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In vitro Anticancer investigation of new synthetic hybrid quinolines

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ABSTRACT

Three novel synthetic Hybrid Quinolines **(6-8)** with different tags on the triazole moiety were synthesized *via* copper-catalyzed azide alkyne cycloaddition (CuAAC) reaction, which gave high yields of the products without undesirable byproducts, the so-called click reaction, between 4-Azido-2,6-dimethylquinoline **2** and three different terminal alkynes **(3-5)**. The synthesized 1,4-hybridized-1,2,3-triazoles were characterized using various spectroscopic tools such as IR, MS, and multi NMR techniques (^{1}H NMR ,^{13}C NMR, COSY, HSQC and DEPT-135°) to elucidate their structure, and the spectral analysis of the compounds was in agreement with the proposed structures. The cytotoxicity of these compounds was assayed using MTT colorimetric technique against mammalian breast cancer (MCF-7) and prostate cancer (PC-3) cell lines using Doxorubicin (Dox.) as positive control. Compound 7 was the most active against both cell lines and especially displayed promising cytotoxic activity against PC-3 cell line as it was 17% (0.2-fold) more active than Dox., while conjugate 6 was much less active than doxorubicin in both cell lines.

Key Words: COVID-19; Biomarkers; Combination; Interleukin-6; C-reactive protein (CRP); Ferritin; Severity

1. INTRODUCTION

Lung cancer is still associated with extremely high morbidity and mortality rates, among other malignancies, about one-quarter of all cancer-related deaths [1,2]. Prostate cancer PCa is the second most privileged cancer and accounts for 13.7% of diagnosed cancer in men with estimated 1.3 million new cases annually. By far, PCa is the fifth leading cause of cancer death worldwide, almost; it causes 6.7% of cancer deaths in men [3].

Approximately the next few decades, demographic considerations are expected to drive up the worldwide cancer incidence rate; by 2025, approximately 20 million additional cases of cancer annually are expected [4]. In front of the estimated factors responsible for the prognosis of cancer are genetic variables, environmental conditions, and human behaviors such as alcohol consumption, tobacco use, and the consumption of particular red meat varieties. Additionally, certain types of cancer are brought on by microbial illnesses such as H. pylori, HPV, and HBV infections. [5]. Despite, an array of commercial

cytotoxic drugs are available from natural as well as synthetic origins are effective in chemotherapy, besides radio and immunotherapy, treatment with these drugs is currently accompanied with side effects and detoxification by the cancer cell immune system. Therefore, renewable drugs are occasionally required. Hybridization of pharmacophores in one entity is still a route to discover new drugs.

The 1,2,3-triazole motif emerged as a joker both in drug design and materials science as well. Pharmacologically, the ring is not common in nature and it is metabolically stable and of low health risk factors [6,7]. The 1,4-disubstituted 1,2,3-triazoles got embarrassed interest due to the ease of manipulation *via* the copper azide alkyne cycloaddition reaction, Click chemistry [8-10]. Besides, the pharmacological benefits of this ring, it is used as a linker to develop new endless structural architectures of huge significance in drug design and discovery [11].

Quinoline-triazole, chalcone-triazole, and ferrocene-triazole conjugates are amongst the interesting structural designs that displayed interesting promising anticancer activities. These conjugates brought their anti-proliferative effect by a variety of methods, such as inducing apoptosis and cell cycle arrest. These conjugates displayed also antimitotic activities through inhibition of aromatases, P-glycoprotein, and the enzymes involved in blood vessel development like: tubulin, NFκB, histone deacetylase (HDAC), vascular endothelial growth factor, and vascular endothelial growth factor receptor 2 (VEGFR-2) kinase [12].

Theophylline, as xanthine derivative, has antitumor effect against MCF-7 cell line through down regulation of SRSF-3 expression and switched p53 from alpha into a beta isoform, induces cellular apoptosis, senescence and reduces colony formation [13]. Theophyllines and their Mn complexes are able to bind to DNA and induce apoptosis [14]. Theophylline 1,2,3-triazol conjugates also used for curing of large cell lung cancer [15]. They were able to be cytotoxic the cancer cells through the inhibition of the epidermal growth factor receptor 2 [16].

