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Amandeep Singh · Vishu Mehra · Neda Sadeghiani · Saghar Mozaffari · Keykavous Parang · Vipan Kumar*

Abstract Huisgen's azide-alkyne cycloaddition reaction was employed to synthesize a series of 1*H*-1,2,3-triazole-tethered uracil-ferrocenyl chalcone conjugates with the aim of evaluating their *in vitro* anti-proliferative efficacy on human leukemia (CCRF-CEM) and human breast adenocarcinoma (MDA-MB-468) cell lines. Cytotoxic evaluation studies identified a number of synthesized conjugates that inhibited the proliferation of leukemia cancer cells by ~70% after 72 h. The selected synthesized conjugates were found to be significantly less cytotoxic against normal kidney cell line (LLC-PK1) when compared with CCRF-CEM cancer cells.

Key Words Click Chemistry**.** Cytotoxic evaluation **.** Ferrocenylchalcone **.** Uracil

Introduction

Cancer, an uncontrolled growth and rapid proliferation of abnormal cells, is one of the most formidable challenges in the world (Fadeyi *et al*., 2008). Most cancers are recognized by the uninhibited growth of cells without demarcation due to the deregulation of crucial enzymes and proteins controlling cell division and proliferation (Mareel and Leroy, 2003; Wesche, Haglund and Haugsten, 2011). According to the World Health Organization (WHO) report, 8.2 million people died of cancer all over the world in 2016 (https://www.cancer.org/research/cancer-facts-statistics.html). A recent survey by American Cancer Society of epidemiologists revealed that out of 595,690 cancer deaths in the US, 188,800 was because of cigarette smoking. In addition, 20% of all cancers are associated with body fatness, physical inactivity, excess alcohol consumption, and/or poor nutrition while certain cancers are related to infections caused by human papillomavirus (HPV), hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), and *Helicobacter pylori*. Although much progress has aspired from the diagnosis to the treatment of cancer, the factors like poor patient compliance, drug resistance, and drug-induced toxicities have provided a strong impetus for the discovery and development of novel cancer chemotherapeutic agents of clinical significance (Grant 2009; Solyanik 2011; Vijayaraghavalu *et al*., 2012).

The recognition of ferrocene and its derivatives for biological explorations may be ascribed to their stability in aqueous and aerobic media, aptness of derivatization, and appropriate electrochemical properties (Allardyce *et al*., 2005; Fouda *et al*., 2007; Ornelas *et al*., 2011). The anticancer activities of ferrocene derivatives were first reported by Fiorina and co-workers against lymphocytic leukemia P-388 (Fiorina, Dubois and Brynes, 1978). Ferrocene derivative of the anti-estrogen tamoxifen has also displayed interesting antiproliferative properties, which have been attributed to its redox behavior (Hillard *et al*., 2006; Hillard *et al*., 2006; Duivenvoorden *et al*., 2005).

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The anticancer activities of 2-ferrocenyl-1,1-diphenylbut-1-ene against HL-60, HCT-8, SF-295, MDA-MB-435, OVCAR-8 and GBM non-cancerous cells were reported by de Oliveira and co-workers, (Oliveira *et al*., 2014) suggesting the potential of conjugating ferrocene with other anticancer agents. A series of ferrocenyl-catechol conjugates were synthesized by Tan and co-workers and evaluated for their cytotoxicity against MDA-MB-231 cancer cell lines. The conjugates exhibited better anticancer activities (IC_{50} = 0.48-1.21 μM) compared to their corresponding phenolic analogs (0.57-12.70 μM) (Tan *et al*., 2012). One of the privileged structures in drug discovery is uracil that has diverse pharmacological profiles, synthetic accessibility, and the ability to bestow drug-like properties to the compound libraries appended to them (Newkome 1982). 5-Fluorouracil (5-FU), an anti-metabolism drug, is widely used in the treatment of malignancies, such as colorectal, stomach and breast cancer. However, its poor selectivity along with high incidences of bone marrow, gastrointestinal tract, and central nervous toxicity has limited its therapeutic importance (Pan *et al*., 2011). To address these problems, modifications of 5-FU have been accomplished resulting in the development of 5-FU derivatives *viz.* floxuridine and tegafur exhibiting improved pharmacological and pharmacokinetic properties (Ohwada *et al*., 2009). 5-FU-Camptothecin (CPT) conjugate, a topoisomerase cytotoxic alkaloid has exhibited comparable cytotoxicity to irinotecan (semi-synthetic analog of CPT) with enhanced selectivity, efficacy and safety (Li *et al*., 2012).

The prevalence of chalcones as a major constituent or a substituent in varied biologically active compounds represent another important structural motif, synthetically manoeuvered for the development of efficacious drugs against tropical diseases and cancer (Singh, Anand and Kumar, 2014). Chalcones have congregated considerable attention for their cytotoxic activities because of their similar mode of action to the structurally related combretastatin (Pettit *et al*., 2005). Chalcone is considered a promising template for the development of HIF-1 inhibitors, a major mechanism for the survival and evasion of tumor cells (Srinivasan, Johnson and Xing, 2011).

The recent revelation from our laboratory has shown the synthesis and cytotoxic potential of 1*H*-1,2,3-triazole-tethered *β*-lactam-chalcone conjugates with activity mainly dependent upon the nature of substituents present at aryl rings of chalcone as well as at *N*-1 position of the *β*-lactam ring (Singh *et al*., 2012). In continuation of our efforts for the synthesis of biologically relevant heterocycles using molecular hybridization, (Singh *et al*., 2017; Raj *et al*., 2015; Kumar *et al*., 2012; Kumar *et al*., 2013., Singh *et al.* 2014) the present work describes the synthesis of uracil-ferrocenyl chalcone conjugates, along with an evaluation of their *in vitro* antiproliferative efficacy on human leukemia (CCRF-CEM,) and human breast adenocarcinoma (MDA-MB-468) cell lines.

Experimental Section

Melting points were determined by an open capillary using a Veego Precision Digital Melting Point apparatus (MP-D) and are uncorrected. ^{1}H NMR spectra were recorded in DMSO with a BRUKER AVANCE II (500 MHz) spectrometer using TMS as an internal standard. Chemical shift values are expressed as parts per million downfield from TMS and J values are in hertz. Splitting patterns are indicated as s: singlet, d: doublet, t: triplet, m: multiplet, dd: double doublet, ddd: doublet of a doublet of a doublet, and br: broad peak. ^{13}C NMR spectra were recorded in DMSO with a BRUKER AVANCE II (125 MHz) using TMS as internal standard. Mass spectra were recorded on a BRUCKER high-resolution mass spectrometer (micrOTOF-QII). Elemental analyses were performedon a Heraus CHN-O-Rapid Elemental Analyzer. Column chromatography was performed on silica gel (60–120 mesh) using ethyl chloroform:hexane mixture as eluent.

