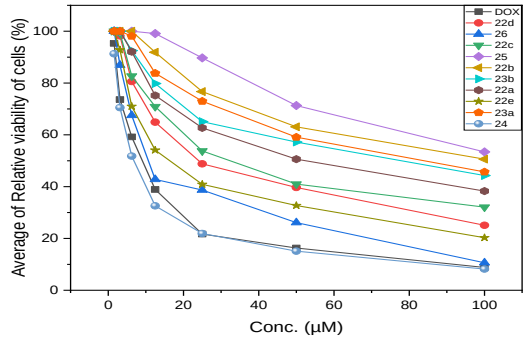


(B)

Fig. 2. IC_{50} (µM) of compounds 22e, 24 and 26 against MCF-7 cell line (A) and PC-3 cell line (B) compared to Dox.



(A)



(B)

Fig. 3. *In vitro* relative viability of MCF-7 (A) and PC-3 (B) cells against compounds **22-26** compared to the standard doxorubicin.

3.3.Molecular docking studies

Molecular docking was used to acquire understanding of the binding affinity and the interaction of the synthesized ligands **22-26** with the 5EF5 (Chaetomium thermophilum Raptor) protein which belongs to TORC1 complex of mTOR and with 3HB5 (breast cancer protein). The results were so promising with 5EF5 and 3HB5 proteins as the structure of the synthesized 1,4-disubstituted 1,2,3-triazole ligands **22-26** is characteristic with the presence of various active sites such as nitrogen of triazole ring, nitrogen of quinoline ring, oxygen of carbonyl group of chalcone, Sulphur of thiophene ring and the various five and six-membered rings, that can interact in different ways with different proteins such as through hydrogen bonding, arene-arene interaction, arene-H

interaction..... *etc.*, This advantage gives them the ability to bind strongly with the active sites of different proteins which give them a strong potential to inhibit those proteins and consequently act as anticancer agents.

3.3.1. Docking with 5EF5

A protein kinase known as Target of Rapamycin (TOR) is an essential regulator of cell development. It acts in two physically and functionally different complexes, TORC1 and TORC2. Pathologies such as diabetes, cancer, and neurodegeneration are associated with dysregulation of mammalian TOR (mTOR) signaling [16]. Mammalian target of rapamycin (mTOR) participates in a variety of biological signaling pathways to control cell division, autophagy, and apoptosis. Studies have revealed that the mTOR signaling pathway is linked to a number of disorders, including osteoporosis, insulin resistance, cancer, and rheumatoid arthritis. The mTOR signaling system, which is frequently active in malignancies, not only controls protein synthesis and gene transcription to control immune cell differentiation and cell division, but it also has a significant impact on tumor metabolism. Consequently, the mTOR signaling system is an important topic in the research of anti-tumor therapies [17].

The docking studies revealed that all the tested ligands **22-23** and **26** reached the binding sites of the protein and showed high binding affinity to the protein with excellent docking energy scores ranged from **– 8.6946** to **– 6.4526** Kcal/mol as shown in (**Table 2**). The results revealed that the ligands **22c**, **23b** and **26** had the potent affinity and interaction with 5EF5 protein; **22c** showed **-7.1127** kcal/mol and formed two arene-H bonds with UNK 594 and UNK 1519 residues of protein and one hydrogen bond between O of C=O group of chalcone and UNK 1514, **26** showed **-6.8387** Kcal/mol binding score and formed two arene-H bonds with UNK 55 and UNK 232 and one hydrogen bond between O of C=O group of chalcone and UNK 238, while **23b** showed **-6.4526** Kcal/mol binding score and formed three hydrogen bonds with UNK 4797, UNK 4755 and UNK 4741 and one arene-H bond with UNK 4739. The ligands **22e** and **22d** showed great affinity and interaction with 5EF5 protein with binding energy scores of **-7.7371** and **-7.6748** Kcal/mol and formed two arene-H bonds. **22a**, **22b** and **23a** showed great binding energy scores of **-6.7596**, **-8.6946** and **-6.6704** Kcal/mol respectively but with one interaction of arene-H bond; **22a**, **22b** through 6-membered ring of chalcone and **23a** through 5-membered ring of triazole. The ligands **24** and **25** showed no results with this protein [18].

Table 2. The interaction data of **22**, **23** and **26** ligands with 5EF5 protein.

