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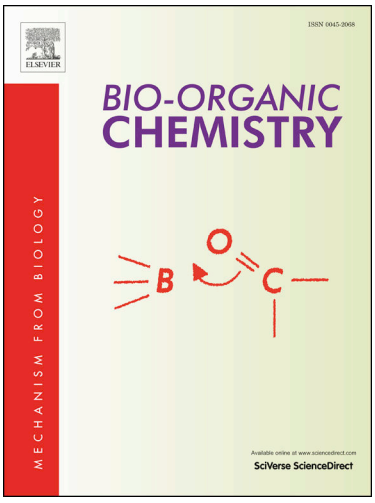

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Anticancer, Antimicrobial Activities of Quinoline Based Hydrazone Analogues: Synthesis, Characterization and Molecular Docking

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Abstract

Based on the biologically active heterocycle quinoline, a series (**18a-p**) of quinoline hydrazone analogues were prepared, starting from 6-bromo/6-chloro-2-methyl-quinolin-4-yl-hydrazines. For all the newly synthesized compounds cytotoxic activities were carried out at the National Cancer Institute (NCI), USA, against full NCI 60 human cancer cell lines. Amongst all the tested compounds, nine compounds (**18b**, **18d**, **18e**, **18f**, **18g**, **18h**, **18i**, **18j**, **18l**) exhibited important anti-proliferative activity at 10 μM concentration and were further screened at 10-fold dilutions of five different concentrations (0.01, 0.1, 1, 10 and 100 μM) with GI_{50} values ranging from 0.33-4.87 μM and LC_{50} values ranging from 4.67 μM to >100j μM. Further, the mean values of GI_{50}, TGI and LC_{50} of the most potent compound **18j** were compared with the clinically used anticancer agents bendamustine and chlorambucil, revealed that the quinolyl hydrazones holds promise as a potential anticancer agents. Further all the newly prepared compounds were screened for their antimicrobial activity. All the quinolyl hydrazones displayed good to excellent antimicrobial activity with MIC values ranging from 6.25 - 100 μg/mL against the tested pathogenic strains. Molecular docking of the synthesized compounds into the active binding site of human DNA topoisomerase I (htopoI) was carried out to predict the binding mode to the DNA topoisomerase I inhibitors. Hopefully in future, compounds based on quinoline core could be used as a lead compounds for designing new anticancer agents.

Keywords: Quinoline; Hydrazones; Alkoxy aldehydes/acetophenones; Anticancer activity; Antimicrobial activity; Docking studies.

1. Introduction

Cancer is a collection of various fatal diseases. Uncontrolled cell growth results in invasion of surrounding tissue and spreading further to other parts of the human body are the characteristics of the deadly disease cancer. According to the world cancer report released by WHO, in the upcoming years the death rates due to cancer will upsurge twice its present-day percentage[1]. Consequently, discovery and development of novel effective drugs for the treatment of cancer have become an urgent need and the key attention of lots of researchers and pharmaceutical companies. Although previously reported chemotherapeutic agents efficiently kill cancer cells, several times this closes to develop multi-drug resistance [2] and hence search and development of novel anticancer drugs possessing heterocyclic core continues globally at a number of research laboratories [3].

Compounds possessing heterocyclic core play significant role in designing and developing new class of structural entities for pharmaceutical applications. Quinoline and its derivatives, as a significant class of pharmaceutically active heterocyclic compounds, demonstrated different pharmaceutical activities[4–10]. Quinoline framework played a crucial role in designing of anticancer agents as their analogues have revealed tremendous results through various mechanism involving disruption of cell migration, growth inhibitors by cell cycle arrest, inhibition of angiogenesis, apoptosis, and modulation of nuclear receptor responsiveness[11]. Moreover, many novel derivatives of quinoline have been reported to display significant anticancer activity through DNA intercalation, causing interference in the replication process[12]. Because of the pharmacological significance of quinoline derivatives, their structures and pharmaceutical properties have been well documented[11,13]. Even though a number of quinoline derivatives and its applications have been reported so far, however only few synthetic quinoline derivatives are available based on quinoline pharmacophore[14].

Halogen-substituted quinoline compounds have been of particular interest, because the halogen atom plays a key role in the exhibition of biological activities[15,16]. Many halo-quinolines have been studied for their potential anticancer activity (**Figure 1(A)**). Moreover, it was reported that 2,4-disubstituted and 2,4,6-trisubstituted quinoline ring play a key role in the designing of novel anti-cancer agents as these derivatives have shown outstanding results through different mechanism of action[17].

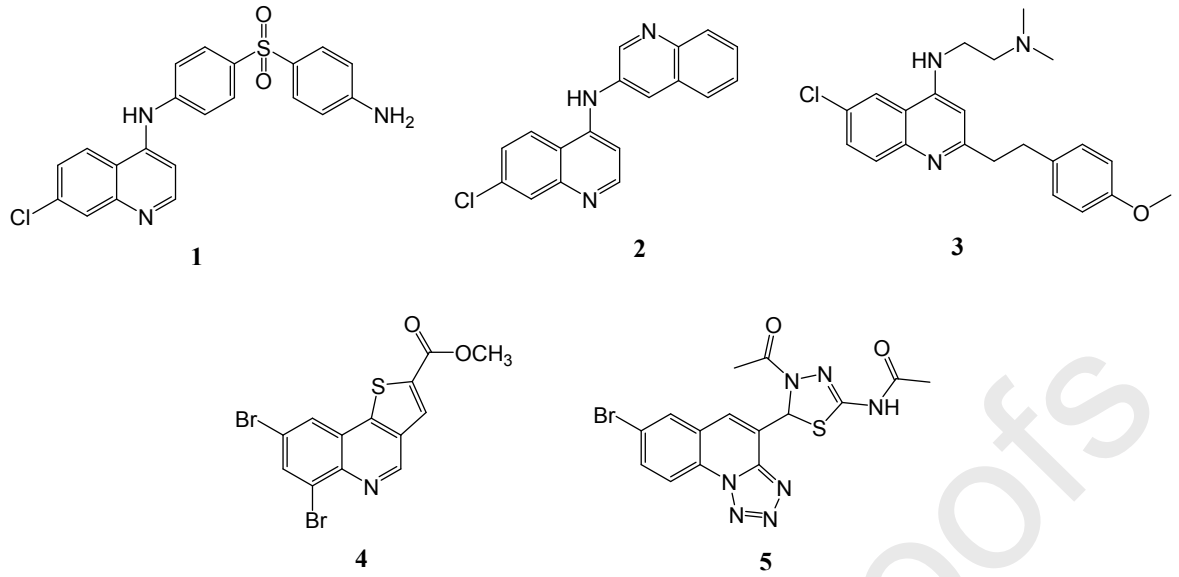


Figure 1(A). Halo-quinolines as a potential anticancer agets.

Since the approval of pelitinib for cancer therapy, a number of the 4-aminoquinoline derivatives have been developed as the skeleton, which is considered as promising framework for antitumor drug development (**Figure 1(B)**)[18]. Chloroquine (CQ), an immunostimulatory agent with 4-aminoquinoline skeleton, has aroused increasing attention due to its antiproliferative potency on different cancer cells[18]. Likewise, Bispo *et al*. prepared a series of 4-quinolinylhydrazones derivatives (**Figure 1(B)**) as anticancer agents[19], which brought us new faith in the design and study of quinoline based anticancer agents. Under the inspiration of CQ and CQ-derivatives, we assumed that newly synthesized compounds with 2,4-substituted-6-halogenated quinolines should possess promising anticancer activity as well.

