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Anticancer activity of novel Schiff bases and azo dyes derived from 3-amino-4-hydroxy-2H-pyrano[3,2-c]quinoline-2,5(6H)-dione

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Abstract: New Schiff bases and azo dyes derivatives have been synthesized *via* appropriate conventional methods using pyranoquinolinone as a starting material. The compounds obtained were characterized by spectral analysis and evaluated for anticancer activity in several human tumor cell lines: MCF-7 breast cancer, HepG2 liver cancer and HCT-116 colon carcinoma. 5-fluorouracil was used as a reference drug. The *in vitro* cytotoxicity screening results revealed that all tested compounds showed promising activity against MCF-7 cells. In particular, compounds **6a, 6b,** and **7b** showed excellent activity against the three human tumor cell lines. Structure-activity relationship studies indicated that the azo derivative with a trifluoromethoxy group (compound **7b**) was the most potent candidate against the three human tumor cell lines (IC_{50}, 1.82-8.06 μg/mL). Our findings highlight pyranoquinolinone analogues as a promising class of compounds for new anticancer therapies.

Keywords: Schiff bases, azo dyes, pyran, quinolinone, anticancer

Introduction

Heterocyclic systems containing a quinolone nucleus represent an important group of compounds in medicinal and pharmaceutical chemistry. In particular, pyranoquinoline alkaloid compounds containing pyrano[3,2-c]quinolone as the parent ring structure have received significant attention due to their broad spectrum of antimicrobial [1-3], anti-inflammatory [4], antimalarial [5], antifungal [6] and anticancer properties [7]. In addition to these bioactivities, 6-*n*-butylpyranoquinolone derivatives are highly selective Topoisomerase II beta (TOP2B) inhibitors [8] which inhibit growth of different tumor cell lines [9] and are potential anti-cancer agents [10]. Schiff bases form an important class of widely used organic compounds with numerous applications in many fields, including analytical, biological and inorganic chemistry. Schiff bases are characterized by the presence of an azomethine linkage that underlies a broad spectrum of anti-inflammatory [11], antimicrobial [12], antitubercular [13], anticancer [14] and antioxidant activities [15]. Additionally, some pyranoquinolinone-based Schiff bases exhibit significantly better TOP2B inhibitory activity against MCF-7 breast cancer cells compared to doxorubicin [8]. Azo dyes are widely used in different applications, such as textile dyeing [16], biomedical studies and advanced organic synthesis. These dyes have multiple interesting biological functions including antibacterial, antifungal [17], antimicrobial [18], antiviral and cytotoxic activities [19]. In addition, organofluorine compounds are gaining interest for their uses in medicinal applications, chemical biology and drug discovery [20], as evidenced by the fact that a large number of fluorine-containing compounds have been approved by the FDA for medical and agricultural use [21]. The unique properties that make fluorine and fluorinated compounds attractive in chemical biology include a small atomic radius, high electronegativity, nuclear spin of ½ and low polarizability of the C–F bond [21]. Continuing our previous work in the field of quinolone chemistry [22,23] and considering all the evidence mentioned so far, the synthesis of novel Schiff bases and azo dyes, especially those appended with *n*-alkylpyranoquinolinone, is of great importance for drug development studies and chemical biology. Accordingly, novel pyranoquinolinone-derived Schiff bases and azo dyes were prepared in one framework to improve the biological activities of the parent compound. Likewise, fluorine and organofluorine groups were incorporated

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into the newly synthesized Schiff bases and azo dyes to improve the desirable pharmacological properties of these compounds.

Material and methods

General

All starting materials and reagents were obtained from Sigma-Aldrich and used without additional purification. Monitoring of reactions was carried out using Thin-layer chromatography (TLC) utilizing precoated Fluka analytical silica gel 60 F_{254} nm TLC plates and visualized under a UV lamp (254 nm). Purity of the compounds was checked using column chromatography over Kielselgel 60 silica gel (40–60 μm). Melting points were recorded with a Sanyo Gallen Kamp MPD 350 B.M 3.5 meting point instrument and were uncorrected. Infrared analysis was carried out with a Thermo Nicolet Nexus 470 FT-IR spectrophotometer. Nuclear magnetic resonance spectra (NMR) were recorded using a Varian-400 MHz spectrometer (^{1}H-NMR at 400 MHz, ^{13}C-NMR at 100 MHz, and ^{19}F-NMR at 376 MHz) using DMSO-d_6 and $CDCl_3$ as solvents. Tetramethylsilane (TMS) was used as an internal reference and chemical shifts are stated in parts per million; (δ values, ppm). Elemental analysis was carried out on a Perkin-Elmer CHN-2400II at the Chemical War Department, Ministry of Defence, Cairo, Egypt. Mass spectra were acquired on a Gas Chromatographic GCMSqp 1000 ex Shimadzu device at 70 eV, a triple-quadrupole tandem mass spectrometer (Micromass W Quattro micro™, Waters Corp., Milford, MA, USA) or Waters ZMD Quadrupole with an electrospray ionization (ESI) chamber. Morphological changes were visualized under an Olympus CKX41 inverted microscope (Tokyo, Japan).

General procedure for preparation of Schiff bases (4a-c)

An equivalent amount of compound 3 (10 mmol) and aldehyde derivatives (10 mmol) were mixed in tetrahydrofuran (25 mL) and the reaction mixture was refluxed for 10 h. The reaction progress was observed using TLC. After completion, the reaction mixture was allowed to cool. The solid obtained was filtered, washed with water twice and the desired Schiff bases **(4a-c)** were obtained after recrystallization from acetic acid.

(E)-6-butyl-3-((2-fluorobenzylidene)amino)-4-hydroxy-2H-pyrano[3,2-c]quinoline-2,5(6H)-dione (4a). Yellow powder; yield 49% and 2 g; mp 222 °C; IR (KBr, cm^{-1}): 3415 (OH), 3123 (C-$H_{aromatic}$), 2953, 2928, 2860 (C-$H_{aliphatic}$), 1709 (C=$O_{\alpha\text{-}pyrone}$), 1656 (C=$O_{quinolone}$), 1590 (C=N), 1537 (C = $C_{aromatic}$); ^{1}H-NMR [$CDCl_3$, 400 MHz]: (*δ, ppm*) 0.99 (t, *J* = 8.00 Hz, 3 H, C4`), 1.46 - 1.51 (m, 2 H, C3`), 1.77 - 1.81 (m, 2 H, C2`), 4.33 (t, *J* = 8.00 Hz, 2 H, C1`), 7.09 (t, *J* = 8.00 Hz, 1 H_{phenyl}), 7.20 (t, *J* = 8.00 Hz, 1 H, C9-H), 7.37 - 7.43 (m, 1 H_{phenyl}), 7.44 (d, *J* = 8.00 Hz, 1 H_{phenyl}), 7.49 (d, *J* = 9.00 Hz, 1 H, C7-H), 7.75 (t, *J* = 8.00 Hz, 1 H_{phenyl}), 8.23 (t, *J* = 8.00 Hz, 1 H, C8-H), 8.34 (dd, *J* = 8.02, 1.37 Hz, 1 H, C10-H), 9.68 (s, 1 H, CH=N), 13.85 (brs, 1 H, OH, exchanges with D_2O); ^{19}F NMR (400 MHz, DMSO-d_6) *δ* ppm: -121.96 (s, 1F_{ortho}); ^{13}C-NMR [DMSO-d_6, 100 MHz]: (δ, *ppm*) 13.75 (C4`), 20.17 (C3`), 29.56 (C2`), 42.49 (C1`), 100.47 (C3), 113.80 (C_{phenyl}), 113.90 (C4a), 115.11 (C10a), 115.81 (C7), 124.13 (C_{phenyl}), 124.83 (C9), 124.90 (C10), 127.66 (C_{phenyl}), 127.69 (C8), 133.12 (C_{phenyl}), 133.83 (C6a), 137.93 (C10b), 154.93 (C_{phenyl}), 156.19 (s, C4), 157.60 (CH=N), 161.48 (C2), 163.17 ($C_{phenyl\text{-}F}$), 164.07 (C5); Anal. Calcd for $C_{23}H_{19}FN_2O_4$ (406.41): C, 67.97%; H, 4.71%; F, 4.67%; N, 6.89%; Found: C, 67.94%; H, 4.65%; F, 4.60%; N, 6.84%.