Several Ferrocenes displayed potential cyctoxicitiy activities, for instance, ferrocene complexes can target both breast and lung cancer [17]. The same was reported for ferrocene-quinoline hybrides which act as topoisomerase poisoning agents. Ferrocene-chalcone, ferrocene-azoles, ferrocene steroids, ferrocene-triazoles, and even ferrocene-sugar conjugates showed interesting cytotxoic activities [18,19].

In terms of the cyctotoxic activities of the aforementioned nuclei, and based on previous work [20-24] which has been done in our lab with these entities, it was envisioned to ligate new hybride molecules on a quinolone scaffold tethered with, chalcone, theophylline, cholesterol, and ferrocene *via* the CuAAC reaction. The syntheses of these compounds and the *in vitro* investigation of their antipoliferative potency in MCF7 and PC3 cell lines are described here.

2. MATERIALS AND METHODS

Purchased from commercial companies, all of the reagents and solvents were utilized without additional purification. Melting points were measured using an electrothermal device. Flash chromatography was performed on a silica gel (30–60 μm) for purification. Thi layer chromatography 60 F245 (0.2 mm) and acquired from Merck were used for Monitoring tests. The spots may be seen by their fluorescence under a UV lamp at λ 245 and 366 nm, or they could be stained with Cerium ammonium molybdate stain (Mostain), KMnO4, 15% H_2SO_4, iodine vapor, or Hanessian's stain. NMR spectra were captured using a Bruker 400 MHz spectrometer at the El Mansoura University Faculty of Pharmacy's NMR facility. Thermo Fisher FT-IR Spectrophotometer at the microanalytical unit, Faculty of Science, El Mansoura University, was used to record IR spectra between 500 and 4000 cm^{-1}. The synthetic part was done at the laboratories of the Chemistry Department, Faculty of Science at Port said University.

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

A mixture of 4-chloro-2,6-dimethylquinoline [20] (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 ($CH_{3Rock.}$). ^{1}H 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).

2.1.1. General procedure for the Click coupling Reactions

A mixture of 1.2 equivalent of the terminal alkyne **3** or **4** [18,19] or **5** [20], and the azidoquinoline **6**, $CuSO_4.5H_2O$ (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.

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

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 157.8 (C=$N_{Quin.}$), 140.5, 138.4, 133.7, 127.9, 124.2, 122.0, 121.5, 120.7, 117.2 (8C$_{Quin.}$, 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).

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

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).

***(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* (8)**

Red crystals (0.33 g, 97%) by (petroleum ether/acetone, 2:1); R_f 0.26 (petroleum ether/acetone, 2:1); Mp 172-174 °C; IR ($\acute{\nu}$, 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_{Ar}-$O_{str.}$); ^{1}H NMR (400 MHz, DMSO): δ_H in ppm = 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_C in ppm = 192.5 (C=O), 160.1, 158.9, 147.7, 143.6, 140.3, 140.0, 137.7, 133.2, 130.9, 129.1, 128.5, 127.5, 122.1, 121.6, 120.7, 118.3, 116.3, 115.7, 81.3, 73.0, 72.6, 69.8, 61.5 (30 C), 25.1, 21.8 (2 CH_3); EI-MS (*m/z*, %) for $C_{33}H_{29}FeN_4O_2$ (568.16): 566.3 (M-2, 32.50), 539.8 (61.5), 488.5 (84.1), 390.6 (83.1), 315.5 (45.0), 249.2 (100), 217.5 (55.9), 173.3 (57.1), 137.7 (37.8), 94.4 (46.9), 48.63 (67.4); UV-vis (DMSO): λ_{max} (nm); 269, 334, 488.