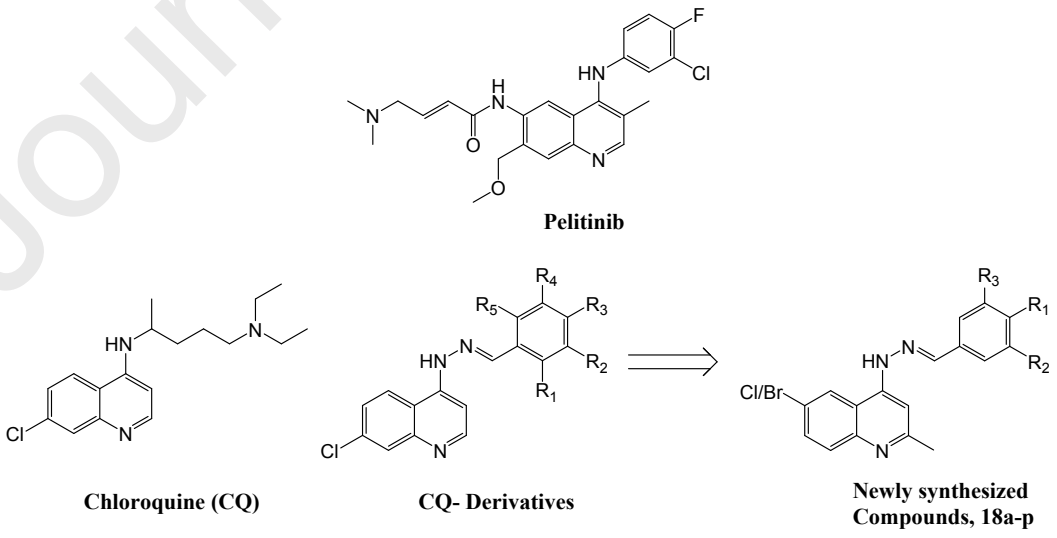


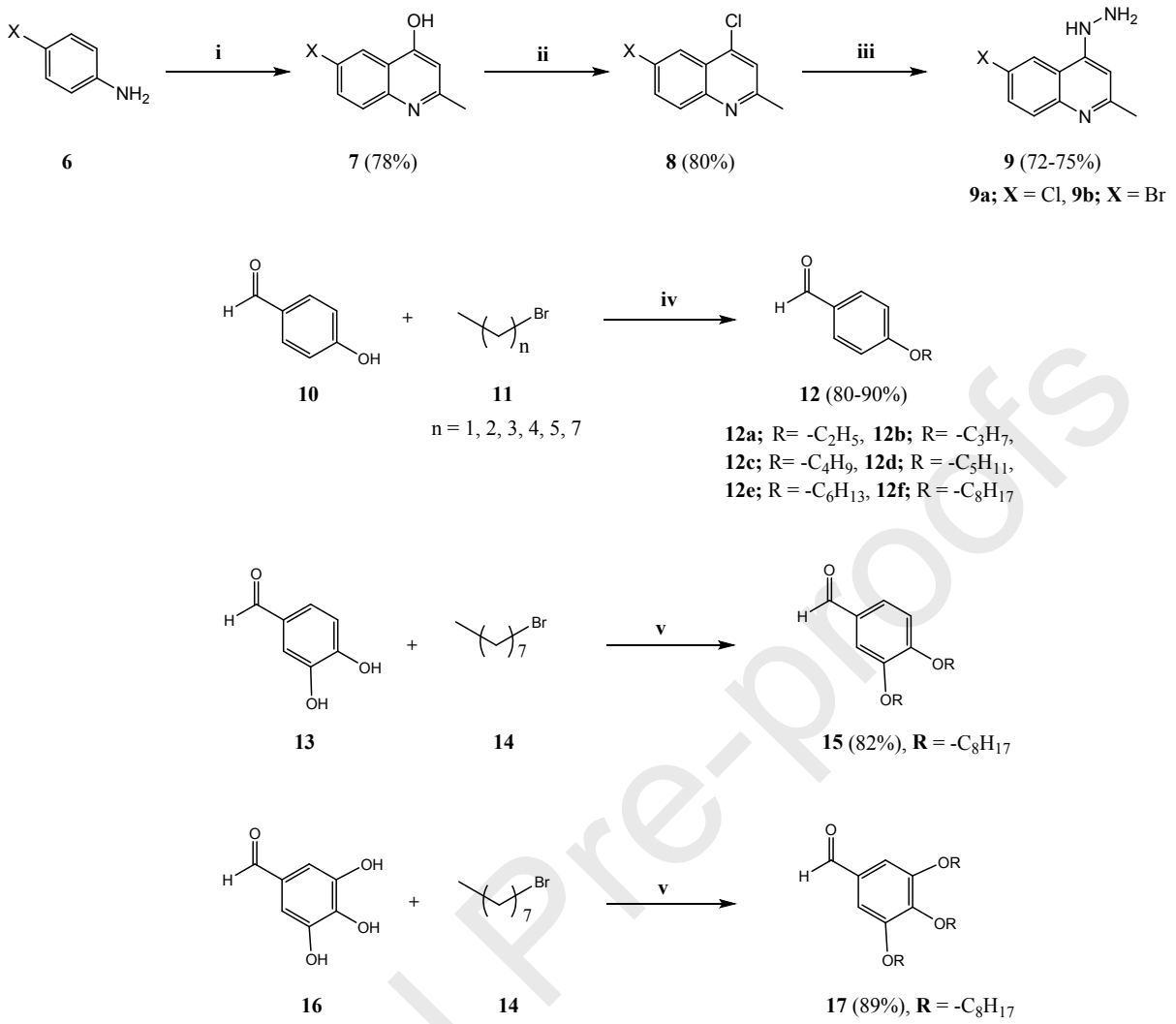
Figure 1(B). Structures of pelitinib, CQ, halo-quinoline derivatives and target compounds **18a-p**.

These observations motivated us to synthesize new 2,4-substituted-6-halogenated quinoline derivatives. In our efforts to synthesize compounds with improved electron affinity and better biological interactions, keeping halo-quinoline ring intact, hydrazinyl (-NH-N=) group introduced at C-4 positions. The introduction of halogen at the C-6 position might adjust lipophilicity. All target molecules were screened for their in vitro anticancer activity against a panel of sixty cancer cell lines in the Developmental Therapeutics Program (DTP) at the National Cancer Institute (NCI). Further, all the newly synthesized compounds were also screened for *in vitro* antifungal, antibacterial activities. Active compounds were also studied for their molecular docking.

2. Results and Discussion

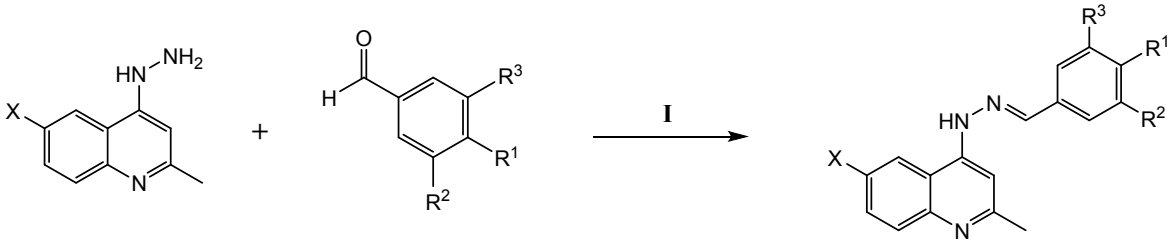
2.1 Chemistry

The synthesis of the target quinolyl hydrazones **18a-p** is as represented in **scheme 1-2**. The synthesis of the final quinolyl hydrazones was started with the preparation of the intermediate 6-bromo/6-chloro-2-methyl-quinolin-4-yl-hydrazines **9a/b** from corresponding 4-bromo/chloro anilines **6** by the reaction with ethyl acetoacetate in the presence of polyphosphoric acid as per the reported procedure [20] (**Scheme 1**). The structures of the quinolyl hydrazines **9a/b** were confirmed using different spectroscopic techniques and are in accordance with the reported once [21].



Reagents and conditions: i) Ethyl acetoacetate, Polyphosphoric acid (PPA), 150 ^{o}C, 2 h ii) $POCl_3$, 80 ^{o}C, 4 h, iii) NH_2NH_2. H_2O, Ethanol, 80 ^{o}C, 4 h, iv) Acetone, K_2CO_3, 60-70 ^{o}C, 6-7 h, **v**) DMF, K_2CO_3, 100 ^{o}C,12h.

Scheme 1. Synthesis of intermediate compounds **9a/9b**, **12a-e**, **15** and **17**.