(E)-6-butyl-3-((3-fluorobenzylidene)amino)-4-hydroxy-2H-pyrano[3,2-c]quinoline-2,5(6H)-dione (4b). Yellow powder; yield 64% and 2.6 g; mp 211 °C; IR (KBr, cm^{-1}): 3428 (OH), 3203 ($CH_{aromatic}$), 2958, 2928, 2876 ($CH_{aliphatic}$), 1738 (C=$O_{\alpha\text{-}pyrone}$), 1676 (C=$O_{quinolone}$), 1613 (C=N), and 1600 (C=$C_{aromatic}$); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 0.92 (t, *J* = 8.00 Hz, 3 H, C4`), 1.36 - 1.47 (m, 2 H, C3`), 1.59 - 1.69 (m, 2 H, C2`), 4.32 (t, *J* = 8.00 Hz, 2 H C1`), 7.29 (t, *J* = 8.00 Hz, 1 H, C9-H), 7.50 (t, *J* = 7.40 Hz, 1 H_{phenyl}), 7.53 (s, 1 H_{phenyl}), 7.65 (dd, *J* = 8.61, 1.17 Hz, 1 H_{phenyl}), 7.68 (d, *J* = 7.83 Hz, 1 H, C7-H), 7.82 (d, *J* = 8.00 Hz, 1 H_{phenyl}), 7.86 (t, *J* = 7.4, Hz, 1 H, C8-H), 8.14 (dd, *J* = 8.02, 1.37 Hz, 1 H C10-H), 9.27 (s, 1 H, CH=N), 13.96 (bs, 1 H, OH, exchanges with D_2O); ^{19}F NMR (400 MHz, DMSO-d_6) δ ppm: -112.86 (s, 1F_{meta}); ^{13}C NMR (100 MHz, DMSO-*d6*) δ ppm: 14.12 (C4`), 19.93 (C3`), 29.65 (C2`), 42.35 (C1`), 100.60 (C3), 112.85 (C_{phenyl}), 113.54 (C4a), 114.20 (C10a), 116.75 (C7), 118.20 (C_{phenyl}), 124.19 (C10), 124.75 (C9), 125.18 (C_{phenyl}), 131.26 (C8), 134.68 (C_{phenyl}), 138.11 (C6a), 139.92 (C10b), 156.43 (C_{phenyl}), 157.36 (C4), 161.68 (CH=N), 162.11 (C2), 163.23 ($C_{phenyl\text{-}F}$), 164.11 (C5); Anal. Calcd for $C_{23}H_{19}FN_2O_4$ (406.41): C, 67.97%; H, 4.71%; F, 4.67%; N, 6.89%. Found: C, 67.91%; H, 4.69%; F, 4.69%; N, 6.80%.

(E)-6-butyl-3-((4-fluorobenzylidene)amino)-4-hydroxy-2H-pyrano[3,2-c]quinoline-2,5(6H)-dione (4c). Yellow powder; yield 68.9 % and 2.8 g; mp 257 °C; IR (KBr, cm^{-1}): 3415 (OH), 3076 ($CH_{aromatic}$), 2948, 2918, 2860 ($CH_{aliphatic}$), 1714 (C=$O_{\alpha\text{-}pyrone}$), 1668 (C=$O_{quinolone}$), 1609 (C=N), and 1598 (C=$C_{aromatic}$); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.02 (t, *J* = 8.00 Hz, 3 H, C 4`), 1.40 - 1.64 (m, 2 H, C 3`), 1.72 - 1.82 (m, 2 H, C 2`), 4.35 (t, *J* = 8.00 Hz, 2 H, C 1`), 7.12

(d, J = 8.80 Hz, 2 H_{phenyl}), 7.44 (t, J = 7.60 Hz, 1H, C9-H), 7.52 (d, J = 8.60 Hz, 1 H, C7-H), 7.76 (t, J = 7.8 Hz, 1 H, C8-H), 7.94 (dd, J = 8.61, 5.48 Hz, 2 H_{phenyl}), 8.34 (dd, J = 8.02, 1.37 Hz, 1 H, C10-H), 9.41 (s, 1 H, CH=N), 12.36 (bs, 1 H, OH, exchanges with D_2O); ^{19}F NMR (400 MHz, $CDCl_3$) δ ppm: -108.6 (s, $1F_{para}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 13.77 (C4`), 20.18 (C3`), 29.58 (C2`), 42.50 (C1`), 100.57 (C3), 113.70 (C4a), 113.84 (C10a), 115.11 (C7), 115.61 (C_{phenyl}), 115.83 (C_{phenyl}), 124.12 (C10), 124.81 (C9), 130.64 (C_{phenyl}), 130.73 (C8), 133.77 (C_{phenyl}), 137.89 (C6a), 156.02 (C10b), 157.82 (C4), 160.25 (CH=N), 161.30 (C2), 163.17 (C5), 165.89 ($C_{phenyl\text{-}F}$); Anal. Calcd for $C_{23}H_{19}FN_2O_4$ (406.41): C, 67.97%; H, 4.71%; F, 4.67%; N, 6.89%. Found: C, 67.99%; H, 4.61%; F, 4.66%; N, 6.92%.

Synthesis of Schiff bases (6a,b)

(E)-6-butyl-3-(ethoxymethylene)-2H-pyrano[3,2-c] quinoline-2,4,5(3H,6H)-trione (5). A mixture of compound **1** (2.85 g, 10 mmol) and triethyl orthoformate (8 mL, 50 mmol) was heated under solvent-free conditions for 10 h. Reaction progress was observed by TLC. The solid obtained was filtered, washed with diethyl ether (3 × 5 mL) and crystallized from dry THF to yield compound **5** as yellow crystals, mp 190–192°C, yield (2.0 g, 58.8%); IR (KBr, cm^{-1}): 3090 ($CH_{arom.}$), 2950 ($CH_{aliph.}$), 1747 ($C{=}O_{\alpha\text{-}pyrone}$), 1679 ($C{=}O_{\gamma\text{-}pyrone}$), 1639 ($C{=}O_{quinolone}$), 1620 (C=C), and 1297 (C–O); ^1H NMR (400 MHz, DMSO-d_6) δ ppm: δ 0.89 (t, J = 4.00 Hz, 3 H, C 4`), 0.93 (t, 3H, $J$ = 4.00 Hz, $OCH_2CH_3$), 1.25 - 1.48 (m, 2 H, C3`), 1.55 - 1.65 (m, 2 H, C2`), 4.14 (t, $J$ = 8.00 Hz, 2 H C1`), 4.20 (q, 2H, J = 8.00 Hz, OCH_2CH_3), 7.21 (t, 1H, J = 8.00 Hz, H-9), 7.35 (d, 1H, J = 8.00 Hz, H-7), 7.96 (t,1H, J = 8.00 Hz, H-8), 8.10 (d, 1H, J = 8.4 Hz, H-10), 9.63 (s, 1H, =CH); Anal. Calcd for $C_{19}H_{19}NO_5$ (341.37): C, 66.85%; H, 5.61%; N, 4.10%; Found: C, 66.78%; H, 5.65%; N, 4.15%.

General procedure for preparation of Schiff bases 6a,b. An equivalent amount of the appropriate aromatic amine (10 mmol) and compound **5** (3.41 g, 10 mmol) were mixed in dry THF (25 mL) and the reaction mixture was refluxed for 10-11 h. The progress of the reaction was monitored by TLC. After the completion of the reaction, the resulting compound was cooled, filtered and crystallized from acetic acid to obtain Schiff bases **6a,b**.

(E)-6-butyl-4-hydroxy-3-(((4-(trifluoromethoxy) phenyl)imino)methyl)-2H-pyrano[3,2-c]quinoline-2,5(6H)-dione (6a). Yellow powder; yield 68.2 % and 3.2 g; mp 222 °C; IR (KBr, cm^{-1}): 3406 (OH), 3085 ($CH_{aromatic}$), 2953, 2924, 2872 ($CH_{aliphatic}$), 1717 ($C{=}O_{\alpha\text{-}pyrone}$), 1665 ($C{=}O_{quinolone}$), 1612 (C=N) and 1600 ($C{=}C_{aromatic}$); ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 0.91 (t, J = 8.00 Hz, 3 H, C4`), 1.44 - 1.49 (m, 2 H, C3`), 1.61 - 1.71 (m, 2 H, C2`), 4.34 (t, $J$ = 8.00 Hz, 2 H, C1`), 7.28 - 7.36 (m, 3 H, ($2H_{phenyl}$ + 1H, C9-H)), 7.54 (d, J = 8.00 Hz, 1 H, C7-H), 7.86 (t, J = 8.00 Hz, 1 H, C8-H), 8.03 (d, J = 8.00 Hz, 2 H_{phenyl}), 8.16 (d, J = 8.00 Hz, 1 H, C10-H), 9.69 (s, 1H, CH=N), 13.74 (s, 1H, OH, exchanges with D_2O); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 14.13 (s, 1 C 4`), 19.96 (C3`), 33.79 (C2`), 42.28 (C1`), 100.89 (C3), 112.44, 113.72, 114.03, 115.05, 116.37, 119.43, 122.71, 123.26, 124.38, 124.63, 132.20, 133.35, 136.11, 136.92, 144.47, 151.27, 152.14, 159.23, 162.84; Anal. Calcd for $C_{24}H_{19}F_3N_2O_5$ (472.41): C, 61.02; H, 4.05; F, 12.06; N, 5.93%; Found: C, 61.05; H, 4.11; F, 12.09; N, 5.88%.

(E)-6-butyl-4-hydroxy-3-((3-(trifluoromethyl) phenyl)diazenyl)-2H-pyrano[3,2-c]quinoline-2,5(6H)-dione (6b). Yellow powder; yield 77.9 % and 3.6 g; mp 257 °C; IR (KBr, cm^{-1}): 3415 (OH), 3150 ($CH_{aromatic}$), 2958, 2928, 2876 ($CH_{aliphatic}$), 1729 ($C{=}O_{\alpha\text{-}pyrone}$), 1665 ($C{=}O_{quinolone}$), 1615 (C=N), and 1605 ($C{=}C_{aromatic}$); ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 0.91 (t, J = 8.00 Hz, 3 H, C 4`), 1.35 - 1.49 (m, 2 H, C 3`), 1.62 - 1.68 (m, 2 H, C 2`), 4.33 (t, $J$ = 8.00 Hz, 2 H, C 1`), 7.48-7.51 (m, 2 H, (C9-H+ $1H_{phenyl}$)), 7.53 (s, 1 H_{phenyl}), 7.75-7.89 (m, 3 H, (C7-H, 1 H_{phenyl}, C8-H)), 8.08 (d, J = 8.00, 1 H_{phenyl}), 8.12 (dd, J = 8.02, 1.37 Hz, 1 H C10-H), 9.80 (s, 1 H, CH=N), 13.75 (s, 1 H, OH, exchanges with D_2O); Anal. Calcd for $C_{24}H_{19}F_3N_2O_4$ (456.41): C, 63.16; H, 4.20; F, 12.49; N, 6.14%; Found: C, 63.19; H, 4.15; F, 12.40; N, 6.19%.