2.2. Biological evaluation of Cytotoxicity

2.2.1. Cytotoxic activity against MCF-7 and PC-3 cell lines.

Using the MTT test, the inhibitory effects of compounds **6–8** on cell growth were assessed in human prostate cancer (PC3) and mammary gland breast cancer (MCF7) cell lines. This colorimetric assay is based on the observation that, in live cells, mitochondrial succinate dehydrogenase converts the yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) to a purple formazan derivative. 10% fetal bovine serum was added to the RPMI-1640 medium used to cultivate the cell lines. At 37°C in an incubator with 5% CO_2, 100 units/ml of penicillin and 100µg/ml of streptomycin were introduced as antibiotics. The cell lines were seeded at 1.0x104 cells/well on a 96-well plate at 37°C for 48 hours with 5% CO_2. Following incubation, the cells were subjected to various concentrations of each of the tested compounds and left for a full day of incubation. Then 20 µl of a 5 mg/ml MTT solution was added and incubated for 4 hours after the drug treatment lasted for 24 hours. Each well received 100 µ of dimethyl sulfoxide (DMSO) in order to dissolve the purple formazan that had developed [25]. Using a plate reader (EXL 800, USA), the colorimetric test is measured and recorded at absorbance of 570 nm. % cytotoxicity = (average of control − average of compound)/(average of control − average of blank) × 100 was the formula used to determine the relative cell viability in percentage terms. Control represents the culture media including cells and DMSO, while blank represents the culture medium devoid of cells. The proportion of survival was plotted against the IC_{50} values [26]. Via the Holding Company for Biological Products and Vaccines (VACSERA), Cairo, Egypt, the cell lines were acquired from ATCC. Fetal bovine serum was obtained from GIBCO, UK, and the chemical reagents (RPMI-1640 medium, MTT, and DMSO) were bought from Sigma Co., St. Louis, USA.

3. RESULTS AND DISCUSSION

3.1. Chemistry

This work aimed at conjugation of quinolone with different tags comprising cholesterol, theophylline and ferrocene to discover the potential cytotoxic effect of new conjugates. The synthetic protocol relies on CuAAC of azido-tagged qninoline **2** with the complement pharmacophores **3-5** which are dopped with terminal alkyne groups (Scheme).

The quinoline scaffold 4-azido-2,6-dimethylquinoline **2** was prepared by azidolysis of 4-chloro-2,6-dimethylquinoline **1** [20] with NaN_3 in DMF in a sand bath at 95-100 °C with stirring overnight (Scheme 1). Compound **2** displayed a strong $N_{3str.}$ band at 2111 cm^{-1}.

The chemical shift (^{1}H NMR) of the two CH_3 groups (**Figure 1**) was assigned based on a comparison with the known 4-azido-6-chloro-2-methylquinoline [21]. The ^{13}C NMR spectrum (**Figure 2**) accounted for the theoretical number of carbon atoms and the 135°-DEPT spectrum (**Figure 5**) displayed only

positive signals. The 2 methyl groups were assigned in the ^{13}C NMR spectrum based on the HSQC spectrum (**Figure 4**).

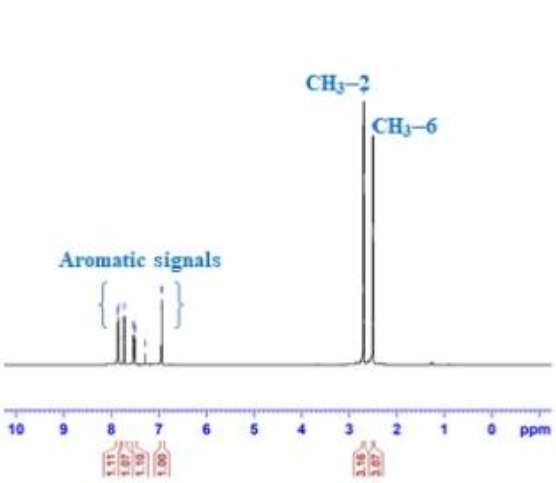


Fig. 1. ^{1}H NMR spectrum of compound **2**.