9 **12/15/17** **18** (76-82%)

X = Br

18a; $\mathbf{R_1}$ = -OC_2H_5, $\mathbf{R_2}$ = -H, $\mathbf{R_3}$ = -H
18c; $\mathbf{R_1}$ = -OC_3H_7, $\mathbf{R_2}$ = -H, $\mathbf{R_3}$ = -H
18e; $\mathbf{R_1}$ = -OC_4H_9, $\mathbf{R_2}$ = -H, $\mathbf{R_3}$ = -H
18g; $\mathbf{R_1}$ = -OC_5H_{11}, $\mathbf{R_2}$ = -H, $\mathbf{R_3}$ = -H
18i; $\mathbf{R_1}$ = -OC_6H_{13}, $\mathbf{R_2}$ = -H, $\mathbf{R_3}$ = -H
18k; $\mathbf{R_1}$ = -OC_8H_{17}, $\mathbf{R_2}$ = -H, $\mathbf{R_3}$ = -H
18m; $\mathbf{R_1}$ = -OC_8H_{17}, $\mathbf{R_2}$ = -H, $\mathbf{R_3}$ = -OC_8H_{17}
18o; $\mathbf{R_1}$ = -OC_8H_{17}, $\mathbf{R_2}$ = -OC_8H_{17}, $\mathbf{R_3}$ = -OC_8H_{17}

X = Cl

18b; $\mathbf{R_1}$ = -OC_2H_5, $\mathbf{R_2}$ = -H, $\mathbf{R_3}$ = -H
18d; $\mathbf{R_1}$ = -OC_3H_7, $\mathbf{R_2}$ = -H, $\mathbf{R_3}$ = -H
18f; $\mathbf{R_1}$ = -OC_4H_9, $\mathbf{R_2}$ = -H, $\mathbf{R_3}$ = -H
18h; $\mathbf{R_1}$ = -OC_5H_{11}, $\mathbf{R_2}$ = -H, $\mathbf{R_3}$ = -H
18j; $\mathbf{R_1}$ = -OC_6H_{13}, $\mathbf{R_2}$ = -H, $\mathbf{R_3}$ = -H
18l; $\mathbf{R_1}$ = -OC_8H_{17}, $\mathbf{R_2}$ = -H, $\mathbf{R_3}$ = -H
18n; $\mathbf{R_1}$ = -OC_8H_{17}, $\mathbf{R_2}$ = -H, $\mathbf{R_3}$ = -OC_8H_{17}
18p; $\mathbf{R_1}$ = -OC_8H_{17}, $\mathbf{R_2}$ = -OC_8H_{17}, $\mathbf{R_3}$ = -OC_8H_{17}

Reagents and conditions: i) Ethanol, Sulphuric acid, 80 °C, 4 h

Scheme 1. Synthetic protocol for quinolyl hydrazones, **18a-p**.

Further, synthesis of several alkoxy substituted aromatic aldehydes **12/15/17** was carried out by the reaction of 4-hydroxybenzaldehydes with varying alkyl chain lengths to have different lipophilicity of the final compounds. The 4-alkyloxybenzaldehydes **12a-f** were prepared by alkylation of 4-hydroxy benzaldehyde **10** with different alkyl halides **11a-f** with chain length varying from 2 to 8 carbons in the presence of K_2CO_3 as a base in acetone following the reported procedure [22] (**Scheme 1**). Similarly, 3,4-bis-octyloxy benzaldehyde **15** and 3,4,5-tris-octyloxy benzaldehyde **17** were prepared by reacting 3,4-dihydroxy benzaldehyde **13** and 3,4,5-trihydroxybenzaldehyde **16** with 1-bromooctane **14** in dry DMF using K_2CO_3 as a base to afford 3,4-bis-octyloxy benzaldehyde **15** and 3,4,5-tris-octyloxy benzaldehyde **17** respectively[23,24] (**Scheme 1**). The final target quinolyl hydrazones **18a-p** were synthesized by the condensation of various alkoxy benzaldehydes **12a-f/15/17** with 6-bromo/6-chloro-2-methyl-quinolin-4-yl-hydrazine **9a/b** in ethanol using a catalytic amount of sulphuric acid following reported procedure[20] (**Scheme 2**).

All the quinolyl hydrazones were characterized by various spectroanalytical techniques. In the IR spectrum of quinolyl hydrazones (**18a-p**), the N–H stretching was observed between 3270-3200 cm^{-1}. The presence of long alkyl chains is marked by the C–H stretching observed at ~2977 cm^{-1}. The strong bands observed at ~1605 cm^{-1} and ~1571 cm^{-1} are corresponding to the

aromatic C=C stretching. Strong absorption bands at frequency range ~850 cm^{-1} and ~770 cm^{-1} are due to the presence of C-Cl and C-Br stretching. In proton NMR of the quinolyl hydrazones (**18a-p**), the terminal methyl protons were observed most upfield near δ 0.9-1.1 ppm as a triplet with coupling constant *J* = 7.2 Hz. The $–CH_3$ group protons on quinoline ring were observed at δ 2.73 ppm as a singlet. For compounds (**18a/18b, R_3** = $-C_2H_5$), $–OCH_2$ protons were observed at δ 4.1 ppm as a quartet with coupling constant *J* = 7.2 Hz while for other compounds $–OCH_2$ protons were appeared as triplet. The methylene proton of imine linkage (-N═CH-) was observed as a singlet at δ 7.5 ppm. The –NH proton was observed most downfield at δ 13.0-14.0 ppm as a singlet. The other aromatic protons were observed in between δ 7.0-8.8 ppm value as per the substitution pattern at various positions of the aromatic ring. In ^{13}C NMR of the new quinolyl hydrazones (**18a-p**) the most downfield carbon was hydrazone carbon observed near δ 160 ppm while the other aromatic carbons were observed between δ 152 to 100 ppm and the oxygen attached carbon was observed near δ 63 ppm and other aliphatic carbons are observed up field with the chemical shift value up to δ 15 ppm. In the mass spectral analysis of the newly synthesized quinolyl hydrazones (**18a-p**), the molecular ion peaks for these compounds were observed as $(M+H)^+$ for all the compounds. The physical data of all the quinolyl hydrazones **18a-p** are as summarized in **Table 1**.

Table 1. Physical data of the newly synthesized quinolyl hydrazones.

ID	Substitution pattern				NSC number	Molecular formula	Yield	mp
	X	R_1	R_2	R_3				
18a	-Br	$-OC_2H_5$	-H	-H	D-804234/1	$C_{19}H_{18}BrN_3O$	82 %	202-203 °C
18b	-Cl	$-OC_2H_5$	-H	-H	D-804233/1	$C_{19}H_{18}ClN_3O$	74 %	210-211 °C
18c	-Br	$-OC_3H_7$	-H	-H	D-804236/1	$C_{20}H_{20}BrN_3O$	78 %	204-215 °C
18d	-Cl	$-OC_3H_7$	-H	-H	D-804235/1	$C_{20}H_{20}ClN_3O$	74 %	200-201 °C
18e	-Br	$-OC_4H_9$	-H	-H	D-804238/1	$C_{21}H_{22}ClN_3O$	76 %	198-199 °C
18f	-Cl	$-OC_4H_9$	-H	-H	D-804237/1	$C_{21}H_{22}BrN_3O$	80 %	186-187 °C
18g	-Br	$-OC_5H_{11}$	-H	-H	D-804240/1	$C_{22}H_{24}BrN_3O$	68 %	194-195 °C
18h	-Cl	$-OC_5H_{11}$	-H	-H	D-804239/1	$C_{22}H_{24}ClN_3O$	72 %	190-191 °C
18i	-Br	$-OC_6H_{13}$	-H	-H	D-804242/1	$C_{23}H_{26}BrN_3O$	74 %	194-195 °C
18j	-Cl	$-OC_6H_{13}$	-H	-H	D-804241/1	$C_{22}H_{24}ClN_3O$	76 %	188-189 °C
18k	-Br	$-OC_8H_{17}$	-H	-H	D-804271/1	$C_{25}H_{30}ClN_3O$	82 %	186-187 °C
18l	-Cl	$-OC_8H_{17}$	-H	-H	D-804270/1	$C_{25}H_{30}ClN_3O$	79 %	182-183 °C

18m	-Br	$-OC_8H_{17}$	-H	$-OC_8H_{17}$	D-804273/1	$C_{33}H_{46}BrN_3O_2$	78 %	184-185 °C
18n	-Cl	$-OC_8H_{17}$	-H	$-OC_8H_{17}$	D-804272/1	$C_{33}H_{46}ClN_3O_2$	71 %	182-183 °C
18o	-Br	$-OC_8H_{17}$	$-OC_8H_{17}$	$-OC_8H_{17}$	D-804275/1	$C_{41}H_{62}ClN_3O_3$	74 %	180-181 °C
18p	-Cl	$-OC_8H_{17}$	$-OC_8H_{17}$	$-OC_8H_{17}$	D-804274/1	$C_{41}H_{62}BrN_3O_3$	72 %	178-189 °C

2.2 Anticancer activity

2.2.1 *In vitro* preliminary anticancer activity at a single dose (10 μM) against full NCI 60 cell panel