General procedure for preparation of azo dyes 7a-e

Diazonium chloride was freshly produced from aniline derivatives (5 mmol) and hydrochloric acid (60 mL, 1M). To the corresponding diazonium chloride, a cooled solution (0-5°C) of compound **1** (1.4 g, 5 mmol) in pyridine (25 mL) was added dropwise and stirred for 30 min. The reaction mixture was stirred at room temperature for around 2 h. The resulting solid was filtered, washed with cold water (3 x 10 mL), dried and crystallized from suitable solvents to give azo compounds **7a-e**.

(E)-6-butyl-3-((3-fluorophenyl)diazenyl)-4-hydroxy-2H-pyrano[3,2-c]quinoline-2,5(6H)-dione (7a). Yellow powder; yield 65 % and 1.3 g; mp 245 °C; IR (KBr, cm^{-1}): 3428 (OH), 3203 ($CH_{aromatic}$), 2958, 2928, 2876 ($CH_{aliphatic}$), 1738 ($C{=}O_{\alpha\text{-}pyrone}$), 1676 ($C{=}O_{quinolone}$), and 1613 (C=C); ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 0.98 (t, J = 8.00 Hz, 3 H, C4`), 1.43 - 1.49 (m, 2 H, C3`), 1.73 - 1.77 (m, 2 H, C2`), 4.29 (t, $J$ = 7.80 Hz, 2 H, C1`), 6.99 - 7.07 (m, 1 H_{phenyl}), 7.32 (t, J = 7.63 Hz, 1 H_{phenyl}), 7.37 (d, J = 9.00 Hz, 1 H_{phenyl}), 7.42 (t, J = 8.02 Hz, 2 H ($1H_{phenyl}$+ C9-H), 7.47 (d, J = 8.61 Hz, 1 H, C7-H), 7.76 (t, J = 8.00 Hz, 1 H, C8-H), 8.30

(dd, *J* = 8.02, 1.37 Hz, 1 H, C10-H), 13.25 (s, 1 H, OH, exchanges with D_2O); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 13.75 (C4`), 20.15 (C3`), 29.57 (C2`), 42.31 (C1`), 90.90 (C3), 99.96 (C4a), 105.24 (C10a), 113.96 (C_{phenyl}), 115.07 (C_{phenyl}), 122.74 (C7), 124.04 (C10), 124.99 (C9), 125.90 (C_{phenyl}), 134.07 (C_{phenyl}), 135.58 (C8), 138.14 (C6a), 141.16 (C_{phenyl}), 159.27 (C10b), 161.66 (C-F_{phenyl}), 162.80 (C2), 169.06 (C4), 178.23 (C5); Anal. Calcd for $C_{22}H_{18}FN_3O_4$ (407.39): C, 64.86; H, 4.45; F, 4.66; N, 10.31%; Found: C, 64.81; H, 4.35; F, 4.52; N, 10.21%.

(E)-6-butyl-4-hydroxy-3-((4-(trifluoromethoxy) phenyl)diazenyl)-2H-pyrano[3,2-c]quinoline-2,5(6H)-dione (7b). Yellow powder; yield 59 % and 1.3 g; mp 252 °C; IR (KBr, cm^{-1}): 3418 (OH), 3054 ($CH_{aromatic}$), 2952, 2922, 2872 ($CH_{aliphatic}$), 1758 (C=$O_{\alpha\text{-}pyrone}$), 1666 (C=$O_{quinolone}$), 1619 (C=C), and 1566 (N=N*azo*.); ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 0.96 (t, *J* = 8.00 Hz, 3 H,C4`), 1.42 - 1.47 (m, 2 H, C3`), 1.69 - 1.74 (m, 2 H, C2`), 4.26 (t, *J* = 8.00 Hz, 2 H, C1`), 7.33 (t, *J* = 8.00 Hz, 3 H, (2 H_{phenyl} + 1 H, C9-H), 7.38 (d, *J* = 8.00 Hz, 1 H, C7-H), 7.67 (d, *J* = 8.00 Hz, 2 H_{phenyl}), 7.76 (t, *J* = 8.00 Hz, 1 H, C8-H), 8.29 (d, *J* = 8.00 Hz, 1 H, C10-H), 13.23 (s, 1 H, OH, exchanges with D_2O); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 13.79 (C4`), 20.19 (C3`), 29.45 (C2`), 42.14 (C1`), 106.28 (C3), 112.41 (C4a), 114.61 (C7), 119.02 (C10a), 119.35 (C_{phenyl}), 121.58 (C10), 122.22 (C_{phenyl}), 122.50 (C_{phenyl}), 122.70 (C9), 125.89 (C8), 135.52 ($COCF_3$), 138.92 (C6a), 141.16 (C-N_{phenyl}), 148.58 (C10b), 157.06 (C-O_{phenyl}), 157.86 (C4), 161.91 (C2), 178.28 (C5); Mass Spectrum, *m/z* (I_r%): 474 [M^++1; 25], 473 [M^+; 100], 445 (22), 389 (30), 284 (40), 285 (80%), 257 (30), 144 (60), 116 (48), 91 (12), 92 (28), 77 (30); Anal. Calcd for $C_{23}H_{18}F_3N_3O_5$ (473.4): C, 58.35; H, 3.83; F, 12.04; N, 8.88%; Found: C, 58.42; H, 3.87; F, 12.09; N, 8.73%.

(E)-6-butyl-3-((4-chlorophenyl)diazenyl)-4-hydroxy-2H-pyrano[3,2-c]quinoline-2,5(6H)-dione (7c) [24]. Yellow powder; yield 60.2 % and 1.25 g; mp 245 °C; IR (KBr, cm^{-1}): 3404 broad band (OH), 3094 ($CH_{aromatic}$), 2955, 2925, 2869 ($CH_{aliphatic}$), 1735 (C=$O_{\alpha\text{-}pyrone}$), 1676 (C=$O_{quinolone}$), and 1616 (C=C); ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 0.99 (t, *J* = 8.00 Hz, 3 H, C4`), 1.43 - 1.49 (m, 2 H, C3`), 1.73 - 1.77 (m, 2 H, C2`), 4.28 (t, *J* = 8.00 Hz, 2 H, C1`),7.31 (t, *J* = 8.02 Hz, 1 H, C9-H), 7.37 (d, *J* = 8.61 Hz, 1 H, C7-H), 7.39 - 7.44 (m, 2 H_{phenyl}), 7.47 (d, *J* = 8.61 Hz, 1 H_{phenyl}), 7.57 (d, *J* = 9.00 Hz, 1 H_{phenyl}), 7.76 (t, *J* = 7.83 Hz, 1 H, C8-H), 8.29 (dd, *J* = 7.83, 1.57 Hz, 1 H, C10-H), 13.24 (s, 1 H, OH, exchanges with D_2O); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 13.75 (C4`), 20.15 (C3`), 29.56 (C2`), 42.31 (C1`), 90.89 (C3), 99.93 (C4a), 114.61 (C10a), 115.07 (C7), 119.22 (C_{phenyl}), 122.69 (C_{phenyl}), 124.03 (C10), 124.97 (C9), 125.85 (C_{phenyl}), 130.12 (C8), 134.07 (C_{phenyl}), 135.50 (C_{phenyl}), 138.14 (C6a), 159.26 (C_{phenyl}), 161.59 (C10b), 162.80 (C2), 169.02 (C4), 178.20 (C5); Anal. Calcd for $C_{22}H_{18}ClN_3O_4$ (423.85): C, 62.34; H, 4.28; Cl, 8.36; N, 9.91%; Found C, 62.29; H, 4.19; Cl, 8.39; N, 9.95%.

(E)-6-butyl-3-((2,4-dichlorophenyl)diazenyl)-4-hydroxy-2H-pyrano[3,2-c]quinoline-2,5(6H)-dione (7d). Orange powder; yield 55.6 % and 1.3 g; mp 263 °C; IR (KBr, cm^{-1}): 3411 (OH), 3087 ($CH_{aromatic}$), 2952, 2928, 2866 ($CH_{aliphatic}$), 1758 (C=$O_{\alpha\text{-}pyrone}$), and 1666 (C=$O_{quinolone}$), 1616 (C=C), 1560 (N=N*azo*); ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 0.96 (t, *J* = 8.00 Hz, 3 H, C4`), 1.43 - 1.48 (m, 2 H, C3`), 1.66 - 1.77 (m, 2 H, C2`), 4.26 (t, *J* = 8.00 Hz, 2 H, C1`), 7.32 (t, *J* = 7.60 Hz, 2 H, C9-H + H_{phenyl}), 7.38 (d, *J* = 7.83 Hz, 1 H, C7-H), 7.45 (d, *J* = 2.35 Hz, 1 H_{phenyl}) 7.75 (t, *J* = 8.00 Hz, 1 H, C8-H), 8.03 (d, *J* = 9.00 Hz, 1 H_{phenyl}), 8.31 (dd, *J* = 8.22, 1.57 Hz, 1 H, C10-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 13.80 (C4`), 20.26 (C3`), 29.43 (C2`), 42.29 (C1`), 106.39 (C3), 112.34 (C4a), 114.61 (C10a), 119.04 (C7), 122.69 (C10), 123.30 (C9), 124.60 (C8), 125.92 (C_{phenyl}), 128.75 (C_{phenyl}), 129.73 (C_{phenyl}), 133.73 (C_{phenyl}), 135.61 (C_{phenyl}), 136.40 (C6a), 141.26 (C_{phenyl}), 156.90 (C10b), 157.73 (C2), 161.88 (C4), 177.90 (C5); Anal. Calcd for $C_{22}H_{17}Cl_2N_3O_4$ (458.29): C, 57.66; H, 3.74; Cl, 15.47; N, 9.17%; Found C, 57.59; H, 3.76; Cl, 15.39; N, 9.11%.