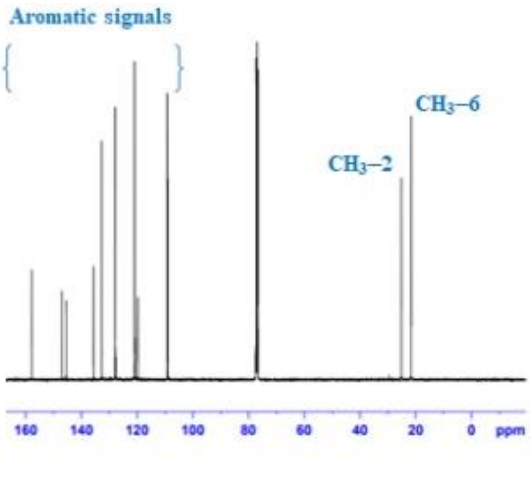


Fig. 2. ^{13}C NMR spectrum of compound **2**.

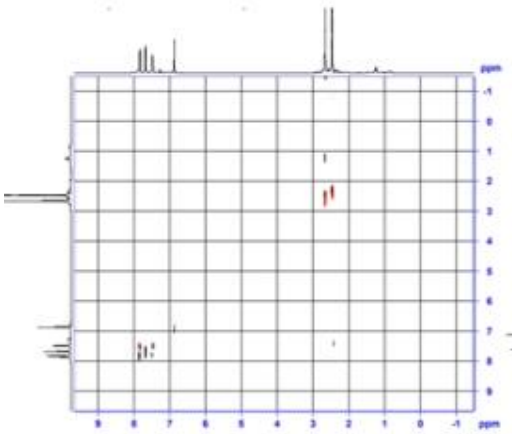


Fig. 3. COSY-NMR spectrum of compound **2**.

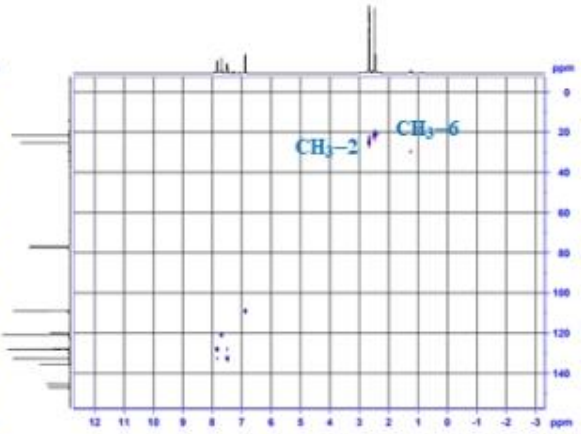


Fig. 4. HSQC-NMR spectrum of compound **2**.

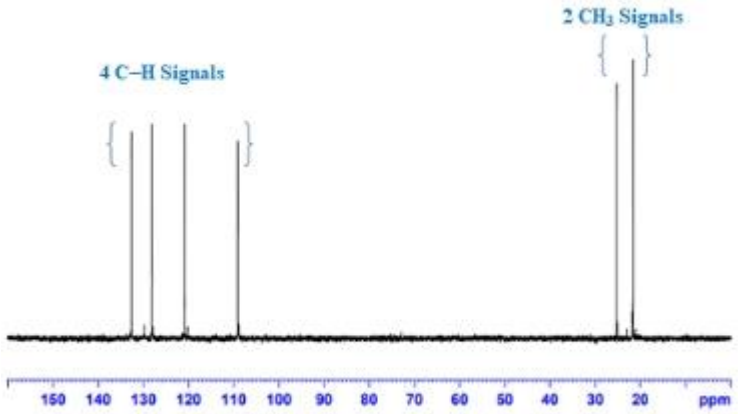


Fig. 5. DEPT-135° NMR spectrum of compound **2**, positive signals only were observed.

Cycloaddition of azidoquinoline **2** under Copper-Catalyzed Azide Alkyne cycloaddition (CuAAC) conditions "Clicking" in the presence of CuSO4.5H2O, L-ascorbic acid, THF–H2O 4:1 under reflux, and the known propargylated pharmacophores **3**, **4** [21], and **5** [23] afforded the required triazoles **6-8** in very good yields as described in (**Scheme 1)**.