The preliminary anticancer screening of the newly prepared quinolyl hydrazones **18a-p** was carried out at NCI, USA under the screening project at the National Cancer Institute (NCI), USA. All the newly synthesized quinolyl hydrazones were selected and evaluated for preliminary *in vitro* one dose anticancer screening at National Cancer Institute (NCI) against full NCI 60 cell line panels representing nine different kinds of cancer comprising leukemia, non-small cell lung cancer, melanoma, CNS cancer, ovarian cancer, renal cancer, prostate cancer, breast cancer in agreement with the protocol of the NCI, USA (http://dtp.nci.nih.gov). Results of each tested compound **18a-p** were reported as a mean graph of the percent growth of the treated cells when compared to the untreated control cells which gives both inhibition values (between 0 and 100) and cytotoxicity values (less than 0). The single dose evaluation results of all the quinolyl hydrazones **18a-p** against sixty cancer cell lines were analysed by COMPARE program. The screening results of all quinolyl hydrazones at 10 μM exhibited excellent anticancer activity with broad spectrum of cytotoxic activity against several cancer cell lines tested. Nine of the quinolyl hydrazones (**18b**, **18d**, **18e**, **18f**, **18g**, **18h**, **18i**, **18j**, **18l**) were selected for further detailed study at five different dilutions due to outstanding results in the single dose study and found to be lethal to a greater extent (see supporting information).

2.2.2 *In vitro* anticancer screening at 5 dose full NCI 60 cell panel

In the second stage, the selected nine compounds including **18b** (D-804233/1), **18d** (D-804235/1), **18e** (D-804238/1), **18f** (D-804237/1), **18g** (D-804240/1), **18h** (D-804239/1), **18i** (D-804242/1), **18j** (D-804241/1), **18l** (D-804270/1) satisfied predetermined threshold inhibition criteria, were screened and evaluated against all the 60 cell lines at 10-fold dilutions of five different concentrations (0.01, 0.1, 1, 10 and 100 μM)[25,26]. The percentage of growth was examined spectrophotometrically against not treated with test agents and using SRB

(sulforhodamine-B) protein assay cell viability was determine according to the experimental protocols described [25,26]. The results of the five dose assay are represented in terms of response parameters GI_{50} (Required molar concentration to inhibit 50% of the growth of cancer cell lines) and LC_{50} (Required molar concentration necessary to kill 50% of the cells at the end of the incubation period of 48 h, cytotoxic activity) for each cell line tested [25,26]. The calculated GI_{50} and LC_{50} values for each sixty cancer cell lines of these nine compounds over the nine cancer types are summarized in **Table 2**. The five dose anticancer screening data revealed that, all nine tested compounds (**18b**, **18d**, **18e**, **18f**, **18g**, **18h**, **18i**, **18j**, **18l**) exhibited outstanding activity with great value of GI_{50} against several cancer cell lines, a number of them lower than 2.0 μM.

Compound **18b** displayed outstanding activity with GI_{50} values from 1.12 to 2.37 μM (48 of them < 2.00 μM) and LC_{50} values from 5.47 to 33.0 μM. The highest cytostatic activity of **18b** was observed against SR leukemia cell line with GI_{50} = 1.12 μM and the best cytotoxic activity was shown against the UO-31 renal cancer cell line with LC_{50} = 5.47 μM. Compound **18d** displayed good activity with GI_{50} values from 1.17 to 2.31 μM (50 of them < 2.00 μM) and LC_{50} values from 5.39 to 38.6 μM. The best cytostatic activity was observed against SR leukemia cell line with GI_{50} values from 1.17 μM and the best cytotoxic activity was displayed against the SK-MEL-5 melanoma cancer cell line with LC_{50} = 5.39 μM. Compound **18e** displayed potent activity with GI_{50} values ranging from 1.47 to 2.64 μM (52 of them < 2.00 μM) and LC_{50} values from 5.39 to >100 μM. Compound **18e** displayed best cytostatic activity against the HOP-62 non–small cell lung cancer cell line and SK-MEL-2 melanoma cancer cell line with GI_{50} = 1.47 μM and the best cytotoxic potency was observed against the UO-31 renal cancer cell line with LC_{50} = 5.39 μM. Compound **18f** displayed noteworthy activity with GI_{50} values ranging from 0.50 to 2.13 μM (55 of them < 2.00 μM) and LC_{50} values from 5.34 to >100 μM. Compound **18f** displayed highest cytostatic activity against the SR leukemia cancer cell line with GI_{50} = 0.50 μM and the best cytotoxic activity was observed against the RXF 393 renal cancer cell line with LC_{50} = 5.34 μM. Compound **18g** showed good anticancer activity with GI_{50} values ranging from from 0.44 to 2.13 μM (56 of them < 2.00 μM) and LC_{50} values from 5.47 to >100 μM. Compound **18g** displayed highest cytostatic activity against the SR leukemia cancer cell line with GI_{50} = 0.44 μM and the best cytotoxic activity was observed against the CAKI-1 and UO-31 renal cancer cell lines with LC_{50} = 5.47 μM. Compound **18h** exhibited excellent activity with GI_{50} values ranging from from 0.36 to 2.23 μM (53 of them < 2.00 μM) and LC_{50} values from 5.02 to 42.0 μM. Compound **18h** displayed best cytostatic

activity against the SR and K-562 leukemia cancer cell lines with GI_{50} = 0.36 μM 0.69 μM respectively and the best cytotoxic activity was observed against the HCT-116 colon cancer cell lines with LC_{50} = 5.02 μM. Compound **18i** exhibited GI_{50} values from 0.39 to 4.87 μM (50 of them < 2.00 μM) and LC_{50} values from 4.67 to >100 μM. Compound **18h** displayed best cytostatic activity against the SR leukemia and HCT-116 colon cancer cell lines with GI_{50} = 0.39 μM and 0.85 μM respectively and the best cytotoxic activity was observed against the HCT-116 colon cancer cell lines with LC_{50} = 4.67 μM.

Among all new compounds, the highest activity was observed for compound **18j** with GI_{50} values ranging from 0.33 to 2.05 μM (52 of them < 2.00 μM) and LC_{50} values from 5.15 to 24.4 μM (**Table 2** and **Figure 3**). The plots of percentage growth in 60 cancer cell lines vs sample concentration at five different dose levels (log dilution from 10^{-4} mol/L to 10^{-8} mol/L) after treatment with compound **18j** are shown in **Figure 2**. The dose response curve of compound **18j** plotted for six subpanels of leukaemia (**Figure 3**), illustrates the endpoint calculations for GI_{50}, TGI and LC_{50} at five dose concentrations. It displayed best cytostatic activity against the some of the cancer cell lines including (SR leukemia cancer cell line with GI_{50} = 0.33 μM), (K-562 leukemia cancer cell line with GI_{50} = 0.46 μM), (HCT-116 cancer cell line with GI_{50} = 0.34 μM), (SW-620 cancer cell line with GI_{50} = 0.97 μM) and the best cytotoxic activity was observed against the HCT-15 colon cancer cell lines with LC_{50} = 5.15 μM (**Figure 2** and **Figure 3**). Compound **18l** demonstrated good activity with GI_{50} values ranging from 1.48 to 2.21 μM (44 of them < 2.00 μM) and LC_{50} values from 5.53 to >100 μM. Compound **18l** displayed highest cytostatic activity against the HOP-62 non-small cell cancer cell line with GI_{50} = 1.48 μM and the best cytotoxic activity was observed against the UO-31 renal cancer cell line with LC_{50} = 5.53 μM.

Further, The *in vitro* anticancer activity of the most potent compound **18j**, among the entire series of newly synthesized compounds was also compared with the activity data obtained from the NCI for the clinically used anticancer agents (Bendamustine and Chlorambucil)[27] in terms of potency (μmol/L) by three response parameters and the results are illustrated in **Table 3**. The results revealed that the compound **18j** has lower mean values of log molar concentration for response parameters (GI_{50}, TGI and LC_{50}) as compared to clinically used anticancer drugs, bendamustine and chlorambucil. Also, the mean graph midpoint (MG-MID) GI_{50} value of the most active compound **18j** was observed to be only 1.58 μM, which is considerably lesser than the standard marketed anticancer drugs (60 and 52 μM, respectively) suggesting that the quinolyl hydrazones holds promise as a potential anticancer agents.