(E)-6-butyl-4-hydroxy-3-((3-nitrophenyl) diazenyl)-2H-pyrano[3,2-c]quinoline-2,5(6H)-dione (7e). Yellow powder; yield 62% and 1.3 g; mp 280 °C; IR (KBr, cm^{-1}): 3414 (OH), 3077 ($CH_{aromatic}$), 2952, 2925, 2866 ($CH_{aliphatic}$), 1755 (C=$O_{\alpha\text{-}pyrone}$), and 1669 (C=$O_{quinolone}$), 1623 (C=C), and 1563 (N=N *azo*); ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 0.98 (t, *J* = 8.00 Hz, 3 H, C4`), 1.44 - 1.54 (m, 2 H, C3`), 1.68 - 1.77 (m, 2 H, C2`), 4.31 (t, *J* = 8.00 Hz, 2 H, C1`), 7.35 (t, *J* = 7.63 Hz, 1 H, C9-H), 7.40 (d, *J* = 8.61 Hz, 1 H, C7-H) 7.66 (t, *J* = 8.02 Hz, 1 H_{phenyl}), 7.79 (t, *J* = 7.80 Hz, 1 H, C8-H), 7.92 (dd, *J* = 8.02, 1.37 Hz, 1 H_{phenyl}), 8.17 (dd, *J* = 7.83, 1.57 Hz, 1 H, C10-H), 8.33 (dd, *J* = 8.22, 1.57 Hz, 1 H_{phenyl}), 8.50 (t, *J* = 1.96 Hz, 1 H_{phenyl}); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 13.79 (C4`), 20.20 (C3`), 29.45 (C2`), 42.21 (C1`), 106.27 (C3), 112.31 (C4a), 112.74 (C10a), 114.70 (C7), 122.26 (C_{phenyl}), 122.83 (C10), 123.29 (C9), 126.02 (C_{phenyl}), 130.89 (C8), 135.86 (C6a), 141.40 (C_{phenyl}), 141.78 (C_{phenyl}), 149.35 (C_{phenyl}), 156.48 (C_{phenyl}), 157.76 (C10b), 162.27 (C2), 176.17 (C4), 178.51 (C5); Analysis calculated for $C_{22}H_{18}N_4O_6$ (434.4): C, 60.83; H, 4.18; N, 12.90%; Found: C, 60.79; H, 4.12; N, 12.85%.

Anticancer activity

Chemicals: for cytotoxicity assay, Dimethyl sulfoxide (DMSO), crystal violet 1% and trypan blue dye were obtained from Sigma (St. Louis, Mo., USA). Fetal Bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25 % Trypsin-EDTA were purchased from (Bio Whittaker ® Lonza, Belgium).

Cell culture

Three cell lines: HCT-116 (colon carcinoma), MCF-7 (breast carcinoma) and HePG-2 (hepatocellular carcinoma) were obtained from VACSERA Tissue Culture center. All cells lines were cultured in a humidified atmosphere of 5% CO_2 and 95% air at 37°C in DMEM containing 10% heat-inactivated fetal bovine serum (FBS), 1% L-glutamine, HEPES buffer and 50 µg/mL gentamycin. The growth medium was renewed twice a week.

Antiproliferative assays and *in vitro* experiments

Antiproliferative activity screening of tested compounds was performed according to a previously described method [25]. *In vitro* antiproliferative activity was measured using HCT-116 (colon carcinoma), MCF-7 (breast carcinoma) and HePG-2 (hepatocellular carcinoma) cancer cell lines. The cell lines were cultured in 96-well plates, at a concentration of 1×10^4 cells per well, in 100 µL growth medium. After 24 h of seeding, the plates were washed with fresh medium including different concentrations of the test compounds. Then cultures that were immediately treated with two-fold serial dilutions of the tested compounds were added to confluent cell monolayers in 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) with a multi-channel pipette. Following this, they were incubated at 37 °C for 48 h. Three wells were used for each test compound concentration. Control cells were incubated without test compounds and with or without DMSO. The low percentage of DMSO present in the wells (maximal 0.1%) did not affect the experiment. After incubating the cells at 37 °C, different sample concentrations were added, and the incubation was continued for 24 h. Then, the viable cell yield was detected using a colorimetric method [26,27]. After the incubation period, the media was aspirated and then the cells were stained with a 1% crystal violet solution for 30 min. Afterward, all excess stain was removed by rinsing the plates with water. The plates were dried and then 30% glacial acetic acid was added to all wells and mixed carefully. The absorbance of the plates was measured after moderate shaking on a microplate reader (Tecan Inc., Morrisville, NC, USA), at 490 nm. Treated samples were correlated with the cell control in the absence of the tested compounds. All experiments were done in triplicate. Cytotoxicity of the tested compound was measured and the percentage viability was calculated as follows:

$$(ODt/ODc) \times 100\%$$

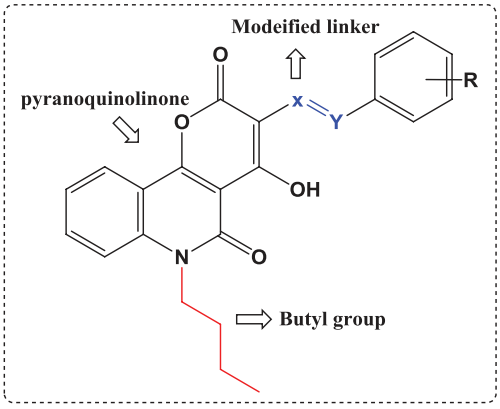
where ODt is the mean optical density of wells treated with the tested compounds and ODc is the mean optical density of untreated cells.

The relationship between compound concentration and surviving cells was plotted to find the survival curve of each tumor cell line after treatment with each compound. The 50% inhibitory concentration (IC_{50}) was assessed from graphic plots of the dose response curve for each concertation using Graphpad Prism software (San Diego, CA. USA).

Results and discussion

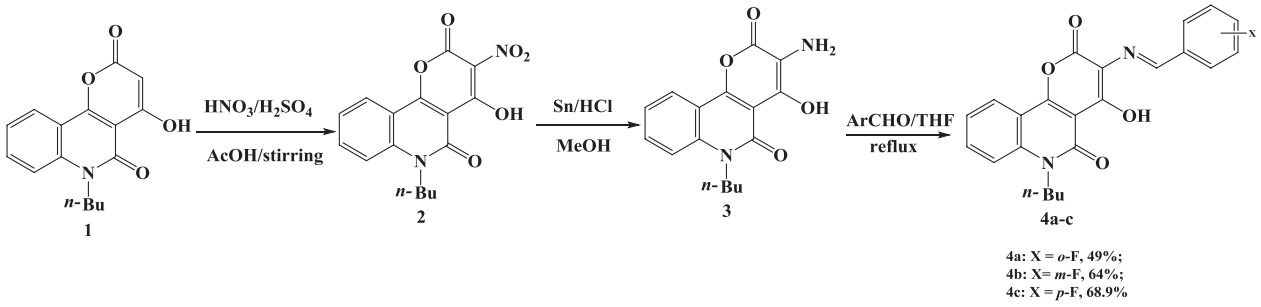
Chemistry

Quinoline analogues exhibited expanded biological activities depending on the structure type. Several natural products with a pyrano[3,2-c]quinolone structural moiety and patented chromenes were reported as promising cytotoxic agents. Thus, based on a hybrid pharmacophore design, it is proposed that applying the structural features of pyrano[3,2-c]quinolone might provide new derivatives for the development of novel anticancer agents (Figure 1). An *N*-butyl group was introduced onto *N*1 in the quinolone ring to enhance the lipophilicity and cell penetration ability. Fluorine-containing compounds have attracted much interest, since the introduction of fluorine atoms or fluoroalkyl moieties to an organic compound can bring about remarkable changes in physical, chemical and biological properties [28]. Fluoro compounds are also traditionally associated with potent antitumor properties. Other than the well-established fluoronucleosides such as 5-fluorouracil, fluorine-containing anticancer molecules include flutamide, an anti-androgen, which was launched in 1983 for the treatment of prostate cancer. Therefore, our new design was further modified by incorporating substituted F, CF_3 and OCF_3 groups to achieve more potent anticancer molecules with multi-targeted molecular mechanisms. Schiff bases and azo compounds are important structures in the medicinal and pharmaceutical fields [29] and the azomethine linkage might be responsible for the biological activities displayed by Schiff bases [30]. In light of the interesting biological activities seen in compounds containing azo groups, pyrano[3,2-c]quinolone, azomethine linkages, and organofluorine groups, the effect of having all these functionalities present simultaneously in one structure was examined, with the hypothesis that incorporating