General procedure for the synthesis of uracil-ferrocenyl chalcone conjugates **(*5a-r*):** To a stirred solution of *O*-propargylated ferrocenylchalcone 4 (1 mmol) and *N*-alkylated-azido-uracil 3 (1 mmol) in an ethanol:water mixture was added copper sulfate (0.05 mmol) and sodium ascorbate (0.13 mmol). The reaction mixture was allowed to stir at room temperature for 10−12 h, and the progress was monitored using TLC. After the completion of reaction, water (20 mL) was added, and the reaction mixture was extracted twice with dichloromethane (2 × 30 mL). The combined organic layers were dried over

anhydrous sodium sulfate and concentrated under reduced pressure to yield a crude product, which was purified *via* column chromatography using a 5:95 (methanol:chloroform, v/v) mixture.

1-(2-{4-[4-(3-ferrocenyl-acryloyl)-phenoxymethyl]-[1,2,3]triazol-1-yl}-ethyl)-1H-pyrimidine-2,4-dione **(5a)**: Yield 85%; Dark red solid, m.p: 210-211^{o}C; ^{1}H NMR (DMSO-d_6, 500 MHZ): 4.10 (s, 5H, H^1), 4.16 (s, 2H, H^9), 4.42 (s, 2H, H^3), 4.58 (s, 2H, H^2), 4.65 (s, 2H, H^8), 5.20 (s, 2H, H^6), 5.30 (d, *J*=7.5Hz, 1H, H^{11}), 6.79 (d, *J*=7.5Hz, 1H, H^{10}), 7.01-7.12 (m, 2H, Ar-H), 7.14 (d, *J*=15.1Hz, 1H, H^4), 7.61 (s, 1H, H^7), 7.71 (d, *J*=15.1Hz, 1H, H^5), 7.99-8.02 (m, 2H, Ar-H), 7.92 (s, 1H, H^7), 11.19 (NH-exchangeable with D_2O); ^{13}C NMR (DMSO, d^6, 125 MHz): 48.2, 48.5, 61.7, 69.0, 69.7, 71.4, 84.8, 102.1, 114.6, 114.7, 118.8, 125.0, 130.5, 143.2, 144.6, 145.7, 150.3, 159.8, 163.9, 187.7. HRMS calcd for $C_{28}H_{25}FeN_5O_4$ $[M]^+$ 551.1256, Found: 551.1270 Anal Calcd(%) for: C, 60.99; H, 4.57; N, 12.70: C, 60.82; H, 4.62; N, 12.66.

1-(3-{4-[4-(3-ferrocenyl-acryloyl)-phenoxymethyl]-[1,2,3]triazol-1-yl}-propyl)-1H-pyrimidine-2,4-dione **(5b)**: Yield 83%. Dark red solid, m.p: 195-196 ^{o}C; ^{1}H NMR (DMSO-d_6, 500 MHz): 2.10-2.19 (m, 2H, H^{11}), 3.73 (t, *J*=6.7Hz, 2H, H^{10}), 4.19 (s, 5H, H^1), 4.43 (t, *J*=6.7Hz, 2H, H^8), 4.53 (s, 2H, H^2), 4.85 (s, 2H, H^3), 5.27 (s, 2H, H^6), 5.55 (d, *J*=7.0Hz, 1H, H^{12}), 7.19 (d, *J*=7.5, 2H, Ar-H), 7.45 (d, *J*=15.3Hz, 1H, H^5), 7.61-7.65 (m, 2H, H^4+H^{11}), 8.08 (d, *J*=7.3Hz, 2H, Ar-H), 8.29 (s, 1H, H^7), 11.23 (NH-exchangeable with D_2O); ^{13}C NMR (DMSO-d^6, 125 MHz): 29.5, 45.5, 47.4, 61.8, 69.6, 69.9, 71.5, 79.7, 101.6, 115.1, 119.3, 125.2, 131.0, 131.4, 142.7, 145.7, 145.9, 151.4, 162.1, 164.2, 187.0 $C_{29}H_{27}FeN_5O_4$ $[M]^+$ 565.1412 Found: 565.1489 Anal Calcd(%) for: C, 61.60; H, 4.81; N, 12.39.Found: C, 61.82; H, 4.72; N, 12.50.

1-(4-{4-[4-(3-ferrocenyl-acryloyl)-phenoxymethyl]-[1,2,3]triazol-1-yl}-butyl)-1H-pyrimidine-2,4-dione **(5c):** Yield 79%. Dark red solid, m.p: 187-188 ^{o}C; ^{1}H NMR (DMSO-d_6,500 MHz): 1.57(t, *J*=6.7Hz, 2H, H^{10}), 1.80 (t, *J*=7.2Hz, 2H, H^9), 3.75 (t, *J*=6.4Hz, 2H, H^{11}), 4.13(s, 5H, H^1), 3.38 (t, *J*=6.1Hz, 2H, H^8),4.51 (s, 2H, H^2), 4.82 (s, 2H, H^3), 5.20 (s, 2H, H^6),5.57 (d, *J*=7.3Hz, 1H, H^{13}), 7.13 (d, *J*=8.1Hz, 2H, ArH), 7.40 (d, *J*=15.1Hz,1H, H^5), 7.60 (d, *J*=15.1Hz, 1H, H^4), 7.65 (d, *J*=7.3Hz, 1H, H^{12}), 8.01 (d, *J*=8.5Hz, 2H, Ar-H), 8.20 (s,1H,H^7), 11.73 (NH-exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 25.7, 27.2, 47.6, 49.5, 61.5, 69.5, 69.8, 71.3, 79.6, 101.3, 115.4, 119.4, 125.0, 131.1,131.2, 142.8, 145.7, 150.5, 160.5, 162.4, 187.1. $C_{30}H_{29}FeN_5O_4$ $[M]^+$579.1569. Found: 579.1578. Anal Calcd (%) for: C, 62.19; H, 5.04; N, 12.09.Found: C, 62.29; H, 5.14; N, 12.15.

1-(5-{4-[4-(3-ferrocenyl-acryloyl)-phenoxymethyl]-[1,2,3]triazol-1-yl}-pentyl)-1H-pyrimidine-2,4-dione **(5d)**: Yield 84%. Dark red solid, m.p: 179-180^{o}C; ^{1}H NMR (DMSO-d_6, 500 MHz): 1.20-1.22 (m,2H,H^{10}), 1.67 (t, *J*=6.7Hz, 2H, H^{11}), 1.83 (t, *J*=7.5Hz, 2H, H^9), 3.63 (t, *J*=7.0Hz, 2H, H^{12}), 4.13 (s, 5H, H^1), 4.37 (t,*J*=7.3Hz, 2H, H^8), 4.52 (s, 2H, H^2), 4.83 (s, 2H, H^3), 5.21 (s, 2H, H^6), 5.54 (d, *J*=7.8Hz, 1H, H^{14}), 7.13 (d,*J*=8.2Hz, 2H, Ar-H), 7.41 (d, *J*=15.5Hz, 1H, H^5), 7.65 (d, *J*=7.8Hz, 1H, H^{13}), 7.66 (d, *J*=15.5Hz, 1H, H^4), 8.08 (d, *J*=8.2Hz, 2H, Ar-H), 8.27 (s, 1H, H^7), 11.73 (NH-exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz); 23.3, 28.4, 29.3, 48.3, 49.4, 61.6, 69.7, 69.8, 71.4, 79.5, 101.1, 115.2, 119.2, 125.1, 131.2, 131.3, 142.7, 145.4, 150.4, 160.3, 162.3, 187.2.$C_{31}H_{31}FeN_5O_4$ $[M]^+$ 593.1725. Found: 593.1747. Anal Calcd (%) for: C, 62.74; H, 5.27; N, 11.80.Found: C, 62.81; H, 5.15; N, 11.72.