Compound **18j** was also studied for their cytotoxicity study in NIH/3T3 normal cell line. The cytotoxicity of compound **18j** was evaluated using MTT assay and IC_{50} value was measured. MTT assay is based on the activity of mitochondrial dehydrogenase enzyme activity and represent the metabolic rate or percentage viability of cell[28,29]. In MTT assay, the percentage viability of cell line was decreased with increased concentration of compound **18j**. Compound **18j** showed IC_{50} 132 ± 0.05 μM against NIH/3T3 cell line, which was considerably higher as compare to GI_{50} values of **18j** against NCI 60 cancer cell lines, showed that compound **18j** is very selective towards cancer cells at lower concentration as compared to healthy cell line.

It has been reported in the literature that quinoline derivatives act by inhibiting DNA topoisomerase complex[30,31]. As a result, there is a high probability that antiproliferative potency of compound **18j**, a quinolyl hydrazone could be due to DNA intercalation. Nevertheless, the studies focusing on the mechanism of action of these derivatives are currently under progress. In conclusion, it is conceivable that derivatization of such compounds will be of attention with the hope to get newer effective and selective anticancer agents.

Table 2. Five dose *in vitro* anticancer activity results[a] (cytotoxic activities of compounds expressed as **GI_{50}[b]** (μM) and **LC_{50}[c]** (μM) for compounds **18b-i** against all sixty cancer cell lines.

Panel Cell lines	Compounds									
	18b		18d		18e		18f		18g	
	GI_{50}	LC_{50}	GI_{50}	LC_{50}	GI_{50}	LC_{50}	GI_{50}	LC_{50}	GI_{50}	LC_{50}
Leukemia										
CCRF-CEM	1.14	9.60	1.78	9.32	1.88	29.0	1.86	>100	1.54	7.99
HL-60(TB)	1.86	7.46	1.83	7.78	2.02	8.19	1.76	7.60	1.91	7.48
K-562	1.36	6.52	1.48	6.63	1.71	9.20	1.09	-	1.24	6.45
MOLT-4	1.89	7.84	1.81	7.88	1.91	8.82	1.70	7.66	1.73	7.42
RPMI-8226	1.94	9.62	1.81	8.79	2.03	17.2	1.92	>100	1.91	9.11
SR	**1.12**	15.5	**1.17**	38.6	2.64	>100	**0.50**	>100	**0.44**	21.3
Non–small cell lung cancer										
A549/ATCC	2.00	6.94	1.72	6.49	1.88	6.61	1.86	6.35	1.90	7.15
EKVX	2.18	16.3	1.64	5.68	1.63	5.63	1.64	5.55	1.63	5.93
HOP-62	2.31	27.3	1.58	5.63	**1.47**	5.88	1.62	6.63	1.73	6.57
HOP-92	2.10	33.0	1.51	6.41	1.54	6.27	1.43	6.25	1.36	6.10
NCI-H226	1.85	7.08	1.69	6.64	1.83	8.01	1.63	11.6	1.85	>100
NCI-H23	1.76	6.23	1.81	6.82	1.86	7.61	1.77	6.63	1.85	7.89
NCI-H322M	1.83	5.95	1.69	5.56	1.76	5.65	1.74	5.63	1.74	5.72
NCI-H460	2.06	8.85	2.04	7.97	2.08	7.94	1.99	-	1.94	7.08
NCI-H522	1.73	6.24	1.75	6.68	1.72	6.00	1.72	6.27	2.06	15.1

Colon Cancer										
COLO 205	1.91	9.31	1.82	6.36	1.85	6.50	2.01	8.09	1.96	7.06
HCC-2998	2.19	6.26	2.03	6.15	2.12	6.56	1.91	5.99	2.07	6.98
HCT-116	1.13	-	1.70	6.48	1.77	6.17	2.13	>100	1.78	6.35
HCT-15	1.55	5.59	1.52	5.67	1.61	5.80	1.35	5.54	1.57	6.25
HT29	1.90	6.33	180	6.77	1.55	5.69	1.24	5.51	1.94	9.25
KM12	1.89	7.04	1.80	6.22	1.95	7.62	1.93	7.73	1.74	5.87
SW-620	1.97	8.22	1.96	7.31	2.03	-	1.97	-	1.92	6.85
CNS Cancer										
SF-268	2.05	7.41	2.24	7.81	2.20	-	2.26	-	1.97	7.07
SF-295	1.76	5.97	1.72	5.94	1.82	6.21	1.66	5.79	1.81	6.7
SF-539	1.97	5.87	1.79	5.71	1.84	5.88	1.84	5.87	1.76	5.92
SNB-19	2.37	14.5	1.95	6.41	1.86	6.28	1.81	6.01	1.96	7.03
SNB-75	1.86	7.12	1.94	6.46	1.70	6.17	1.75	6.35	1.77	6.50
U251	1.91	5.96	2.05	6.15	1.80	5.96	1.72	5.90	1.82	6.31
Melanoma										
LOX IMVI	1.73	5.68	1.70	5.66	1.77	5.90	1.69	5.67	1.86	7.12
MALME-3M	2.02	6.40	1.79	5.93	1.91	6.06	1.85	6.18	1.88	6.37
M14	1.53	**5.55**	1.69	6.17	1.74	5.88	1.89	8.07	1.88	6.64
MDA-MB-435	1.75	6.05	1.80	5.83	1.80	5.94	1.73	5.80	1.76	5.74
SK-MEL-2	1.90	6.17	1.73	5.99	**1.47**	5.56	1.85	6.34	1.98	7.69
SK-MEL-28	1.78	5.73	1.73	5.74	1.72	5.71	1.66	5.72	1.65	5.84
SK-MEL-5	1.72	5.56	1.54	**5.39**	1.68	5.53	1.61	5.47	1.74	5.71
UACC-257	1.81	6.10	1.79	6.16	1.66	6.09	1.61	6.01	1.88	6.89
UACC-62	1.79	6.17	1.76	5.94	1.74	9.09	1.61	5.69	1.66	5.90
Ovarian Cancer										
IGROV1	1.93	6.87	1.92	6.85	1.91	6.78	1.93	6.84	1.72	6.60
OVCAR-3	1.93	6.36	2.13	6.44	2.06	7.18	2.03	7.04	1.92	5.92
OVCAR-4	1.79	6.90	1.71	6.01	1.67	5.62	1.56	5.76	1.76	6.06
OVCAR-5	1.97	6.22	1.86	5.76	1.86	5.71	1.82	5.76	1.83	5.88
OVCAR-8	2.34	14.1	2.31	8.88	1.77	7.07	1.78	6.74	2.13	14.0
NCI/ADR-RES	1.90	7.83	1.90	7.65	1.96	7.56	1.73	6.50	1.83	9.59
SK-OV-3	2.86	25.0	1.78	5.71	1.66	5.61	1.80	5.94	1.75	5.77
Renal Cancer										
786-0	1.57	5.86	1.86	6.27	1.83	6.20	2.00	7.06	1.80	6.55
A498	1.70	7.01	1.72	5.79	1.48	5.47	1.52	5.67	1.83	6.02
ACHN	1.79	5.63	1.74	5.59	1.73	5.57	1.73	5.57	1.77	5.72
CAKI-1	1.78	5.77	1.78	5.73	1.71	5.65	1.68	5.62	1.63	5.47
RXF 393	1.66	5.65	1.55	5.58	1.60	5.82	1.41	5.34	1.67	6.10
SN12C	1.82	6.35	1.82	6.02	1.74	6.07	1.63	5.82	1.72	6.21
TK-10	1.91	5.94	2.04	5.92	1.91	5.76	1.87	5.76	1.87	6.25
UO-31	1.63	**5.47**	1.56	5.43	1.48	**5.39**	1.56	5.50	1.57	5.47
Prostate Cancer										
PC-3	1.51	6.54	1.53	6.39	1.75	6.49	1.71	6.55	1.55	5.88
DU-145	1.82	6.30	1.90	6.15	1.94	7.73	1.82	7.57	1.81	5.85
Breast Cancer										
MCF7	1.68	6.60	1.67	6.90	1.83	7.37	1.65	6.87	1.71	7.93

MDA-MB-231/ATCC	1.83	6.23	1.85	6.56	1.88	6.54	1.74	6.09	1.77	6.56
HS 578T	2.15	11.5	2.15	9.96	1.99	>100	1.86	-	2.33	>100
BT-549	1.61	6.15	2.17	1.96	1.77	6.60	1.75	6.71	1.77	6.64
T-47D	1.93	7.46	1.71	7.67	1.69	7.65	1.73	8.18	1.81	8.36
MDA-MB-468	1.73	6.17	1.69	6.04	1.67	6.22	1.53	5.78	1.83	6.96

Table 2 Continue…..