Group No.	Comp.	R	X	Y
Group 1	4a	*o*-F	N	H
	4b	*m*-F	N	H
	4c	*p*-F	N	H
Group 2	6a	*p*- OCF_3	H	N
	6b	*m*-CF_3	H	N
Group 3	7a	*m*-F	N	N
	7b	*p*- OCF_3	N	N
	7c	*p*-Cl	N	N
	7d	2,4-diCl	N	N
	7e	*m*-NO_2	N	N

Figure 1 Template design for Schiff bases and azo dyes of pyranoquinolinone derivatives **4a-c**, **6a,b** and **7a-e**



Scheme 1 Synthesis of compounds **4a-c**

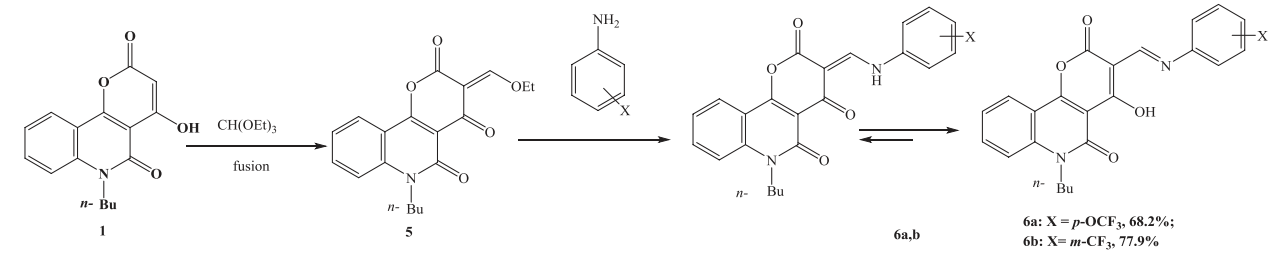
more than one bioactive heterocycle moiety into a single framework may result in novel heterocycles with enhanced/altered bioactivity (Figure 1).

The synthesis of Schiff bases **4a-c** with different specific aldehydes in dry THF as a solvent and a glacial acetic acid catalyst resulted in three new Schiff bases. For the synthesis of fluorinated Schiff base derivatives **4a-c**, it was first attempted to obtain 3-aminopyrano[3,2-*c*]quinolinedione **3** by the nitration of compound **1** using a mixture of concentrated nitric acid and sulfuric acid, which produced 3-nitropyrano[3,2-c]quinolinedione (**2**). The nitro-derivative **2** was reduced to obtain 3-aminopyrano[3,2-*c*] quinolinedione (**3**) according to a synthetic pathway reported in the literature [31] The new Schiff base derivatives **4a-c** were produced by condensing the amino derivative (**3**) with fluorine-containing benzaldehydes in dry THF. This process is summarized in Scheme 1.

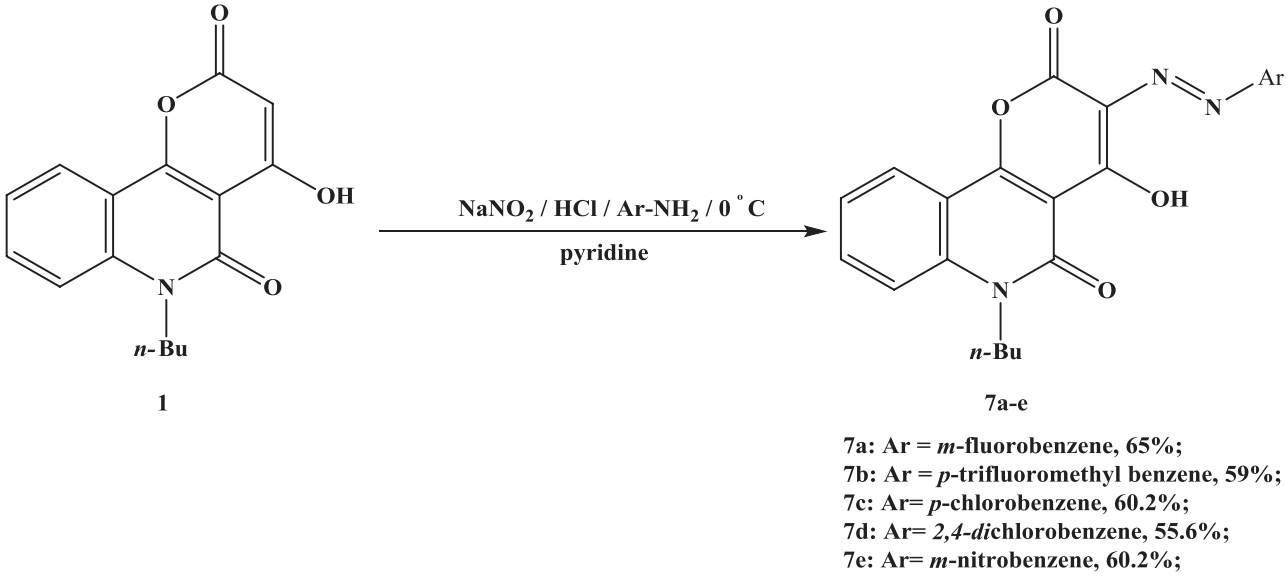
The electronic spectral data of the Schiff bases **4a-c** are described in the experimental section. IR spectra of compounds **4a-c** confirmed the absence of the double stretching absorption bands of the amino group of compound **3** and displayed a characteristic absorption band near 1590-1613 cm^{-1} due to ($HC=N_{imine}$). ^1H NMR spectra of compounds **4a–c** revealed the absence of the characteristic signal indicating the amine functionality observed in compound **3.** One characteristic singlet was observed at δ 9.27-9.68 ppm, which is due to the N=CH proton of the azomethine group. ^{13}C NMR spectra displayed signals in the range of δ 157–161 ppm, corresponding to azomethine carbon atoms. ^{19}F-NMR spectra of compounds **4a-c** showed only one singlet signal with integration=1, which was attributed to the presence of a fluorine atom in the molecules.

Additional new fluoro-substituted Schiff bases **6a,b** were synthesized starting from the ethoxymethylene compound (**5**), which was prepared by treating pyranoquinolinone **1** with triethylorthoformate under solvent-free conditions. The structure of compound **5** was supported by its ^1H NMR spectrum, which exhibited the presence of two new characteristic signals at δ 0.93 ppm and δ 4.20 ppm, which were attributed to ethyl group protons (OCH_2CH_3). Another characteristic singlet signal indicated an oleinic proton at δ 9.63 ppm. Compound **5** was allowed to react with fluoro-substituted anilines in boiling THF to obtain Schiff bases **6a,b**, as outlined in Scheme 2.

The IR spectra indicated the presence of stretching vibration bands at 1612 cm^{-1} in compound **6a** and at 1615 cm^{-1} in **6b**, attributed to (HC=N). The ^1H NMR spectra of compounds **6a** and **6b** revealed a singlet signal assigned to (HC=N) protons at δ 9.69 and 9.80 ppm, respectively. In addition,



Scheme 2 Synthesis of compounds **6a,b**



Scheme 3 Synthesis of compounds **7a-e**

there are eight phenyl and benzo protons in the aromatic region from 7.28 ppm to 8.16 ppm in **6a**, and from 7.48 ppm to 8.12 ppm in **6b**. Another feature was the disappearance of the two characteristic ethyl group proton signals (OCH_2CH_3) which were observed in ethoxymethylene (compound **5**). ^{13}C NMR spectrum of compound **6a** demonstrated the presence of four aliphatic carbon atoms in the region δ 14.1-42.3 ppm, due to the butyl group, and nineteen sp^2 hybridized carbon atoms in the region δ 100-162 ppm belonging to the aromatic carbon atoms and the azomethine carbon atom (HC=N). The highly deshielded aliphatic carbon atom of the trifluoromethoxy group was observed at a high chemical shift in the aromatic region.

A series of azo dyes (**7a-e**) were synthesized by coupling the diazonium salt of aniline derivatives with pyranoquinolinone **1.** Unfortunately, the reaction using diluted sodium carbonate solution failed. The reaction succeeded only when pyridine was used as a solvent and a basic catalyst, as described in Scheme 3. Structure elucidation of compounds **7a-e** was carried out by IR, ^1H NMR, ^{13}C NMR, and mass spectrometry. Elemental analyses were also performed.

The IR spectra of compounds **7a-e** showed a new stretching absorption band from 1560 cm^{-1} to 1570 cm^{-1} which was assigned to the presence of the (N=N) azo group [32]. In addition, carbonyl absorption bands were observed at 1735 cm^{-1} to 1758 cm^{-1} due to the α-Pyrano carbonyl group and from 1666 cm^{-1} to 1676 cm^{-1} as a stretching vibration absorption signal attributed to the quinolinone carbonyl group. ^1H NMR spectra of azo dyes **7a-e** were marked by the disappearance of the aromatic proton at position 3 of *n*-butylpyranoquinolinone (**1**) and confirmed the appearance of new protons in the aromatic region, corresponding to the phenyl ring of aniline derivatives. ^{13}C NMR spectra of the **7a-e** series demonstrated four signals in the aliphatic region attributed to four carbons of the n-butyl group and aromatic carbon signals, which are compatible with the number of carbon atoms in the molecular formula of the azo dyes **7a-e**. The molecular weight of some of these series was confirmed using mass spectrophotometry. For example, the mass spectrum of **7a** revealed the molecular ion peak M^+ is the base peak at m/z = 473 (100%) and M^++1 m/z = 474 (25%), where the peak at m/z = 285 (80%), was attributed to *n*-butylpyranoquinolin-3-one.