1-(6-{4-[4-(3-ferrocenyl-acryloyl)-phenoxymethyl]-[1,2,3]triazol-1-yl}-hexyl)-1H-pyrimidine-2,4-dione **(5e):** Yield 79%. Dark red solid, m.p: 170-171^{o}C; ^{1}H NMR (DMSO-d_6, 500 MHz): 1.26-1.27 (m, 4H, H^{10}+H^{11}), 1.52-1.54 (m, 2H, H^{12}), 1.81-1.83 (m, 2H, H^9), 3.47 (t, *J*=7.3Hz, 2H, H^{13}), 4.04 (s, 5H, H^1), 4.25 (t,*J*=1.5Hz, 2H, H^2), 4.34 (t, *J*=7.2Hz, 2H, H^5), 4.71 (t, *J*=1.7Hz, 2H, H^3), 5.24 (s, 2H, H^6), 5.35 (d, *J*=7.1Hz, 1H, H^{15}), 7.15 (d, *J*=7.3Hz, 2H, Ar–H), 7.43 (d, *J*=15.2Hz,1H, H^5), 7.62–7.63 (m, 2H, H^4+H^{14}), 8.06 (d, *J*=7.3Hz, 2H, Ar-H), 8.27 (s, 1H, H^7), 11.22 (NH-exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 25.1, 25.2, 28.5, 29.6, 48.2, 49.3, 61.6, 69.5, 69.6, 71.3, 79.3, 101.2, 115.3, 119.3, 125.2, 131.2, 131.3, 142.7, 145.7, 146.1, 150.3, 160.2, 162.6, 187.4. HRMS calcd for $C_{32}H_{33}FeN_5O_4$ $[M]^+$ 607.1882. Found: 607.1876. Anal Calcd (%) for: C,63.27; H, 5.48; N, 11.53.Found C,63.33; H,5.38; N,11.43

1-(8-{4-[4-(3-ferrocenyl-acryloyl)-phenoxymethyl]-[1,2,3]triazol-1-yl}-octyl)-1H-pyrimidine-2,4-dione **(5f):** Yield 80%. Dark red solid, m.p: 151-152^{o}C; ^{1}H NMR (DMSO-d_6, 500MHz): 1.21-1.25 (m, 8H, H^{10}+H^{11}+H^{12}+H^{13}), 1.52 (t, *J*=6.7Hz, 2H, H^{14}), 1.79 (t, *J*=6.8Hz, 2H, H^9), 3.60 (t, *J*=7.1Hz, 2H, H^{15}),

4.17 (s, 5H, H^1), 4.34 (t, *J*=6.8Hz, 2H, H^9), 4.51(s, 2H, H^2), 4.83 (s, 2H, H^3), 5.25 (s, 2H, H^6), 5.51 (d, *J*=7.7Hz, 1H, H^{17}), 7.16 (d, *J*=8.6Hz, 2H, Ar-H), 7.43 (d, *J*=15.2Hz, 1H, H^4), 7.59-7.62(m, 2H, H^5+H^{16}), 8.06 (d, *J*=8.5Hz, 2H, Ar-H) ,8.24 (s, 1H, H^7), 11.17 (NH-exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 26.1, 26.2, 28.6, 28.8, 28.9, 30.0, 47.8, 49.8, 61.8, 69.6, 69.9, 71.5, 79.7, 101.2, 115.1, 119.3, 125.0, 131.0, 131.4, 142.6, 145.7, 146.1, 151.3, 162.0, 164.1, 187.0. HRMS calcd for $C_{34}H_{37}FeN_5O_4$ $[M]^+$ 635.2195. Found: 635.2184. Anal Calcd(%) for: C, 64.26; H, 5.58; N, 11.02.Found: C, 64.31; H, 5.74; N, 11.10.

5-Chloro-1-(2-{4-[4-(3-ferrocenyl-acryloyl)-phenoxymethyl]-[1,2,3]triazol-1-yl}-ethyl)-1H-pyrimidine-2,4-dione **(5g):** Yield 83%; Dark red solid, m.p: 186-187^{o}C: ^{1}H NMR (DMSO-d_6, 500MHZ): 4.14 (s, 2H, H^9), 4.18 (s, 5H, H^1), 4.53 (s,2H, H^2), 4.68 (s, 2H, H^3), 4.84 (s, 2H, H^8), 5.25 (s, 2H, H^6), 7.15-7.17 (m, 2H, Ar-H), 7.44 (d, *J*=15.0Hz, 1H, H^5), 7.62 (d, *J*=15.0Hz, 1H, H^4), 7.78 (s, 1H, H^7), 8.06-8.08 (m, 2H, Ar-H), 8.27 (s, 1H, H^{10}), 11.85 (NH-exchangeable with D_2O); ^{13}C NMR (DMSO-d^6, 125 MHz): 48.1, 48.3, 61.7, 61.9, 69.6, 71.6, 79.7, 106.8, 115.1, 119.2, 125.7, 131.0, 131.4, 142.9, 145.6, 145.8, 150.4, 159.8, 162.0, 187.0. HRMS calcd for $C_{28}H_{24}ClFeN_5O_4$ $[M]^+$ 585.0866. Found: 585.0876 Anal Calcd(%) for: C, 57.41; H, 4.13; N, 11.95; Found: C, 57.35; H, 4.19; N, 11.84.