Panel Cell lines	**Compounds**							
	18h		**18i**		**18j**		**18l**	
	GI_{50}	**LC_{50}**	**GI_{50}**	**LC_{50}**	**GI_{50}**	**LC_{50}**	**GI_{50}**	**LC_{50}**
Leukemia								
CCRF-CEM	1.74	-	1.71	9.20	1.53	-	2.04	47.0
HL-60(TB)	1.64	6.90	1.29	8.25	1.39	8.21	2.00	9.95
K-562	**0.69**	6.20	1.02	5.21	**0.46**	24.4	1.66	18.3
MOLT-4	1.65	6.85	1.20	8.42	1.85	8.88	2.05	52.4
RPMI-8226	1.84	-	1.86	8.87	1.81	-	2.01	19.4
SR	**0.36**	4.20	**0.39**	>100	**0.33**	8.11	1.61	>100
Non–small cell lung cancer								
A549/ATCC	1.90	6.52	1.78	6.61	1.93	7.22	1.70	7.26
EKVX	1.67	5.67	1.72	5.73	1.75	-	1.80	6.15
HOP-62	1.62	6.66	1.47	5.89	1.55	6.11	**1.48**	6.42
HOP-92	1.47	5.99	1.45	6.08	11.42	6.67	1.51	6.05
NCI-H226	1.64	8.25	1.60	6.23	1.86	-	1.70	7.74
NCI-H23	1.78	6.76	1.78	6.62	1.88	6.65	1.90	7.76
NCI-H322M	1.74	5.66	1.71	5.55	1.70	5.76	1.79	5.75
NCI-H460	1.95	8.03	1.94	7.74	1.62	-	2.01	7.42
NCI-H522	1.63	5.80	1.70	6.49	1.79	6.87	1.89	7.89
Colon Cancer								
COLO 205	1.89	6.89	1.85	6.65	1.58	5.85	1.82	6.96
HCC-2998	1.95	6.42	2.03	6.24	1.93	6.19	2.03	6.36
HCT-116	1.04	5.02	**0.85**	4.67	**0.34**	-	1.83	7.95
HCT-15	1.43	5.64	1.52	5.46	1.27	5.15	1.85	6.17
HT29	1.44	5.46	1.63	5.98	1.59	5.99	1.81	6.17
KM12	1.79	6.50	1.80	6.81	1.67	6.29	1.85	6.39
SW-620	1.73	7.18	1.56	8.16	**0.97**	-	1.89	6.96
CNS Cancer								
SF-268	2.23	8.94	2.07	8.70	2.10	-	2.21	7.90
SF-295	1.77	6.28	1.72	5.95	1.73	6.07	1.83	6.50
SF-539	1.86	5.87	1.87	5.75	1.84	5.78	1.86	5.81
SNB-19	1.87	6.22	1.83	6.43	1.88	6.12	2.00	7.34
SNB-75	1.63	6.36	1.84	6.50	1.81	6.50	1.86	6.58
U251	1.70	5.87	1.87	6.02	1.90	6.20	1.82	6.30
Melanoma								
LOX IMVI	1.69	5.90	1.53	5.50	1.26	5.25	1.66	6.06
MALME-3M	1.85	6.21	4.87	6.10	1.91	6.22	1.85	6.08
M14	1.76	5.91	1.67	5.67	1.71	6.26	1.75	7.06
MDA-MB-435	1.75	5.91	1.73	5.77	1.71	5.80	1.73	5.79

SK-MEL-2	1.70	5.90	1.78	6.17	1.72	6.10	1.97	6.53
SK-MEL-28	1.69	5.66	1.81	5.81	1.76	5.74	1.78	5.75
SK-MEL-5	1.63	5.54	1.67	5.51	1.59	5.54	1.73	5.79
UACC-257	1.72	6.22	1.95	6.83	1.61	6.59	1.75	9.30
UACC-62	1.68	5.89	1.74	6.01	1.78	6.01	1.87	6.53
Ovarian Cancer								
IGROV1	1.91	6.79	1.75	6.80	1.79	6.96	1.84	7.34
OVCAR-3	1.97	6.51	1.94	6.85	2.05	6.83	2.17	6.70
OVCAR-4	1.72	6.03	1.81	6.59	1.75	5.78	1.82	6.92
OVCAR-5	1.82	5.71	1.94	5.84	1.92	5.82	1.79	5.70
OVCAR-8	1.98	9.08	2.07	8.23	1.91	9.28	2.14	15.0
NCI/ADR-RES	1.89	8.14	1.90	7.73	1.90	7.20	1.97	8.39
SK-OV-3	1.65	5.78	1.65	5.70	1.70	5.70	1.65	5.83
Renal Cancer								
786-0	1.78	6.18	1.71	5.91	1.89	-	1.86	7.19
A498	1.19	5.13	1.58	6.03	1.77	6.55	1.98	6.00
ACHN	1.75	5.60	1.74	5.58	1.71	5.55	1.74	5.60
CAKI-1	1.64	5.62	1.65	5.59	1.65	5.64	1.69	5.70
RXF 393	1.63	5.84	1.55	5.64	1.56	5.65	1.55	5.57
SN12C	1.71	6.06	1.69	5.93	1.69	5.9	1.78	6.30
TK-10	1.77	5.65	1.93	5.84	1.89	5.83	1.91	5.81
UO-31	1.56	5.51	1.53	5.48	1.50	5.56	1.55	**5.53**
Prostate Cancer								
PC-3	1.61	5.98	1.64	6.29	1.57	6.56	1.50	6.16
DU-145	1.88	7.28	1.92	7.13	1.86	6.87	1.76	6.24
Breast Cancer								
MCF7	1.64	7.04	1.64	6.84	1.47	6.97	1.94	7.89
MDA-MB-231/ATCC	1.71	7.28	1.62	5.85	1.67	6.87	1.81	6.64
HS 578T	1.92	-	2.16	9.34	2.19	-	2.23	9.10
BT-549	1.74	6.20	1.91	8.00	1.16	>100	1.42	6.91
T-47D	1.76	7.83	1.82	7.60	1.98	8.00	1.92	8.71
MDA-MB-468	1.70	6.67	1.70	6.11	1.65	5.95	1.79	6.54

[a] Data obtained from the NCI's *in vitro* human cancer cell lines screen.

[b] Required molar concentration to inhibit 50% of the growth of cancer cell lines.

[c] LC_{50}: Required Molar concentration required to kill 50% of the cells.

The most active compounds (**lowest GI_{50}** and **LC_{50} value**) are highlighted in red.

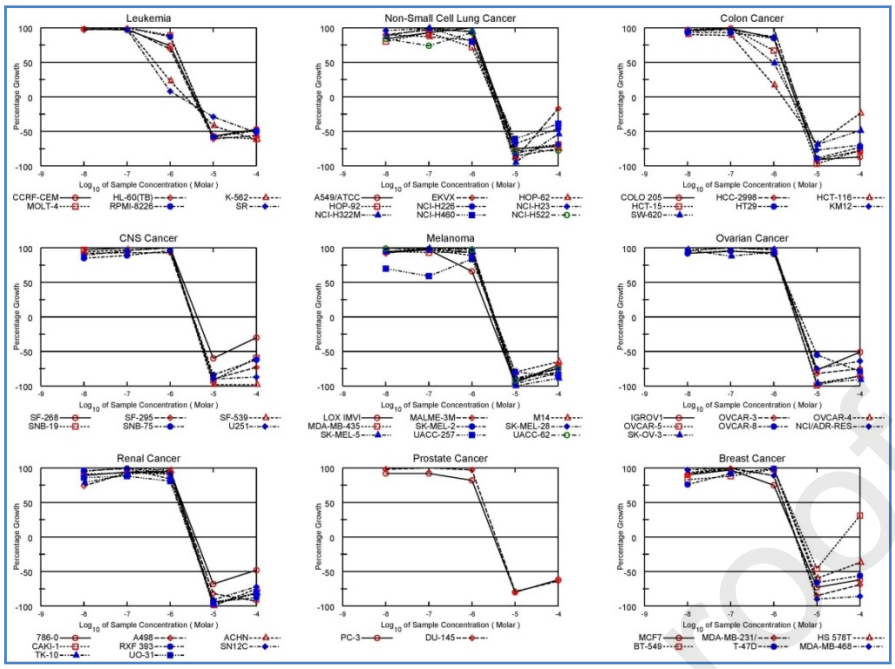


Figure 2. Five dose assay graph of compound **18j** (NSC: D-804241/1) against nine panel cancer cell line at NCI.