The ^{1}H NMR spectrum of compound **6** (**Figure 6**) showed the aromatic protons of the quinoline and triazole rings within the range *d* 7-8 ppm. Besides, the olefinic proton (H-6) of the cholesterol ring at *d* 7-8 ppm. The aliphatic protons of the cholesterol moiety appeared in the high field region of the spectrum with the characteristic doublets of the methyl groups CH_3-19, CH_3-21, CH_3-26, and CH_3-27. The singlet of the most shielded protons of the CH_3-18 at *d* 0.61 ppm was diagnostic too. This panel of signals confirms the coupling reaction and the proposed structure for compound **6**.

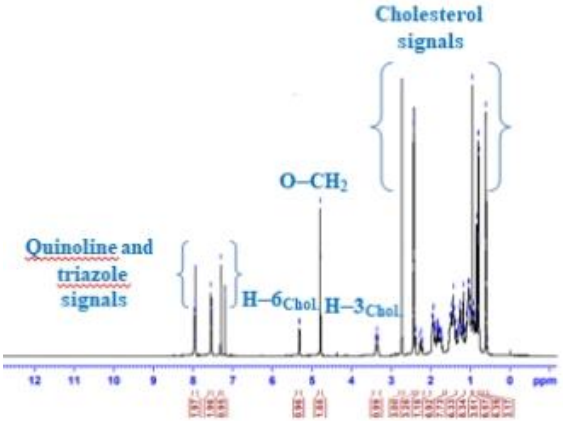


Fig. 6. ^{1}H NMR spectrum of compound **6**.

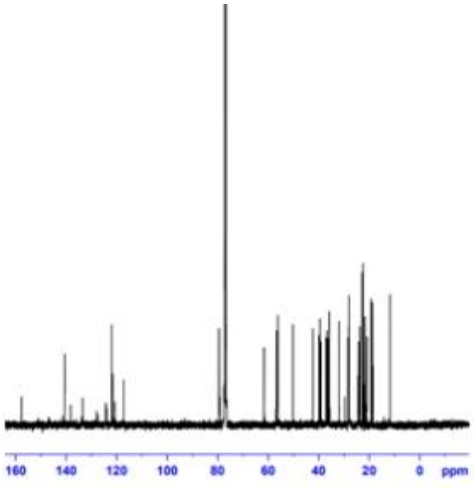


Fig. 7. ^{1}H NMR spectrum of compound **6**.

In case of compound **7**, the aromatic protons of the quinolone ring and the unique aromatic proton in both the purine and the triazole rings were typically observed in the aromatic region (**Figure 8**). While, the four methyl groups were observed in the high field region of the ^{1}H NMR spectrum. The ^{13}C NMR spectrum (**Figure 9**) of this theophylline derivative unambiguously showed the previous aliphatic carbons.

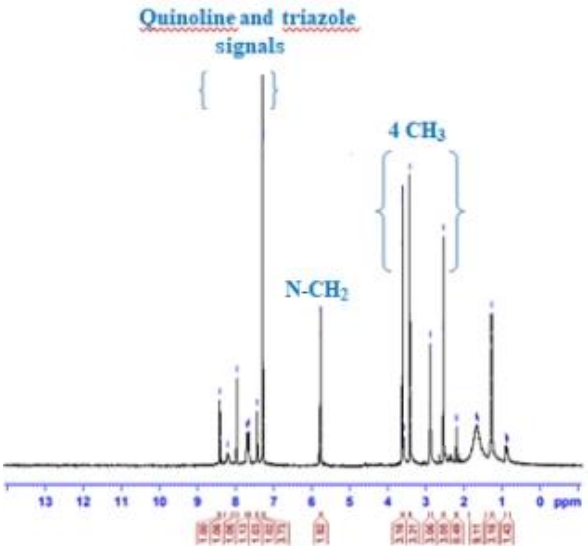


Fig. 8. ^{1}H NMR spectrum of compound **8**.