5-Chloro-1-(3-{4-[4-(3-ferrocenyl-acryloyl)-phenoxymethyl]-[1,2,3]triazol-1-yl}-propyl)-1H-pyrimidine-2,4-dione **(5h)**: Yield 80%. Dark red solid, m.p: 170-171 ^{o}C; ^{1}H NMR (DMSO-d_6, 500 MHz): 2.13-2.21 (m, 2H, H^{11}), 3.75 (t, *J*=6.3Hz, 2H, H^{10}), 4.20 (s, 5H, H^1), 4.45 (t, *J*=6.4Hz, 2H, H^8), 4.55 (s, 2H, H^2), 4.86 (s, 2H, H^3) ,5.29 (s, 2H, H^6), 7.17 (d, *J*=7.2Hz, 2H, Ar-H),7.42 (d, *J*=15.1Hz, 1H, H^5), 7.62-7.66 (m, 2H, H^5+H^{11}), 8.06 (d, *J*=7.3Hz, 2H, Ar-H), 8.27(s, 1H, H^7), 11.25 (NH-exchangeable with D_2O; ^{13}C NMR (DMSO-d_6, 125 MHz): 29.3, 45.3, 47.5, 61.7, 69.5, 69.6, 71.4, 79.5, 105.4, 115.3, 119.4, 125.4, 131.2, 131.5, 142.5, 145.5, 145.8, 151.6, 162.3, 164.5, 187.2. $C_{29}H_{26}ClFeN_5O_4$ $[M]^+$ 599.1023 Found: 599.1072 Anal Calcd (%) for: C, 58.07; H, 4.37; N, 11.68. Found: C, 58.14; H, 4.21; N, 11.54.

5-Chloro-1-(4-{4-[4-(3-ferrocenyl-acryloyl)-phenoxymethyl]-[1,2,3]triazol-1-yl}-butyl)-1H-pyrimidine-2,4-dione **(5i):** Yield 75%. Dark red, solid: m.p 161-162^{o}C; ^{1}H NMR (DMSO-d_6, 500 MHz): 1.54 (t, *J*=6.2Hz, 2H, H^{10}), 1.84 (t, *J*=7.2Hz, 2H, H^9), 3.72 (t, *J*=6.3Hz, 2H, H^{11}), 4.20 (s, 5H, H^1), 4.42 (t, *J*=6.4Hz, 2H, H^8), 4.58 (s, 2H, H^2), 4.81 (s, 2H, H^3), 5.22 (s, 2H, H^6),7.15 (d, *J*=8.3Hz, 2H, Ar-H) ,7.42 (d, *J*=15.4Hz, 1H, H^5), 7.65 (d, *J*=15.4Hz, 1H, H^4), 8.04 (d, *J*=8.3Hz, 2H, Ar-H), 8.24 (s, 1H, H^7), 8.25 (s, 1H, H^{12}) ,11.77 (NH-exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 25.8, 27.3, 47.5, 49.3, 61.7, 69.3, 69.7, 71.4, 79.5, 105.6, 115.3, 119.5, 125.3, 131.2, 131.3, 142.4, 145.5, 150.7, 160.3, 162.5, 187.2. $C_{30}H_{28}ClFeN_5O_4$ $[M]^+$613.1179.Found: 613.1166. Anal Calcd (%) for: C, 58.70; H, 4.60; N, 11.41.Found: C, 58.62; H, 4.49; N.11.34

5-Chloro-1-(5-{4-[4-(3-ferrocenyl-acryloyl)-phenoxymethyl]-[1,2,3]triazol-1-yl}-pentyl)-1*H*-pyrimidine-2,4-dione **(5j):** Yield 81%. Dark red solid, m.p: 152-153 ^{o}C; ^{1}H NMR (DMSO-d_6, 500 MHz): 1.23-1.28 (m, 2H, H^{10}), 1.64 (t, *J*=6.5Hz, 2H, H^{11}), 1.87 (t, *J*=7.1Hz, 2H, H^9), 3.69 (t, *J*=7.0Hz, 2H, H^{12}), 4.20 (s, 5H, H^1), 4.39 (t, *J*=7.2Hz, 2H, H^8), 4.58 (s, 2H, H^2), 4.89 (s, 2H, H^3), 5.23 (s, 2H, H^6), 7.16 (d, *J*=8.4Hz, 2H, Ar-H), 7.48 (d, *J*=15.0Hz, 1H, H^5), 7.65 (d, *J*=15.0Hz, 1H, H^4), 8.10 (d, *J*=8.4Hz, 2H, Ar-H) ,8.25 (s, 1H, H^7), 8.29 (s, 1H, H^{13}), 11.78 (NH-exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 23.1, 28.3, 29.5, 48.2, 49.3, 61.5, 69.4, 69.5, 71.3, 79.4, 105.2, 115.3, 119.4, 125.2, 131.1, 131.3, 142.5, 145.6, 150.6, 160.4, 162.3, 187.3.$C_{31}H_{30}ClFeN_5O_4$ $[M]^+$ 627.1336. Found: 627.1324. Anal Calcd (%) for: C, 59.30; H, 4.82; N, 11.15.Found: C, 59.21; H, 4.71; N, 11.21.

5-Chloro-1-(6-{4-[4-(3-ferrocenyl-acryloyl)-phenoxymethyl]-[1,2,3]triazol-1-yl}-hexyl)-1H-pyrimidine-2,4-dione **(5k):** Yield 82%. Dark red solid, m.p: 140-141^{o}C; ^{1}H NMR (DMSO-d_6, 500 MHz): 1.23-1.24 (m, 4H, H^{10}+H^{11}), 1.51-1.53 (m, 2H, H^{12}), 1.80-1.82 (m, 2H, H^9), 3.44 (t, *J*=7.3Hz, 2H, H^{13}), 4.02 (s, 5H, H^1), 4.25 (t, *J*=1.3Hz, 2H, H^2), 4.35 (t, *J*=7.3, 2H, H^5), 4.73 (t, *J*=1.5Hz, 2H, H^3), 5.22 (s, 2H, H^6), 7.13 (d, *J*=7.1Hz, 2H, Ar-H), 7.41 (d, *J*=15.2Hz, 1H, H^5), 7.62 (d, *J*=15.2Hz, 1H, H^4), 8.05 (d, *J*=7.1Hz, 2H, Ar-H), 8.25 (s, 1H, H^7), 8.29 (s, 1H, H^{14}), 11.20 (NH-exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 25.1, 25.3, 28.4, 29.3, 48.4, 49.5, 61.4, 69.3, 69.4, 71.5, 79.5, 106.2, 115.4, 119.5, 125.4, 131.4, 131.6, 142.2, 145.5, 146.3, 150.1, 160.4, 162.3, 187.2. HRMS calcd for $C_{32}H_{32}ClFeN_5O_4$ $[M]^+$ 641.1492. Found: 641.1478. Anal Calcd (%) for: C, 59.87; H, 5.02; N, 10.91.Found C,59.73; H, 5.14; N, 10.82.