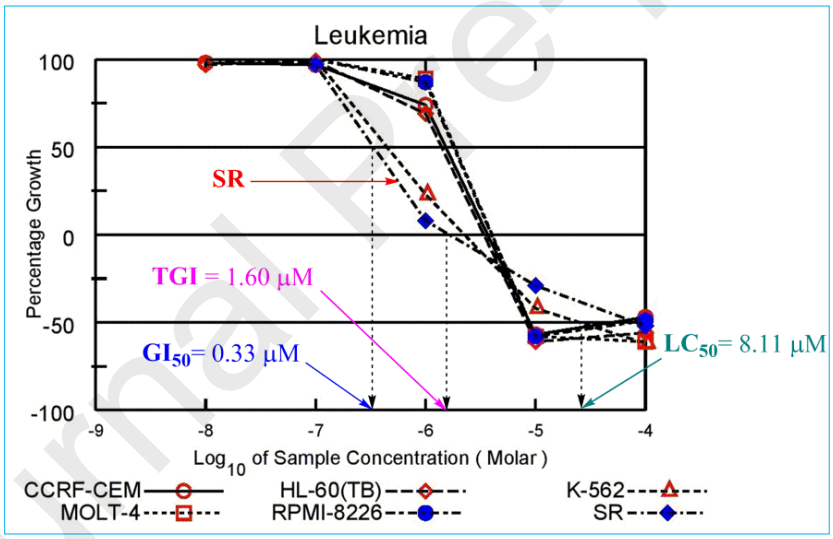


Figure 3. Dose response curve of Leukemia subpanel of compound **18j** (NSC: D-804241/1) showing end point calculations for SR cell line at five dose concentrations (log dilution from 10^{-4} mol/L to 10^{-8} mol/L).

Table 3. *In vitro* anticancer activity comparison of compounds **18j** and clinically used anticancer agents (Bendamustine and Chlorambucil).

Compd.	NSC No.	Log (High Conc.)	Potency in log_{10}(M) unit in µmol/L			No. of expts	N.C.L.[d]	MID[e] GI_{50}
			GI_{50} EDP[c]	TGI EDP[c]	LC_{50} EDP			
18j	804241/1	-5.0	-5.80	-5.49	-5.14	2	60	1.58
BENDA[a]	138783	-4.0	-4.153	-4.018	-4.004	3	60	60

CHLB[b]	3088	-5.0	-4.785	-4.282	-4.062	2	59	52

a Bendamustine hydrochloride (Treanda).

b Chlorambucil (Leukeran).

c Endpoint.

d Number of cell lines.

e Mean graph midpoint (MG-MID). Data were obtained from the Developmental Therapeutics Program, NCI main web site for comparison purpose.

2.3 *In vitro* antimicrobial activity study

In continuation of biological activity, all newly prepared quinolyl hydrazones **18a-p,** were screened for their antimicrobial activity against (*S. aureus* (MTCC 96), *B. subtilis* (MTCC 619) as Gram positive bacteria, *E. coli* (MTCC 739), *P. aeruginosa* (MTCC 741) as Gram negative bacteria. For antifungal activity *A. niger* (MTCC 282) and *C. albicans* (MTCC 183) were used. Zone of inhibition against all the six pathogenic strains were measured using paper disc diffusion technique [32]. MIC of the test compound was determined by agar streak dilution method [33]. The standard antibiotics, Ciprofloxacin and gresiofulvin were taken as the standard references during for antibacterial and antifungal activity respectively.

The results revealed that, all the quinolyl hydrazones demonstrated outstanding antibacterial as well as antifungal activity with MIC value ranging from 6.25 µg/mL to 100 µg/mL as summarized in **Table 4**. Among all quinolyl hydrazones, compound **18p** showed excellent inhibitory activity (MIC = 6.25 µg/mL) against the Gram +*ve* bacteria *S. aureus* while compounds **18g**, **18i**, **18j**, **18o** also displayed noteworthy inhibition with MIC = 12.5 µg/mL against the same bacterial strain. Against *B. subtilis* compounds **18a**, **18e**, **18i**, **18l** and **18n** showed MIC = 12.5 µg/mL with remarkable inhibition (**Table 4**). In case of Gram -*ve* bacteria *E. coli*, the best inhibitory activity was observed for compounds **18i** with MIC = 6.25 µg/mL while compounds **18k**, **18n** and **18p** have shown potency with MIC = 12.5 µg/mL. In case of *P. aeruginosa*, the best inhibitory activity was observed for compounds **18p** with MIC = 6.25 µg/mL and compounds **18i**, **18l** and **18o** demonstrated good potency against the same bacterial strain with MIC = 12.5 µg/mL (**Table 4**). Against the fungal strain *C. albicans,* compounds **18c**, **18e**, **18j**, **18** and **18r** exhibited potent inhibitory activity (MIC = 12.5 µg/mL) while in the case of *A. niger,* compounds **18a**, **18g**, **18i**, **18l** and **18p** demonstrated excellent inhibitory activity with MIC = 12.5 µg/mL (**Table 4**). In conclusion, all the quinolyl hydrazones showed

good to excellent antimicrobial activity and these kinds of compounds possessing quinoline as a core unit along with hydrazone linkages could be develop as an effective antimicrobial agents.

Table 4. *In vitro* antimicrobial screening of compounds, 18a-p.

ID	Zone of inhibition in mm and (MIC in µg/mL)											
	Gram(+*ve*) bacteria				Gram(-*ve*) bacteria				Fungi			
	S. aureus		*B. subtilis*		*E. coli*		*P. aeruginosa*		*C. albicans*		*A. niger*	
	Zone (mm)	MIC (µg/mL)	Zone (mm)	MIC (µg/mL)	Zone (mm)	MIC (µg/mL)	Zone (mm)	MIC (µg/mL)	Zone (mm)	MIC (µg/mL)	Zone (mm)	MIC (µg/mL)
18a	21±0.1	100	25±0.3	12.5	22±0.1	50	19±0.1	100	24±0.4	25	25±0.2	12.5
18b	24±0.2	25	24±0.2	25	19±0.3	125	24±0.3	25	19±0.2	125	20±0.3	100
18c	25±0.1	12.5	21±0.1	100	24±0.2	25	18±0.2	125	25±0.1	12.5	24±0.1	25
18d	21±0.3	100	23±0.2	25	20±0.4	100	22±0.3	50	21±0.3	100	20±0.4	100
18e	22±0.2	50	25±0.3	12.5	20±0.2	100	24±0.1	25	25±0.2	12.5	21±0.3	100
18f	20±0.1	100	21±0.1	100	18±0.2	125	19±0.2	125	20±0.4	100	24±0.2	25
18g	25±0.3	12.5	22±0.3	50	21±0.3	100	19±0.3	100	24±0.3	25	25±0.1	12.5
18h	24±0.2	25	17±0.2	125	20±0.1	125	22±0.2	50	18±0.2	125	17±0.2	100
18i	26±0.1	12.5	25±0.1	12.5	27±0.3	6.25	25±0.3	12.5	22±0.3	50	25±0.3	12.5
18j	25±0.1	12.5	24±0.1	25	24±0.2	25	23±0.1	25	25±0.1	12.5	23±0.2	25
18k	23±0.3	25	22±0.3	50	26±0.1	12.5	24±0.2	25	21±0.2	100	22±0.2	50
18l	24±0.2	25	25±0.2	12.5	24±0.3	25	25±0.1	12.5	24±0.3	25	25±0.2	12.5
18m	22±0.4	50	23±0.1	25	21±0.2	100	22±0.3	50	24±0.1	25	22±0.1	50
18n	24±0.2	25	26±0.3	12.5	25±0.3	12.5	23±0.1	25	25±0.3	12.5	23±0.3	25
18o	25±0.1	12.5	24±0.2	25	24±0.3	25	26±0.3	12.5	23±0.2	25	24±0.3	25
18p	28±0.3	6.25	24±0.1	25	26±0.2	12.5	27±0.2	6.25	24±0.1	25	25±0.1	12.5
A	**33**±0.1	**<3.12**	**30**±0.2	**<3.12**	**32**±0.1	**<3.12**	**31**±0.2	**<3.12**	-----		----	
B	----		----		----		----		**31**±0.1	**<3.12**	**30**±0.2	**<3.12**
DMSO	----		----		----		----		----		----	

A - Ciprofloxacin, standard reference drug for antibacterial screening.
B – Griseofulvin, standard reference drug for antifungal screening.