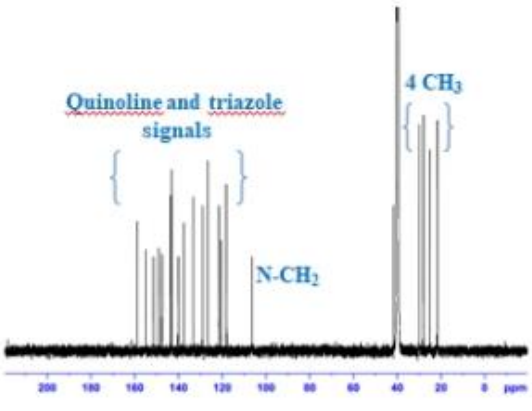


Fig. 9. ^{1}H NMR spectrum of compound **9**.

Finally, the ferrocene conjugate **8** was obtained in excellent yield. The protons of the enone moiety had high coupling constant in ^{1}H NMR indicating an *S*-trans orientation in the chalcone center of the molecule (**Figure 10**). Other protons as well as the ^{13}C signals all were in agree with the proposed structure for compound **8** (**Figure 11**).

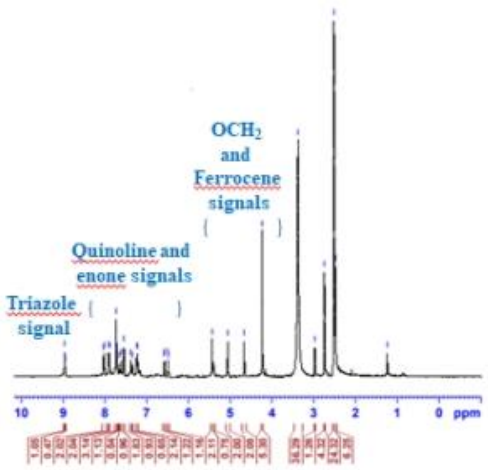


Fig. 10. ^{1}H NMR spectrum of compound **7**.

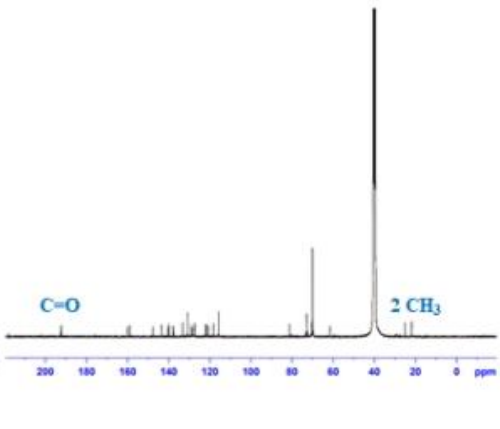
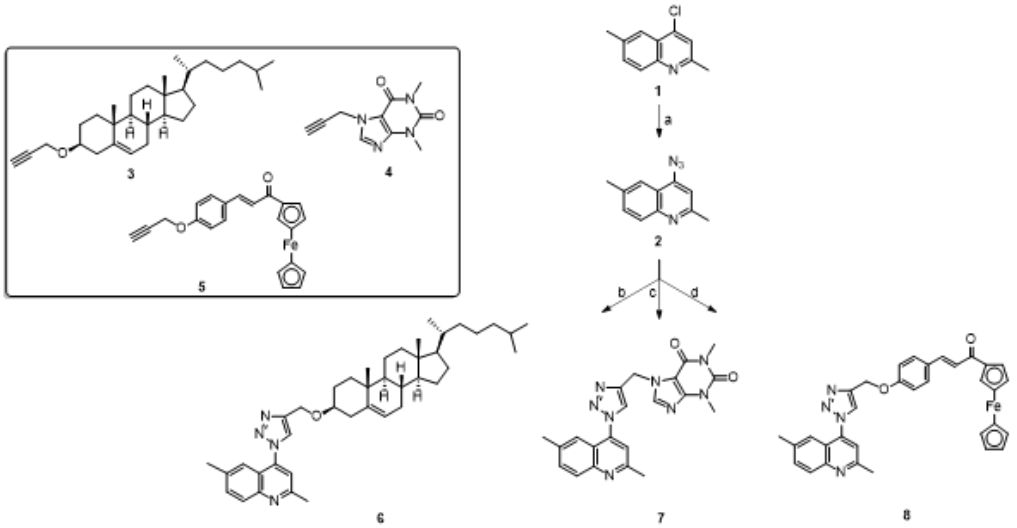


Fig. 11. ^{1}H NMR spectrum of compound **7**.