5-Chloro-1-(8-{4-[4-(3-ferrocenyl-acryloyl)-phenoxymethyl]-[1,2,3]triazol-1-yl}-octyl)-1H-pyrimidine-2,4-dione **(5l) :** Yield 82%. Dark red solid, m.p: 130-131^{o}C; ^{1}H NMR (DMSO-d_6, 500 MHz): 1.17-1.19 (m, 8H, H^{10}+H^{11}+H^{12}+H^{13}), 1.55 (t, *J*=6.3Hz, 2H, H^{14}), 1.82 (t, *J*=6.5Hz, 2H, H^{9}), 3.62 (t, *J*=7.3Hz, 2H, H^{15}), 4.19 (s, 5H, H^{1}), 4.34 (t, *J*=6.5Hz, 2H, H^{9}), 4.55 (s, 2H, H^{2}), 4.82 (s, 2H, H^{3}), 5.28 (s, 2H, H^{6}), 7.19 (d, *J*=8.1Hz, 2H, Ar-H), 7.47 (d, *J*=15.2, 1H, H^{4}), 7.56 (s, 1H, H^{16}), 7.60 (d, *J*=15.2, 1H, H^{5}), 8.04 (d, *J*=8.1, 2H, Ar-H), 8.27 (s,1H,H^{7}), 11.15 (NH-exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 26.2, 26.3, 28.3, 28.5, 28.8, 30.2, 47.5, 49.6, 61.7, 69.3, 69.7, 71.3, 79.4, 106.4, 115.3, 119.1, 125.4, 131.2, 131.5, 142.4, 145.2, 146.3, 151.6, 162.3, 164.3, 187.4. HRMS calcd for $C_{34}H_{36}ClFeN_5O_4$ $[M]^+$ 669.1805. Found: 669.1834. Anal Calcd (%) for: C, 61.95; H, 5.42; N, 10.15.Found: C, 61.87; H, 5.32; N, 10.07.

5-Bromo-1-(2-{4-[4-(3-ferrocenyl-acryloyl)-phenoxymethyl]-[1,2,3]triazol-1-yl}-ethyl)-1H-pyrimidine-2,4-dione **(5m)** : Yield 80%, Dark red solid, m.p: 160-162^{o}C; ^{1}H NMR (DMSO-d_6, 500 MHZ): 4.18 (s, 5H, H^{1}), 4.24 (s, 2H, H^{9}), 4.50 (s, 2H, H^{2}), 4.69 (s, 2H, H^{3}), 4.75 (s, 2H, H^{8}), 5.26 (s, 2H, H^{6}), 7.10-7.12 (m, 2H, Ar-H), 7.25 (d, *J*=15.0Hz, 1H, H^{5}), 7.64 (d, *J* =15.0Hz, 1H, H^{4}), 7.72 (s, 1H, H^{7}), 8.00-8.03 (m, 2H, Ar-H), 8.15 (s,1H, H^{10}), 11.82 (NH-exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 48.0, 48.2, 61.7, 61.8, 69.8, 71.4, 79.3, 95.8, 114.7, 118.9, 125.0, 130.6, 131.5, 143.1, 144.5, 145.6, 150.5, 159.9, 161.8, 187.2; HRMS calcd for $C_{28}H_{24}BrFeN_5O_4$ $[M]^+$ 629.0361, found 629.0375; Anal Calcd (%) for: C, 53.36; H, 3.84; N, 11.11; Found: C, 53.41; H, 3.78; N, 11.22

5-Bromo-1-(3-{4-[4-(3-ferrocenyl-acryloyl)-phenoxymethyl]-[1,2,3]triazol-1-yl}-propyl)-1H-pyrimidine-2,4-dione **(5n)** : Yield 82%. Dark red solid, m.p: 150-151^{o}C; ^{1}H NMR (DMSO-d_6, 500 MHz): 2.15-2.17 (m, 2H, H^{11}), 3.71 (t, *J*=6.3Hz, 2H, H^{10}), 4.23 (s, 5H, H^{1}), 4.41 (t, *J*=6.3Hz, 2H, H^{8}), 4.51 (s, 2H, H^{2}), 4.81 (s, 2H, H^{3}) , 5.22 (s, 2H, H^{6}), 7.18 (d, *J*=7.6Hz, 2H, Ar-H), 7.44 (d, *J*=15.2Hz, 1H, H^{5}), 7.64-7.68 (m, 2H, H^{5}+H^{11}), 8.08 (d, *J*=7.4Hz, 2H, Ar-H), 8.29 (s, 1H, H^{7}), 11.27 (NH-exchangeable with D_2O; ^{13}C NMR (DMSO-d_6, 125MHz): 29.1, 45.2, 47.2, 61.5, 69.4, 69.7, 71.6, 79.2, 95.3, 115.2, 119.5, 125.3, 131.5, 131.7, 142.2, 145.8, 145.9, 151.3, 162.2, 164.5, 187.1; $C_{29}H_{26}BrFeN_5O_4$ $[M]^+$ 643.0518 Found: 643.0524 Anal Calcd(%) for: C, 54.06; H, 4.07; N, 10.87. Found: C, 54.19; H, 3.96; N, 10.74.

5-Bromo-1-(4-{4-[4-(3-ferrocenyl-acryloyl)-phenoxymethyl]-[1,2,3]triazol-1-yl}-butyl)-1H-pyrimidine-2,4-dione **(5o):** Yield 77%. Dark red solid, m.p: 138-139^{o}C; ^{1}H NMR (DMSO-d_6, 500 MHz): 1.59 (t, *J*=6.4Hz, 2H, H^{10}), 1.82 (t, *J*=7.0Hz, 2H, H^{9}), 3.70 (t, *J*=6.7Hz, 2H, H^{11}), 4.19 (s, 5H, H^{1}), 4.40 (t, *J*=6.7Hz, 2H, H^{8}), 4.53 (s, 2H, H^{2}), 4.85 (s, 2H, H^{3}), 5.26 (s, 2H, H^{6}), 7.18 (d, *J*=8.6Hz, 2H, Ar-H) ,7.45 (d, *J*=15.2Hz, 1H, H^{5}), 7.63 (d, *J*=15.2Hz, 1H, H^{4}), 8.08 (d, *J*=8.6, 2H, Ar-H), 8.22 (s, 1H, H^{7}), 8.27 (s, 1H, H^{12}), 11.73 (NH-exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 25.9, 27.1, 47.7, 49.4, 61.8, 69.6, 69.9, 71.5, 79.7, 95.1, 115.1, 119.3, 125.1, 131.0, 131.4, 142.6 , 145.7, 150.8, 160.1, 162.1, 187.0 .$C_{30}H_{27}BrFeN_5O_4$ [M]+ 656.0575. Found: 656.0571. Anal Calcd (%) for: C, 55.82; H, 4.14; N, 10.65. Found: C, 55.75; H, 4.27; N, 10.59.