Comp. No.	Dock score (S) (Kcal/mol)	Interaction	Protein interacting amino acid	Ligand interacting atom or ring
22a	-6.7596	π -H	UNK 1531	6-ring
22b	-8.6946	π -H	UNK B312	6-ring
22c	-7.1127	H-acceptor	UNK 1514	O
		π -H	UNK 594	5-ring
		π -H	UNK 1519	6-ring
22d	-7.6748	π -H	UNK 399	6-ring
		π -H	UNK 402	5-ring
22e	-7.7371	π -H	UNK 559	6-ring
		π -H	UNK 563	5-ring
23a	-6.6704	π -H	UNK 17	5-ring
23b	-6.4526	H-donor	UNK 4797	S
		H-acceptor	UNK 4755	O
		H-acceptor	UNK 4741	O
		π -H	UNK 4739	5-rig
26	-6.8387	π -H	UNK 55	6-ring
		π -H	UNK 232	5-ring
		H-acceptor	UNK 238	O

3.3.2. Docking with 3HB5

The ligands **22e**, **24** and **26** were docked against the breast cancer protein 3HB5 and the results showed a strong interaction between the tested ligands and 3HB5 as shown in (**Table 3**). The ligand **22e** showed a strong binding affinity with **-8.1024** Kcal/mol docking binding score and formed two hydrogen bonds; one with Lys 70 residue of 3HB5 through the nitrogen atom of quinoline ring and the other with Ser 69 residue through the nitrogen atom of triazole ring. The ligand **24** also showed a strong binding affinity with **-7.8423** Kcal/mol docking score and formed two hydrogen bonds with Thr 190 residue of 3HB5 through oxygen of theophylline ring and nitrogen of triazole ring, also formed an arene-arene interaction with Phe 192 residue through 6-membered ring of theophylline nucleus and an arene-H interaction with Phe 194 through triazole ring. Moreover, the ligand **26** exhibited a docking score of **-7.6474** Kcal/mol and formed three hydrogen bonds with Gly 43, Arg 44 and Ala 91 residues of the protein through oxygen atom of carbonyl group of chalcone and nitrogen atom of quinoline ring. Those results interpret their strong cytotoxic activities against the breast cancer cell line MCF-7 with IC_{50} = **15.84±1.2**, **6.61±0.4** and **9.46±0.7** respectively.

Table 3. The interaction data of **22e**, **24** and **26** ligands with 3HB5 protein of breast cancer.

Comp. No.	Dock score (S) (Kcal/mol)	Interaction	Protein interacting amino acid	Ligand interacting atom or ring
22e	-8.1024	H-acceptor	Lys 70	N-Quinoline
		H-acceptor	Ser 69	N-Triazole
24	-7.8423	H-acceptor	Thr 190	O
		H-acceptor	Thr 190	N
		π–π	Phe 192	6-ring
		π -H	Phe 192	5-ring
26	-7.6474	H-acceptor	Gly 43	O
		H-acceptor	Arg 44	O
		H-acceptor	Ala 91	N-Quinoline

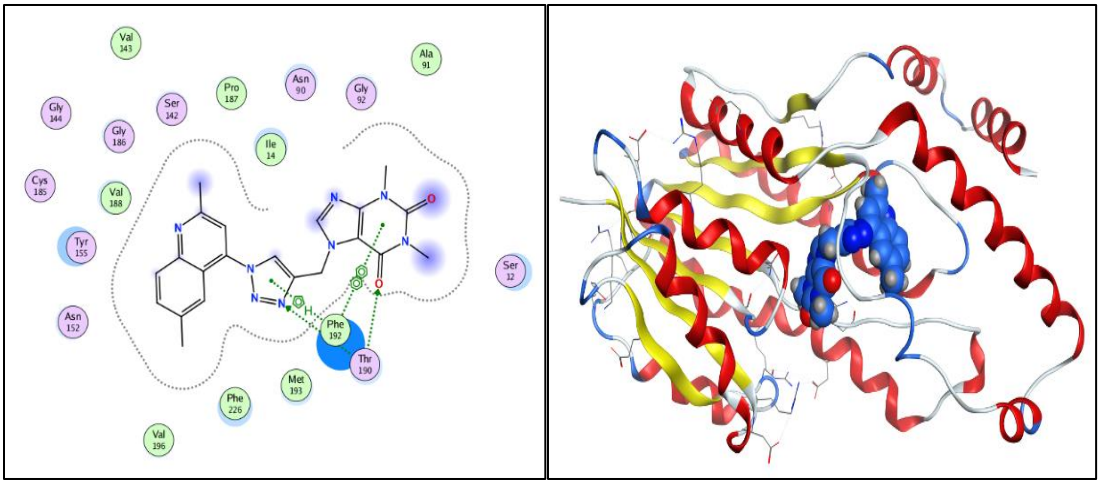


Fig. 4. 2D and 3D protein-ligand interaction of **24** with 3HB5 of breast cancer protein.

3. Conclusion

A new 1,4-Disubstituted 1,2,3-Triazole derivatives **22-26** have been synthesized in good yields via copper-catalyzed azide-alkyne cycloaddition reactions and characterized by FT-IR, ^{1}H NMR and ^{13}C NMR techniques. The synthesized compounds were tested as anticancer agents and exhibited a very strong cytotoxic activity against both breast and prostate cell lines. Molecular docking studies interpreted their strong cytotoxicity with their high binding affinity because of the strong docking binding scores and multiple active sites for interaction.

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