Anticancer activity

The *in vitro* antitumor activity of compounds **4b**, **6a,b**, and **7a,b** was evaluated in three human cancer cell lines, HepG-2, HCT-116 and MCF-7. 5-fluorouracil was used as a reference control drug. The cells were treated with the selected synthesized compounds at variable concentrations and cell viability was calculated. The results showed that increasing the dose of the tested compounds decreased cell viability in all three cancer cell lines. The relationship between cell survival and compound concentration was plotted to obtain the survival curve for each cancer cell line after 24 hours (Figures 2-4). The viability of HepG-2 liver cancer cells treated with compounds **4b**, **6a,b**, and **7a,b** for 24 hours was tested using a colorimetric assay (Figure 2). The results showed that compounds **6a**, **6b, 7a** and **7b** were more potent than the standard drug at 0-500 µg/mL. While Compound **4b** was more active than the standard drug at 125 and 500 µg/mL.

Figure 3 shows the viability of HCT-116 colon cancer cells after treatment with the test compounds for 24 h. All the tested compounds showed activity comparable to reference drug 5-fluorouracil. The three compounds **6a**, **6b**, and **7b** were shown to be the most active, since they were more cytotoxic than the reference drug at 0- 500 µg/mL. Compounds **4b** and **7a** were more potent than 5-fluorouracil at 500 µg/mL.

MCF-7 breast cancer cells were also assessed for viability after 24-h treatment with compounds **4b**, **6a**, **6b**, **7a**, and **7b** (Figure 4). Most of the examined compounds

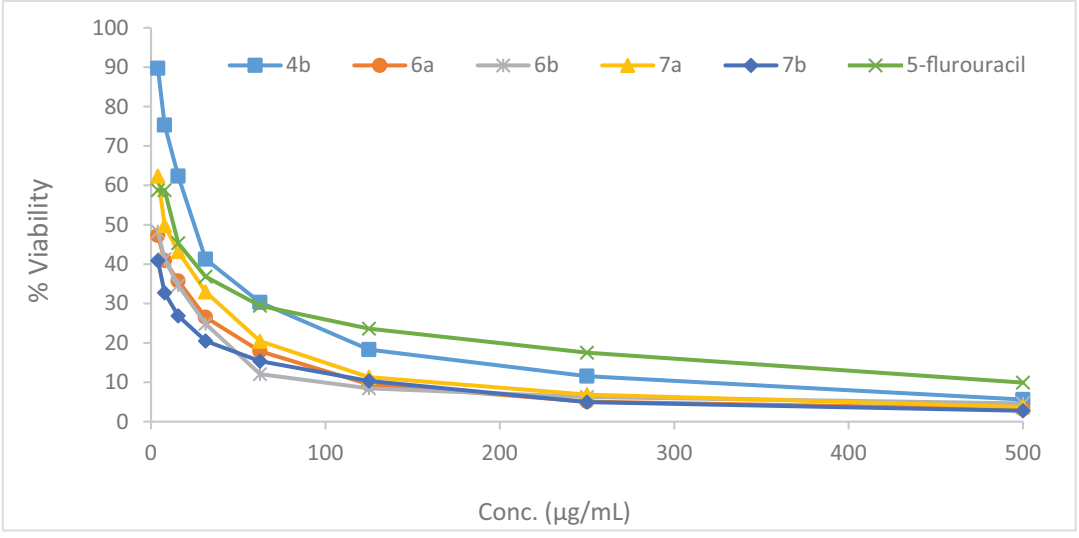


Figure 2 Effect of 0-500 µg/mL of compounds **4b, 6a, 6b, 7a,** and **7b** on HepG-2 cell viability. 5-fluorouracil used as a standard drug

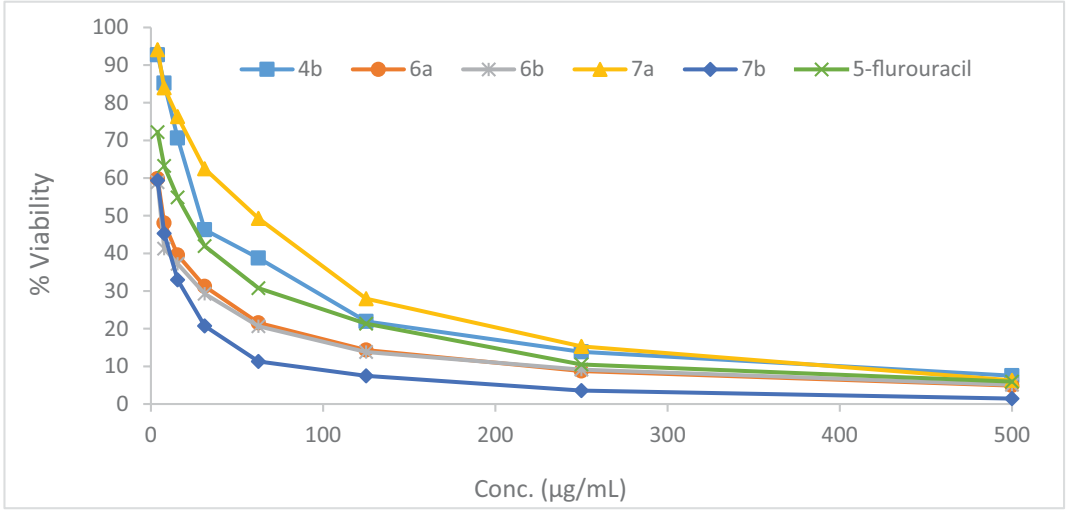


Figure 3 Effect of 0-500 µg/mL compounds **4b, 6a, 6b, 7a,** and **7b** on HCT-116 cell viability. 5-fluorouracil used as the reference drug

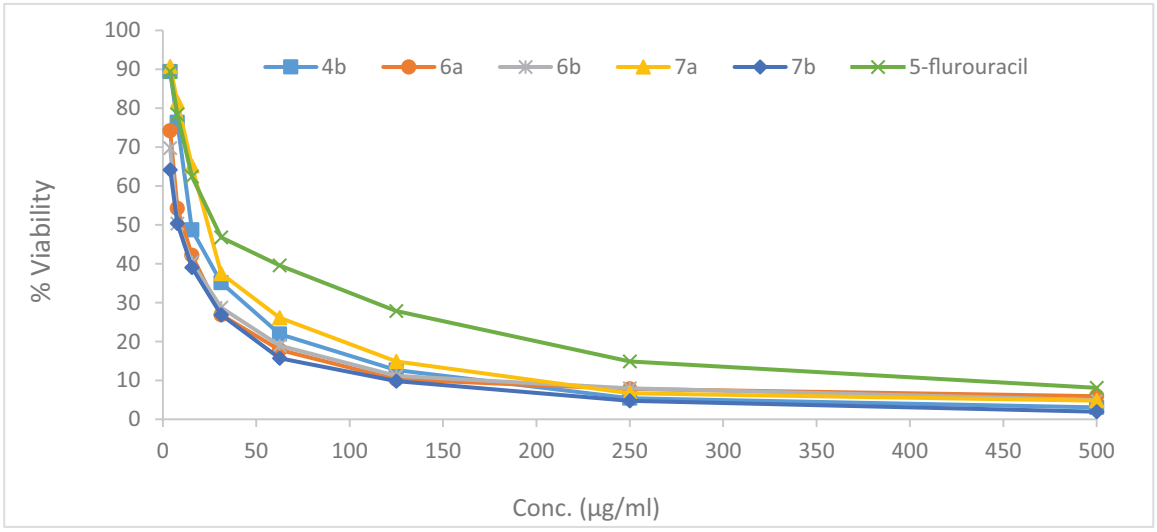


Figure 4 Effect of 0-500 µg/mL compounds **4b, 6a, 6b, 7a**, and **7b** on MCF-7 cell viability. 5-fluorouracil used as the reference drug

displayed notable inhibitory activity for MCF-7 cells. All tested compounds **4b, 6a, 6b, 7a** and **7b** were more active than the standard control (5-fluorouracil) at 0- 500 µg/mL.

The anti-proliferative effects of compounds **4b, 6a,b**, and **7a,b** were assessed using their IC_{50} values compared to the IC_{50} values of a control drug. The results are described in Table 1 and Figure 5. All the tested compounds showed significant cytotoxicity against all cancer cell lines, with IC_{50} values from 1.82-60.9 µg/mL (Table 1, Figure 5). Among the tested compounds, compound **7b** displayed the most potent inhibitory activity on all cancer cell lines (HepG-2, HCT-116, and MCF-7) with IC_{50} values of 1.82, 6.49, and 8.06 µg/mL, respectively. From the initial structure–activity relationships, it was found that the introduction of a (-OCF_3) group to the para position of the phenyl ring somewhat enhances the cytotoxic activity, while the fluorine substitution at the meta position on the phenyl ring (as in **7a**) decreases activity compared to **7b**. Additionally, compound **7b**, which has more fluorine content, was more active than **7a**. Other aspects that may enhance activity are the effect of the alkyl chain length and the presence of an *N*-butyl group. A similar result was obtained in preceding studies that showed that the inhibitory activities against tumor cells increased with longer *N*-alkyl chain length [33-35]. This may be due to improved lipophilicity which thus enhances cell membrane penetration of the evaluated compounds [36].