5-Bromo-1-(5-{4-[4-(3-ferrocenyl-acryloyl)-phenoxymethyl]-[1,2,3]triazol-1-yl}-pentyl)-1H-pyrimidine-2,4-dione **(5p)**: Yield 80%. Dark red solid, m.p: 129-130^{o}C; ^{1}H NMR (DMSO-d_6, 500 MHz): 1.21-1.26 (m, 2H, H^{10}), 1.62 (t, *J*=6.9Hz, 2H, H^{11}), 1.85 (t, *J*=7.3Hz, 2H, H^{9}), 3.65 (t, *J*=7.0Hz, 2H, H^{12}), 4.19 (s, 5H, H^{1}), 4.38 (t, *J*=7.0Hz, 2H, H^{8}), 4.54 (s, 2H, H^{2}), 4.85 (s, 2H, H^{3}), 5.27 (s, 2H, H^{6}), 7.18 (d, *J*=8.7Hz, 2H, Ar-H), 7.45 (d, *J*=15.2Hz, 1H, H^{5}), 7.63 (d, *J*=15.2Hz,1H, H^{4}), 8.08 (d, *J*=8.7Hz, 2H, Ar-H), 8.21 (s, 1H, H^{7}) ,8.26 (s, 1H, H^{13}), 11.73 (NH-exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 23.0, 28.2, 29.7, 48.0, 49.6, 61.8, 69.6, 69.9, 71.5, 79.7, 95.4, 115.1, 119.3, 125.0, 131.0, 131.4, 142.6, 145.7, 150.8, 160.2, 162.1, 187.0. $C_{31}H_{30}BrFeN_5O_4$ $[M]^+$671.0831.Found: 671.0820. Anal Calcd (%) for: C, 55.38; H, 4.50; N, 10.42.Found: C, 55.41; H, 4.38; N, 10.37.

5-Bromo-1-(6-{4-[4-(3-ferrocenyl-acryloyl)-phenoxymethyl]-[1,2,3]triazol-1-yl}-hexyl)-1H-pyrimidine-2,4-dione **(5q):** Yield 85%. Dark red solid: m.p 114-115^{o}C; ^{1}H NMR (DMSO-d_6, 500 MHz): 1.25-1.26 (m, 4H, H^{10}+H^{11}), 1.54-1.56 (m, 2H, H^{12}), 1.83-1.84 (m, 2H, H^{9}), 3.41 (t, *J*=7.3Hz, 2H, H^{13}), 4.04 (s, 5H, H^{1}), 4.23 (t, *J*=1.5Hz, 2H, H^{2}), 4.32 (t, *J*=7.4Hz, 2H, H^{5}), 4.71 (t, *J*=1.5Hz, 2H, H^{3}), 5.25 (s, 2H, H^{6}), 7.16 (d, *J*=7.3Hz, 2H, Ar–H), 7.45 (d, *J*=15.3Hz, 1H, H^{5}), 7.66 (d, *J*=15.3Hz, 1H, H^{4}), 8.03 (d, *J*=7.1Hz, 2H, Ar-H), 8.23 (s, 1H, H^{7}), 8.27 (s,1H, H^{14}), 11.24 (NH-exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz): 25.3, 25.5, 28.3, 29.5, 48.2, 49.2, 61.3, 69.5, 69.6, 71.3, 79.2, 95.4, 115.2, 119.3,

125.5, 131.2, 131.4, 142.5, 145.7, 146.5, 150.3, 160.2, 162.1, 187.4. $C_{32}H_{32}BrFeN_5O_4$ $[M]^+$ 685.0987.Found: 685.0973. Anal Calcd (%) for: C,56.00; H, 4.70; N, 10.20.Found C,56.09; H, 4.62; N, 10.31

5-Bromo-1-(8-{4-[4-(3-ferrocenyl-acryloyl)-phenoxymethyl]-[1,2,3]triazol-1-yl}-octyl)-1H-pyrimidine-2,4-dione **(5r):** Yield 81%. Dark red solid, m.p: 105-106^{o}C; ^{1}H NMR (DMSO-d_6, 500 MHz); 1.12- 1.15 (m, 8H, H^{10}+H^{11}+H^{12}+H^{13}), 1.54 (t, *J*=6.4Hz, 2H, H^{14}), 1.84 (t, *J*=6.3Hz, 2H, H^9), 3.65 (t, *J*=7.5Hz, 2H, H^{15}), 4.20 (s, 5H, H^1), 4.37 (t, *J*=6.3Hz, 2H, H^9), 4.58 (s, 2H, H^2), 4.85 (s, 2H, H^3), 5.27 (s, 2H, H^6), 7.20 (d, *J*=8.5Hz, 2H, Ar-H) ,7.45 (d, *J*=15.1Hz, 1H, H^4), 7.57 (s, 1H, H^{16}), 7.62 (d, *J*=15.1Hz, 1H, H^5) ,8.08 (d, *J*=8.5Hz, 2H, Ar-H), 8.23 (s, 1H, H^7), 11.13 (NH-exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 125MHz): 26.1, 26.2, 28.5, 28.6, 28.8, 30.1, 47.6, 49.7, 61.6, 69.5, 69.6, 71.2, 79.6, 95.4, 115.2, 119.2, 125.3, 131.3, 131.7, 142.5, 145.5, 146.2,151.4, 162.2, 164.2, 187.0 . HRMS calcd for $C_{34}H_{36}BrFeN_5O_4$ $[M]^+$713.1300.Found: 713.1334. Anal Calcd(%) for: C, 57.16; H, 5.08; N, 9.80. Found: C, 57.29; H, 4.91; N, 9.55.

Biological evaluation

Cell Culture

Human leukemia cell line (CCRF-CEM, ATCC No. CCL-119) and human breast adenocarcinoma (MDA-MB-468, ATCC, No. HTB-132) were purchased from American Type Culture Collection. The cells were grown in 75 cm^2 cell culture flasks with RPMI-16 medium for CCRF-CEM and DMEM for MDA-MB-468, supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin solution (10,000 units of penicillin and 10 mg of streptomycin in 0.9% NaCl) in a humidified atmosphere of 5% CO_2, 95% air at 37 °C.

Cytotoxicity Assay

MDA-MB-468 (5,000 cells), and CCRF-CEM cells (50,0000 cells) were seeded in 0.1 mL per well in 96-well plates 24 h prior to the experiment. Cells were treated with Dox (5 µM) and DMSO (16.6%) as positive controls and compounds 5a-r (50 µM). Plates were incubated for 24 or 72 h at 37 °C in a humidified atmosphere of 5% CO_2. Before adding MTS reagent [Cell Proliferation Assay Kit (Colorimetric) (abcam197010)], the medium of MB-MB-468 was replaced with fresh medium. Cell viability was then determined by measuring the fluorescence intensity of the formazan product at 490 nm using a SpectraMax M2 microplate spectrophotometer. The percentage of cell survival was calculated as [(OD value of cells treated with compounds) (OD value of culture medium)] / [(OD value of control cells) (OD value of culture medium)] × 100.