The IR spectra in all cases were agreed with the proposed structures and the mass spectra as well.



Scheme 1. *Reagents and conditions: (a)* NaN_3, DMF, 80-100 °C (57%) [7,10]; *(b)* **3**, $CuSO_4.5H_2O$, L-ascorbic acid, THF–H_2O 4:1, rfx (71%); *(c)* **4**, $CuSO_4.5H_2O$, L-ascorbic acid, THF–H_2O 4:1, rfx (88%); *(d)* **5**, $CuSO_4.5H_2O$, L-ascorbic acid, THF–H_2O 4:1, rfx (97%).

3.2. Anticancer Activity

Using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) colorimetric assay, the *in vitro* anticancer activity of the synthesized quinolines **6-8** was evaluated against two human cancer cell lines: the human prostate cancer cell line PC3 and the human breast cancer cell line MCF7. In this experiment, doxorubicin served as the positive control.

Table 1. Cytotoxic activities of compds. **6-8.**

Compd.	*In vitro* Cytotoxicity IC_{50} (µM)	
	MCF-7	**PC-3**
6	88.56±4.2	>100
7	6.61±0.4	7.33±0.5
8	9.46±0.7	13.89±1.0
Dox.	4.17±0.2	8.87±0.6

(IC_{50} values are expressed in µM ± S.E.). **IC_{50} (µM)**: 1 – 10 (very strong), 11 – 20 (strong), 21 – 50 (moderate), 51 – 100 (weak) and above 100 (non-cytotoxic).

3.2.1. MCF-7 cell line

Table 1 and **Figures 12, 13**, displaying the *in vitro* cytotoxicity of compounds **6-8** against the MCF-7 cell line. The quinolone-triazole cholesterol conjugates **6** showed IC_{50} of 88.56±4.2 that is 21-fold less active than doxorubicin. The conjugate quinolone-triazole theophylline **7** showed better cytotoxity (6.61±0.4) than compound **6** where, it was 0.6-fold less active than **Dox.** Finally, the quinolone-triazole chelcone-ferrocene conjugate **8** was 1.3-fold less active than doxorubicin.

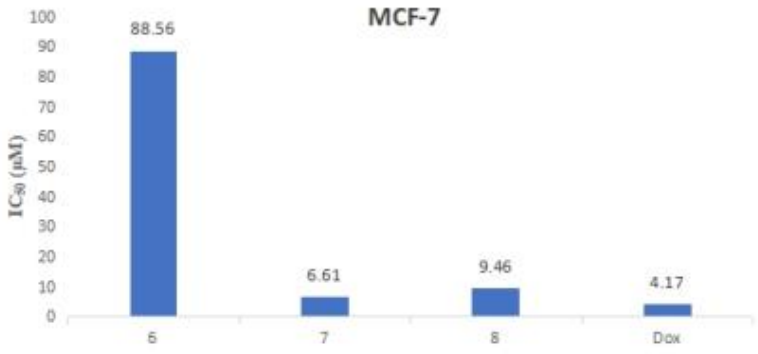


Fig. 12. IC_{50} (μM) of compounds **6-8** against MCF-7 cell line compared to **Dox**.