The cytotoxic activity of the compounds was evaluated against HepG-2 cells for compounds **4b**, **6a**, **6b**, **7a**, and **7b**. Based on the experimental results, the cytotoxicity of the compounds **6a**, **6b**, and **7b** were high, with IC_{50} values of 3.07, 5.29, and 1.82 µg/mL, respectively,

Table 1 Cytotoxicity (IC_{50}) of selected compounds against the three cancer cell lines

Compound	HePG-2 (human liver cancer)	HCT-116 (human colon cancer)	MCF-7 (breast cancer)
4b	11.6	28.6	15.2
6a	3.07	7.25	11.26
6b	5.29	9.92	13.76
7a	7.74	60.9	24.2
7b	1.82	6.49	8.06
5-fluorouracil	6.44	21.5	28

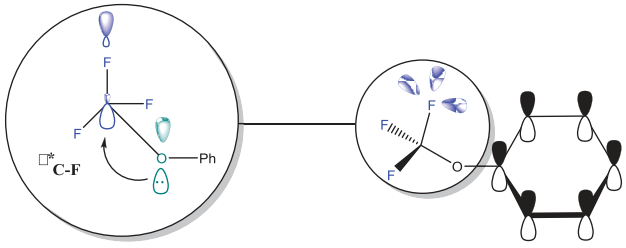


Figure 5 The $n_0 \rightarrow \sigma^*_{C-F}$ hyperconjugative interaction and the preferred conformation of trifluoromethoxyarene, where the O–CF_3 bond lies orthogonally to the aromatic plane

compared with 5-fluorouracil (IC_{50}: 6.44 µg/mL). Compounds **4b** and **7a** showed moderate cytotoxic activity (IC_{50}: 7.74 and 11.6 µg/mL, respectively). The cytotoxicity of compounds **6a**, **6b**, and **7b**, which contained a *p*-OCF_3 group (**6a** and **7b**) or a *p*-CF_3 group (**6b**) at the phenyl ring was higher than **4b** and **7a**, which contained one fluorine atom. These results match with earlier findings that -CF_3 and -OCF_3 groups significantly affect cytotoxicity. Generally, the action of the trifluoromethyl group is caused

by the high electronegativity of fluorine (4.0 on Pauling's electronegativity scale). Combined with the comparatively similar size of fluorine atoms to hydrogen atoms (the van deer waals radii of F and H atoms are 1.47 Å and 1.20 Å, respectively), this results in increasing oxidative, hydrolytic, and thermal stability. In addition, the trifluoromethyl group has a similar electronegativity to oxygen, large hydrophobic parameters and high lipophilicity: $F < CF_3 < OCF_3 < SCF_3$. This enhances absorption rate, improves the transport rate and helps the compound penetrate through lipid membranes more easily than the corresponding non-fluorinated molecules [37].

Among the fluorine-bearing functional groups, the trifluoromethoxy (OCF_3) group is becoming increasingly significant. There is growing attention on the OCF_3-containing compounds, since the OCF_3 group is one of the most lipophilic substituents, as specified by its Hansch–Leo parameter [πx (SCF_3) = +1.44, πx (SF_5) = +1.23, πx (OCF_3) = +1.04, πx (CF_3) = +0.88, πx (OCH_3) = −0.02] [38]. Furthermore, because of the strong electron-withdrawing effect of fluorine, the lone-pair of electrons on the oxygen are drawn towards the trifluoromethyl group and weakly interact with the aromatic π-electron system. This nO → σ* C–F hyperconjugative interaction and the steric bulk of the CF_3 group causes the O– CF_3 bond to lie orthogonally to the aromatic ring [39] (Figure 5). This orientation makes the OCF_3 an electron-withdrawing group (χ = 3.7) [40] and a strong para-directing substituent. Certainly, the repulsion between the lone-pair electrons on fluorine atoms and the aromatic π electrons increases the electron density at the para position [41]. The better conformational flexibility of the OCF_3 group compared to a methoxy group can improve the binding affinity of biologically active aryl trifluoromethyl ethers [42]. With these promising properties, many pharmaceuticals which have the OCF_3 group show enhanced usefulness and decreased side effects.

The *in vitro* growth inhibitory activity of the compounds **4b, 6a, 6b, 7a** and **7b** was examined in HCT-116 cells and compared to the standard anticancer drug, 5-flurouracil. The results showed that all the tested compounds exhibited concentration-dependent inhibitory activity in HCT-116 cells (Table 1, Figure 5). Remarkably, compound **7b** was most active against HCT-116 cells with more activity than the reference drug (IC_{50}, 6.49 µg/mL compared to 5-flurouracil IC_{50}, 21.5 µg/mL). Compounds **6a** and **6b** showed excellent antitumor activity against HCT-116 cells, with better activity than 5-flurouracil (IC_{50}, 7.25 µg/mL and 9.92 µg/mL, respectively). Compound **4b** and **7a** showed a lower ability to inhibit HCT-116 cells (IC_{50}, 28.6 µg/mL and 60.9 µg/mL, respectively). The order of activity against HCT-116 cell line was **7b>6a>6b**. Moreover, compounds **4b** and **7a** were less active against HCT-116 cells.

Compounds **4b**, **6a**, **6b**, **7a**, and **7b** were examined for their *in vitro* antiproliferative activity against MCF7 cells. 5-flurouracil was used as a reference drug. All five compounds exhibited higher antiproliferative activity than 5-flurouracil [IC_{50}, 8.06-24.2 µg/mL (Table 1, Figure 6)]. Compound **7b** was the most potent compound in the series (IC_{50}, 8.06 µg/mL against MCF7). In comparison to the fluoro-substituted derivative **7a** (IC_{50}, 24.2 µg/mL), compound **7b** displayed threefold greater antiproliferative potency against MCF7 cells, indicating that the trifluoromethoxy (-OCF_3) group substitution has better antiproliferative

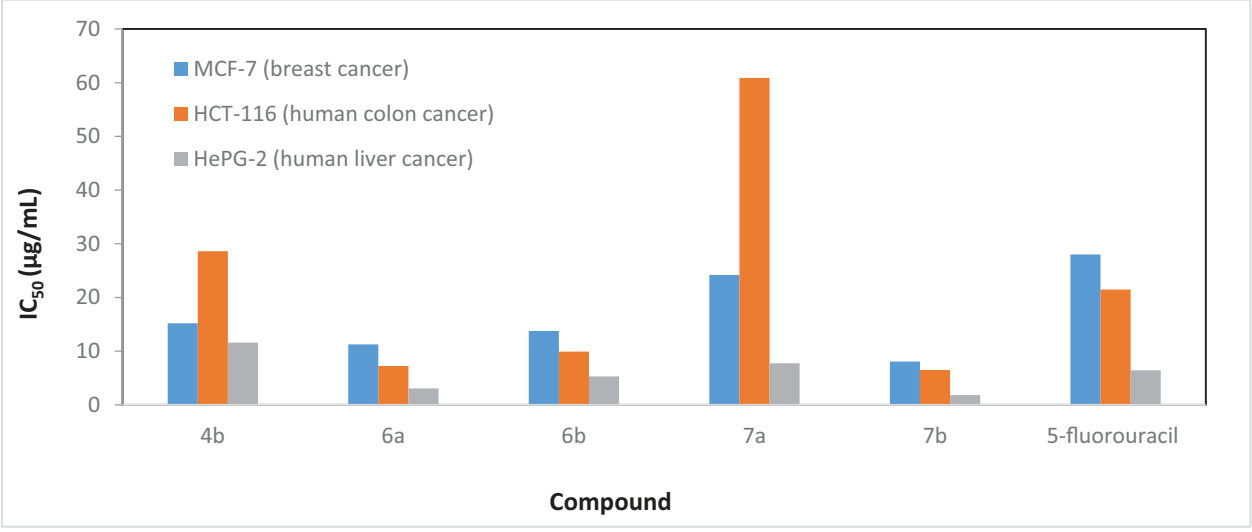


Figure 6 IC_{50} values of compounds **4b, 6a, 6b, 7a, 7b** and 5-fluorouracil in HepG-2, HCT-116 and MCF-7 cells

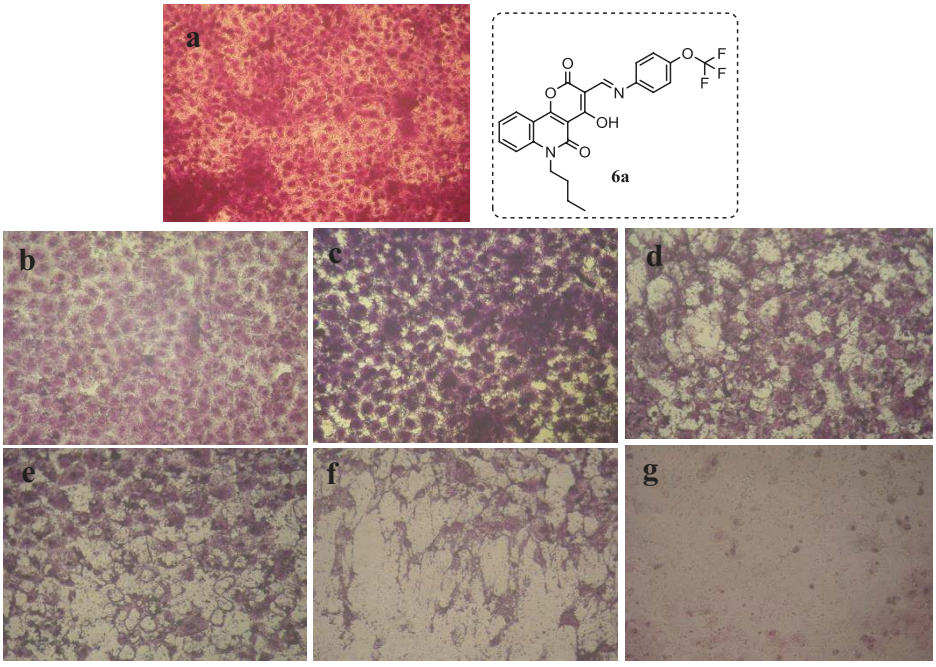


Figure 7 Morphological alterations in HePG-2 cells incubated with compound **6a** for 24 h. (a) negative control; (b) 15.6 µg; (c) 31.25 µg; (d) 62.5 µg; (e) 125 µg; (f) 250 µg ; and (g) 500 µg. The images were taken at 100x magnification using an inverted microscope (CKX41; Olympus, Japan)