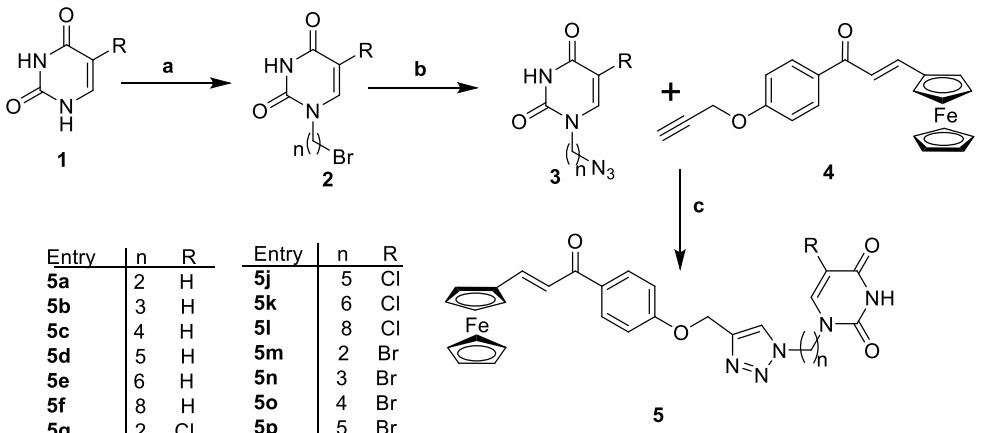
In vitro analysis of cytotoxicity on normal kidney cells

The *in vitro* cytotoxicity of the compounds was evaluated using normal kidney cell line (LLC-PK1, ATCCCL-101), by using the MTS Cell Proliferation Assay Kit. LLCPK cells were seeded at 5,000 cells in 0.1 mL per well in 96-well plates. The cells were seeded in medium (EMEM containing FBS (10%)), 24 h prior to the experiment. The compounds at the concentration of 50 µM were added to each well in triplicate and incubated for 24 h or 72 h at 37 °C in a humidified atmosphere of 5% CO_2. Doxorubicin was used a concentration of 5 µM. Cell viability was then determined by measuring the fluorescence intensity at 490 nm using a SpectraMax M2 microplate spectrophotometer. The percentage of cell survival was calculated as [(OD value of cells treated with the test mixture of compounds) − (OD value of culture medium)]/[(OD value of control cells) − (OD value of culture medium)] × 100%.

Results and Discussion

Chemistry

Huisgen's azide-alkyne cycloaddition reaction was employed for the synthesis of desired series of 1*H*-1,2,3-triazole-tethered uracil-ferrocenyl-chalcone conjugates. The synthetic methodology involved an initial base-promoted propargylation of *p*-hydroxyacetophenone, followed by its condensation with ferrocene-carboxaldehyde in ethanol as reported previously (Kumar *et al.*, 2013). The second precursor *viz*. *N*-alkylazido 5-substituted uracil derivatives were prepared by hydride-promoted alkylation of 5-substituted uracil analogues with dibromoalkane followed by its nucleophilic substitution reaction with sodium azide. Mono-alkylated uracil is accompanied by the formation of dialkylated uracils albeit in poor yields as per literary reports (Dezor-Mazur, Kazmierczak and Golankiewicz, 1984). Cu-promoted azide-alkyne cycloaddition reaction between *N*-alkyl azido-uracils and *O*-propargylated ferrocenyl-chalcone afforded the desired 1*H*-1,2,3-triazole-tethered uracil-ferrocenyl chalcone conjugates that were purified *via* column chromatography using $CHCl_3$:CH_3OH (95:5 v/v) mixture as the eluents (**Scheme 1**).



Entry	n	R
5a	2	H
5b	3	H
5c	4	H
5d	5	H
5e	6	H
5f	8	H
5g	2	Cl
5h	3	Cl
5i	4	Cl

Entry	n	R
5j	5	Cl
5k	6	Cl
5l	8	Cl
5m	2	Br
5n	3	Br
5o	4	Br
5p	5	Br
5q	6	Br
5r	8	Br

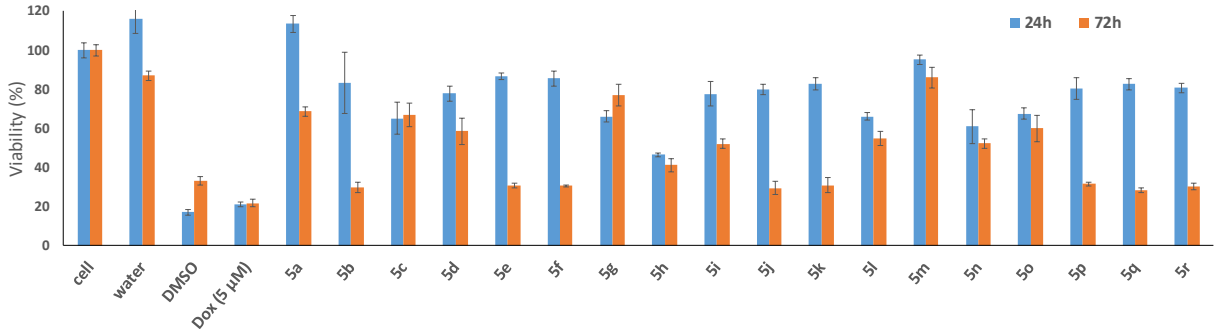
Scheme 1. Synthesis of compounds **5a-r** (a) Dibromo alkanes (1,2 eq), NaH (1.2 eq), DMF, 60 ^oC, 8h (b) NaN_3, DMF, 60 ^oC, 1h (c) $CuSO_4.5H_2O$, Sodium Ascorbate, EtOH:H_2O, rt, 12h

The structures of the synthesized uracil-ferrocenyl chalcone conjugates were assigned on the basis of spectral data and analytical evidence. The conjugate **5p**, for example, showed a molecular ion peak at 671.0831 (m/z) along with a characteristics signals in ^1H and ^{13}C NMR spectra. Its spectrum ^1H NMR showed the presence of singlet at δ 4.19 corresponding to 5H (cyclopentadiene ring of ferrocene) along with two singlets at δ 4.54 (2H) and δ 4.85 (2H) due to the presence of ferrocene ring proton along with the presence of two doublets at δ 7.45 and at δ 7.54 (*J* =15.2 Hz) corresponding to *trans*-olefinic protons. The presence of characteristic signals at δ 162.1 and 187.0 ppm corresponding to the uracil ring carbonyls along with the requisite number of carbons in ^{13}C NMR spectrum further corroborated the assigned structure

The synthesized conjugates were evaluated for their cytotoxic profiles against human leukemia cell (CCRF-CEM) and human breast adenocarcinoma (MDA-MB-468) cells using MTS-assay, and the results are depicted graphically in **Figure 1**. A standard drug, doxorubicin (Dox) was used as a positive control. A number of compounds exhibited substantial cytotoxicity after 72 h incubation. A closer inspection revealed the dependence of cytotoxicity on the length of alkyl chain introduced as a spacer with a preference for longer chain lengths while the nature of substituents at C-5 position of uracil did not seem to influence the activity profiles. The conjugates, having longer alkyl chain lenghts introduced as spacer, *viz*. **5e** (n = 6); **5f** (n =8); **5j** (n = 5); **5k** (n = 6); **5p** (n = 5); **5q** (n = 6), and **5r** (n = 8) reduced the proliferation of CCRF-CEM cells by approximately 70% after 72 h against. A similar inspection of the cytotoxic activities of the synthesized conjugates against MDA-MB-468 revealed that most of the

conjugates were inactive even after 72 h; the exception being **5a,** having an ethyl chain as spacer and **5d,** with a pentyl chain reduced the cell proliferation by 59% and 62%, respectively.