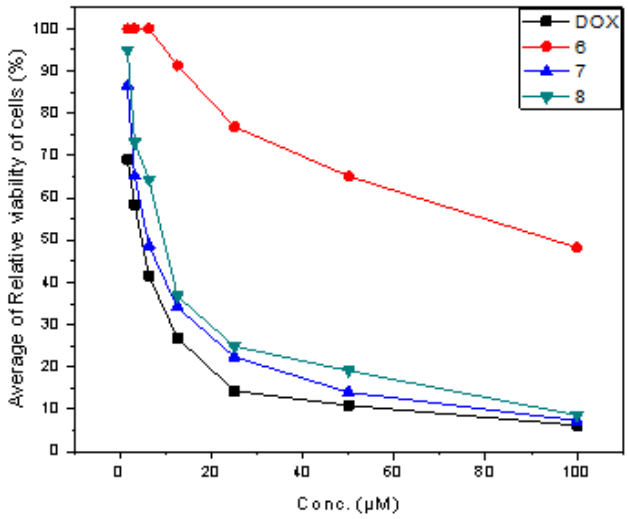


Fig. 13. *In vitro* relative viability of MCF-7 cells toward compounds **6-8.**

3.2.2. PC-3 cell line

In case of the prostate cancer cell line PC-3 (**Table 1** and **Figures 14, 15**) conjugate **6** was much less active than doxorubicin compared to its activity towards the MCF-7 cell line. The collective results of this conjugate shows that conjugation of cholesterol with quinoline through the 1,2,3-triazole linker does not stimulate cytotoxicity, at least against this pair of cell lines.

In line with the result of conjugate **7** against the MCF-7 cell line, this compound displayed promising cytotoxicity (7.33±0.5) aginst the PC-3 cell line which corresponds to 0.2-fold more active than doxorubicin. Generally, these results disclose the significance of tethering the quinolone-triazole moiety with the theophylline tag on ensuing toxicity against these cell lines, as the quinoline-triazole moiety is common in the three compounds, theophylline tag is the only significant in compound 7, and maybe it induced the strong cytotoxic activity as it has many binding sites that could adhere to the proteins through as it has 4 nitrogen atoms and 2 oxygens.

Finally, the ferrocene conjugate **8** retained the same dimensioned activity (1.3-fold) against the PC-3 cell line as it did in case of the MCF-7 cell line.

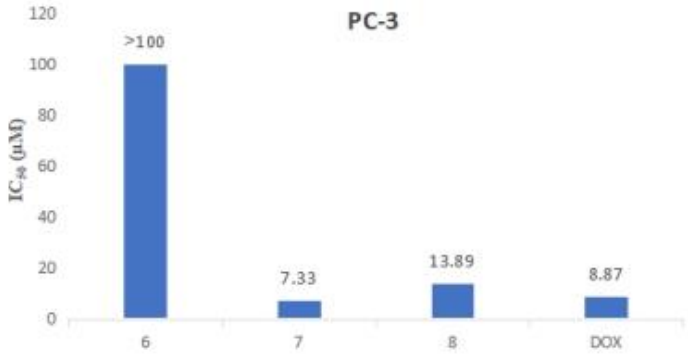


Fig. 14. IC_{50} (µM) of compounds **6-8** against PC-3 cell line compared to **Dox**.

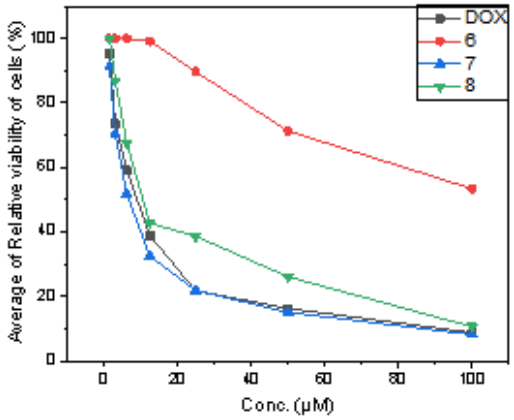


Fig. 15. *In vitro* relative viability of PC-3 cells toward compounds **6-8.**

4. CONCLUSION

Three novel synthetic Hybrid Quinolines **6-8** were synthesized by copper-catalyzed azide-alkyne cycloaddition reactions in favorable yields and characterized by FT-IR, MS and multi NMR techniques. Their *in vitro* cytotoxicity was tasted against the MCF-7 and the PC-3 cell lines, where compound **7** displayed better cyctotoxicity than Doxorubicin against the PC-3 cell line. Further investigation of the mechanism of action of compound **7** against the PC-3 cell line is highly encouraged along with *in vivo* studies.

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