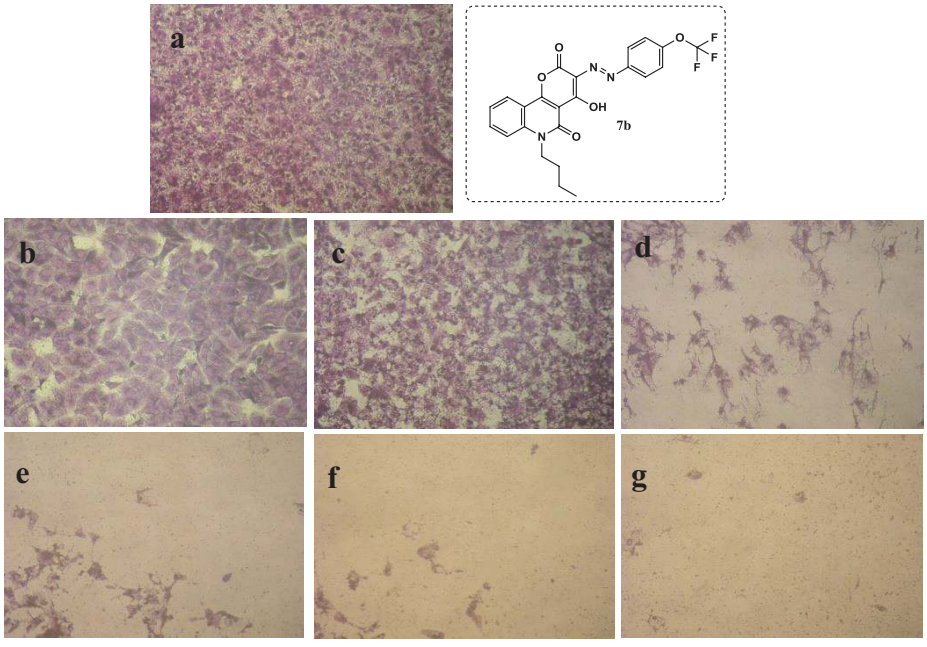


Figure 8 Morphological alterations in MCF-7 cells incubated with compound **7b** for 24 h. (a) negative control; (b) 7.8 µg; (c) 15.6 µg; (d) 31.25 µg; (e) 62.5 µg; (f) 250 µg; and (g) 500 µg. The images were taken at 100x magnification using an inverted microscope (CKX41; Olympus, Japan)

activity than the (-F) group. A similar phenomenon was observed in HePG-2 cells. Generally, these results indicate that the antiproliferative activity for compounds **6a**, **6b**, and **7b** were the best among all those tested. The presence of the trifluoromethoxy (-OCF_3) group or the trifluoromethyl (-CF_3) group in the aryl ring favors antiproliferative activity against all the three cancer cells. In addition, azo compound **7b** exhibited higher anticancer activity than

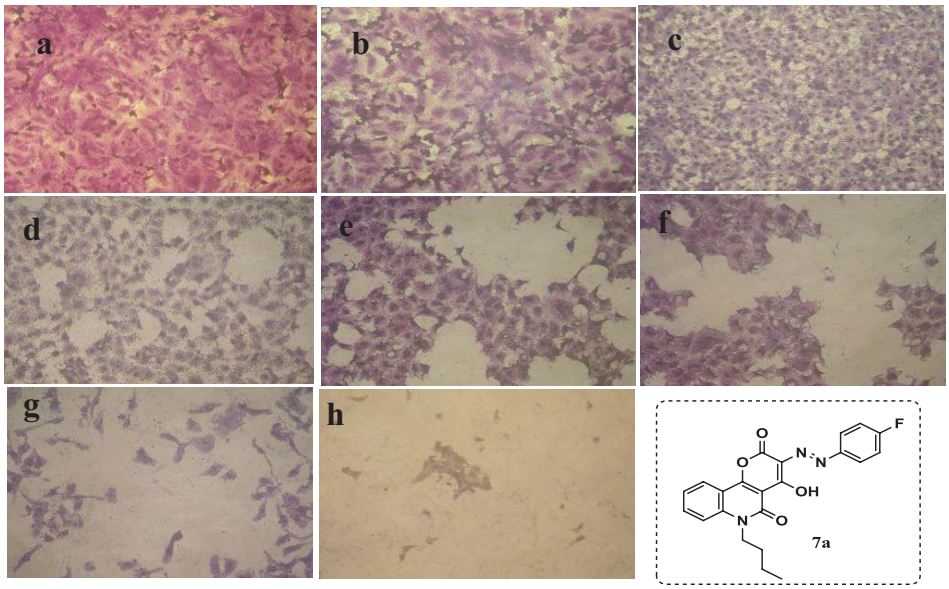


Figure 9 Morphological alterations in HCT-116 cells incubated with compound **7a** for 24 h. (a) negative control; (b) 7.8 µg; (c) 15.6 µg; (d) 31.25 µg; (e) 62.5 µg; (f) 125 µg; (g) 250 µg; and (h) 500 µg. The changes were microscopically observed at 100x magnification using an inverted microscope (CKX41; Olympus, Japan)

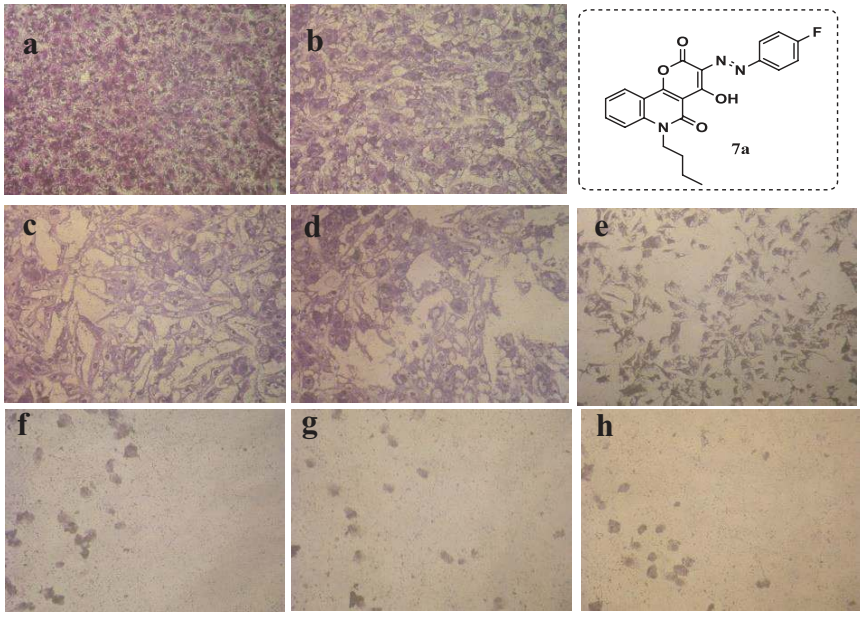


Figure 10 Morphological alterations in MCF-7 cells incubated with compound **7a** for 24 h. (a) negative control; (b) 7.8 µg; (c) 15.6 µg; (d) 31.25 µg; (e) 62.5 µg; (f) 125 µg; (g) 250 µg; and (h) 500 µg. The changes were microscopically observed at 100x magnification using an inverted microscope (CKX41; Olympus, Japan)

Schiff base **6a**, possibly because azo molecules are involved in the inhibition of DNA, RNA, and protein synthesis, as well as hindering carcinogenesis [43]. The presence of (N=N) in the azo molecular structure is accountable for the interaction with the active site of the target protein [44].

After incubating HepG-2, MCF-7 and HCT-116 tumor cells with the selected compounds for 24 h, morphological changes were assessed (Figures 7-10). There were alterations in cell surface morphology between the control (untreated cell) and the drug-treated cells.

Conclusion

In summary, a new series of novel Schiff bases and azo dye derivatives containing a pyranoquinolinone

moiety were designed, synthesized and biologically evaluated. The most potent, compound **7b**, exhibited remarkable inhibitory activity against HepG-2, MCF-7 and HCT-116 tumor cell lines. Therefore, this compound merits further investigation as a drug candidate for cancer therapy. The structure-activity relationship suggests that trifluoromethyl and trifluoromethoxy groups at appropriate positions of an organic molecule dramatically alter their properties in terms of lipophilicity, lipid solubility, oxidative thermal stability, permeability and oral bioavailability, resulting in improved transport. Future research using this compound may detect a lead molecule which can be established for clinical trials.

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