(a)



(b)

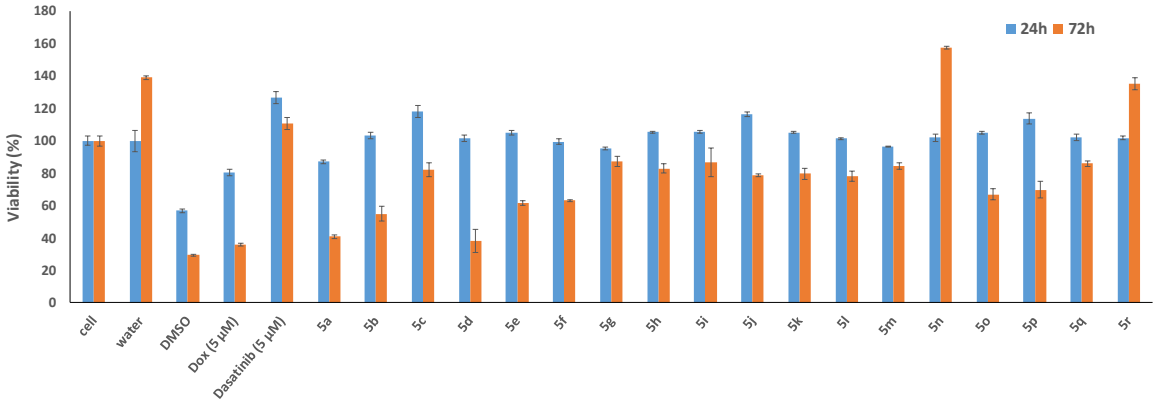


Figure 1. Cytotoxic activity of compounds **5a-r** (50 µM) on (a) CCRF-CEM and (b) MDA-MB-468 cells

Seven of the most potent conjugates *viz*. **5e**, **5f**, **5j**, **5k**, **5p**, **5q**, **5r** were also evaluated for the cytotoxic profiles against normal kidney cell line (LLC-PK1 ATCC CL-101) and the results were compared with doxorubicin. As evident, the conjugates with potent activity against CCRF-CEM cell line did not show any significant toxicity at a concentration of 50 µM after 24 h incubation (**Figure 2**). Furthermore, the compounds were found to be significantly less cytotoxic in non-tumorigenic LLC-PK1 when compared with CCRF-CEM cancer cells after 72 h (**Figure 1**).

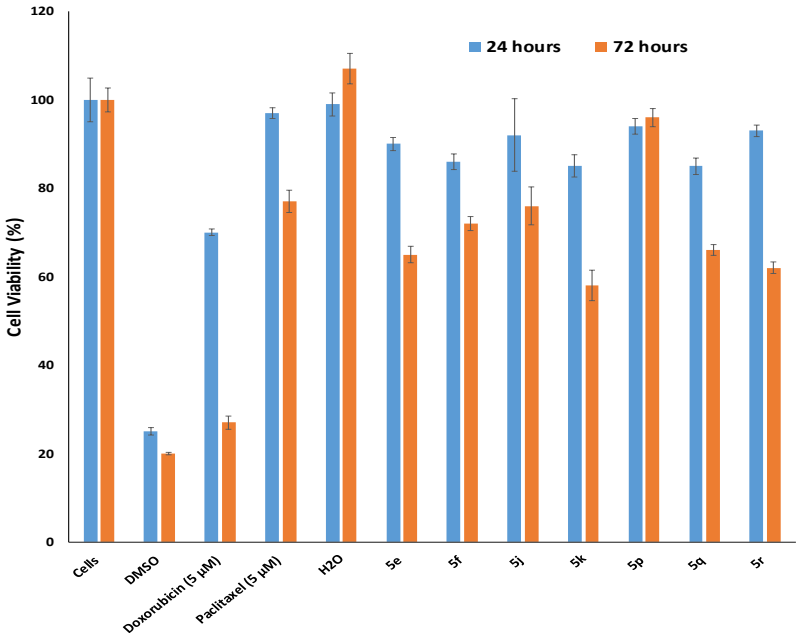


Figure 2. Cytotoxic activity of **5e**, **5f**, **5j**, **5k**, **5p**, **5q** and **5r** (50 µM) against normal kidney cell line (LLC-PK1).

Conclusion

In conclusion, a series of uracil-ferrocenyl chalcone conjugates were prepared *via* Cu-promoted azide alkyne cycloaddition reaction along with an evaluation of their cytotoxic potential against human leukemia and human breast adenocarcinoma cell lines. Structure-Activity Relationship (SAR) studies revealed the dependence of cytotoxic efficacy on the length of alkyl chain introduced as linker with a number of conjugates exhibiting ~70% antiproliferative activity against human leukemia (CCRF-CEM) cell line after 72 hours. The synthesized conjugates were also found to be significantly less toxic to normal kidney cell line (LLC-PK1, ATCC CL-101).

Abbreviations

SAR, Structure Activity Relationship; CCRF-CEM, ATCC No. CCL-119, Human leukemia cell line; MDA-MB-468, ATCC, No. HTB-132, human breast adenocarcinoma; LLC-PK1, ATCCCL-101, normal kidney cell line

Associated Content

Scanned (^{1}H, ^{13}C) NMR spectra for the compounds *viz*. **5f, 5o, 5p.** This material is available free of charge via the Internet at http:// pubs.rsc.org.

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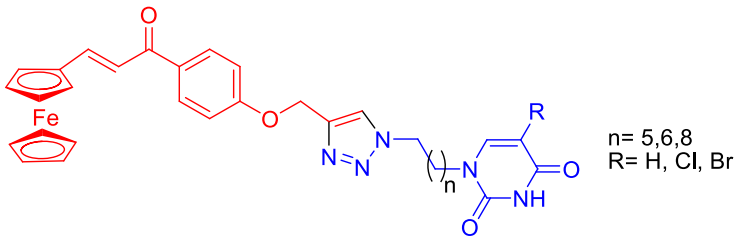

Most potent conjugates against human leukemia cell (CCRF-CEM) cell line

Synthesis and *in vitro* anti-proliferative efficacy of 1*H*-1,2,3-triazole-tethered uracil-ferrocenylchalcone conjugates on human leukemia (CCRF-CEM) and human breast adenocarcinoma (MDA-MB-468) cell lines.