2.4 Molecular docking study

DNA topoisomerase I is a nuclear enzyme crucial for solving topological complications that happen throughout DNA transcription, replication, and chromosome segregation [34]. DNA topoisomerase 1 forms a single-stranded nick onto the DNA by a nucleophilic attack on the DNA phosphodiester bond to make a "cleavage complex" in which the enzyme is covalently linked to the 3′ end of the broken DNA strand followed by a "controlled rotation" of the broken scissile strand nearby the unbroken strand, bring about in the DNA superhelical tension relaxation. Ultimately, the 5′ end of the scissile strand facilitates a nucleophilic attack on the phosphotyrosyl-DNA phosphodiester to transfer the DNA and release the enzyme, which ends the catalytic cycle of the enzyme [35,36]. Trapped Top1ccs generate unfavourable lesions, which lead to the formation of DNA double-strand breaks (DSBs) upon collision with continuing replication forks and/or transcription machinery and are responsible for the killing of proliferating malignant cancerous cells [37,38].

Quinoline containing compounds represent a large number of chemotherapeutic agents demonstrating cytotoxic activity through DNA intercalation and hence affecting the replication of DNA [39]. Topo I play a crucial role in cell proliferation and considered as chief target for the prevention of prompt proliferation of cancerous cells [36]. A number of research studies have been carried out on quinoline derivatives act by inhibiting human DNA topoisomerase complex [11].

Subsequently, the molecular docking of all the active compounds (**18b**, **18d**, **18e**, **18f**, **18g**, **18h**, **18i**, **18j**, **18l**) with the target protein, human DNA topoisomerase-I enzyme (PDB: 1SC7) has been carried out using QIAGEN CLC Drug Discovery Work Bench 3.0. Agreement of Validation of Molecular Docking performed was emphasised by redocking of co-crystallized ligand Indenoisoquinoline (M38 C990) on to the receptor pocket. Validated Docking results are in good concurrence with existing co-crystal at the active site with an RMSD of 0.024 A^{o}[40]. Results of the docking study of the active compounds (**18b**, **18d**, **18e**, **18f**, **18g**, **18h**, **18i**, **18j**, **18l**) into the active site of enzyme revealed different molecular interactions exhibiting hydrogen bonding, π interactions and hydrophobic interactions between the synthesized ligands and topoisomerase enzyme (**Table 5** and **Figure 4**).

All the docked molecules exhibited pronounced interactions both with Nucleotides (DNA, TGP) and topoisomerase subunits Arg-364 (active site interaction) by hydrogen bonding through the nitrogen atoms of hydrazone linkage. The CLC dock score for all the nine active

compounds was observed in between -35.54 to -57. 65. The highest dock score (-57.65) was observed for compound **18l** (**Table 5**).The most potent and active compound of the series **18j** interacted with the amino acid residue Arg-364 and TGP base pair of the DNA through hydrogen bonding with dock score of -40.45 (**Table 5**). Compound **18j** also showed hydrophobic interaction with Asn-722, Leu-721, Thr-718, Lys-425 amino acid residues. Molecules exhibited interactions both with DNA and topoisomerase subunits by hydrogen bonding except compound **18l**, which was intercalated only with DNA by hydrogen bonding and compound **18i,** interacted only with Arg-364 amino acid residue of topoisomerase enzyme by forming a hydrogen bond between NH2 of Arg-364 and nitrogen atom of hydrazone linkage (**Figure 4**). Compounds **18e** and **18g** interacted with NH_2 of Lys-425 amino acid residue by hydrogen bonding through oxygen of alkoxy side chain. Along with the hydrogen bonding all active compounds showed number of hydrophobic interaction with different amino acid residues including Asn-722, Leu-721, Pro-431, Asp-533 Thr-718 and Asp-533.

From the results of docking, all the active compounds approach to DNA towards the DNA cleavage site in the DNA–topoisomerase-I and developing a stable ternary complex through noncovalent interactions and thereby stabilizing it which leads to inhibitory effect on DNA–topoisomerase-I. This prevents DNA re-ligation and therefore causes DNA damage which results in apoptosis[41]. Binding interactions of some active compounds with the DNA–topoisomerase-I receptor are shown in **Figure 4**.

Table 5. Molecular docking results of human DNA topoisomerase-I enzyme (PDB: 1SC7).

Ligand	Hydrogen bonds within $6A^0$	Hydrophobic/Stearic interactions within $6A^0$	CLC Dock Score
18b	Arg-364, (TGP)	Asn-722, Thr-718	-51.83
18d	Arg-364, (TGP)	Asn-722, Asp-533, Thr-718	
18f	Arg-364, (TGP)	Arg-316	-36.11
18e	Lys-425, (TGP)	Arg-364, Asn-352, Glu-356, Lys-425	-45.44
18h	Arg-364, (TGP)	Asn-722, Leu-721, Pro-31, Thr-718, Gln-748, Lys-751, Thr-747	-53.38
18g	Lys-425, (TGP)	Arg-364, Asn-722, Asp-533, Glu-356, Lys-374, Lys-425, Thr-718	-43.43
18j	Arg-364, (TGP)	Asn-722, Leu-721, Thr-718, Lys-425	-40.45
18i	Arg-364	Arg-364, Asn-722, Asp-533, Glu-356, Lys-374, Lys-425, Thr-718	-35.54
18l	(TGP)	Asn-352, Glu-356, Gly-359, Ile-355, Lys-354, Lys-374, Pro-357 Pro-358	-57.65

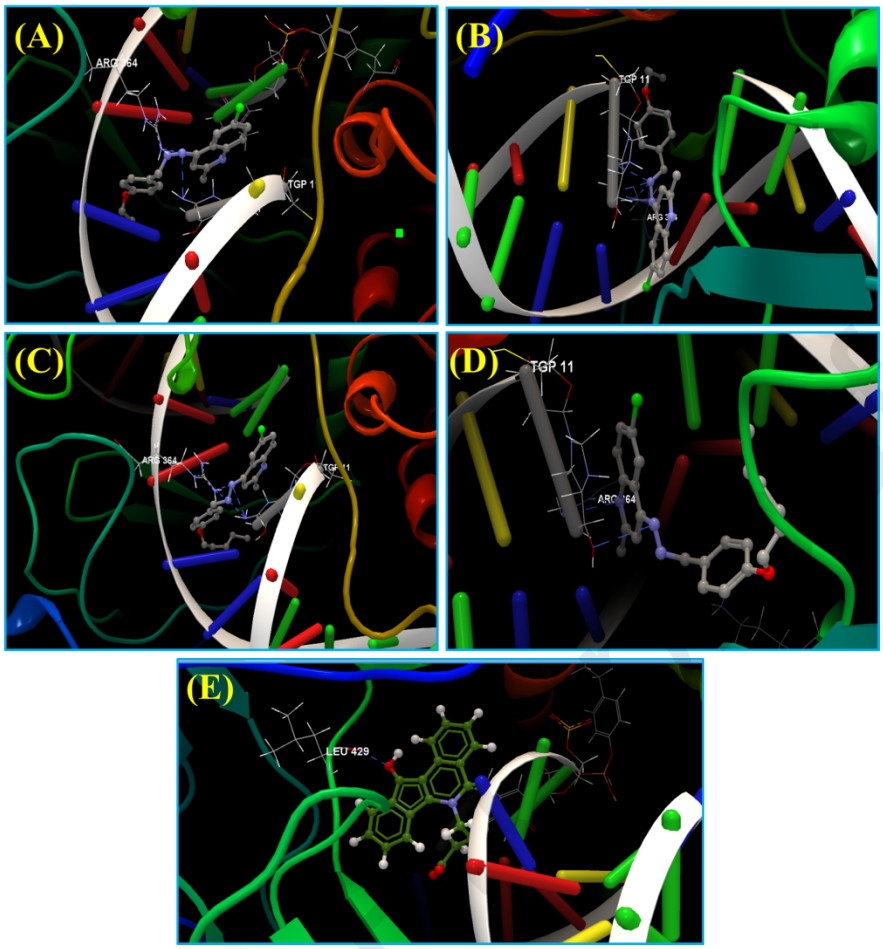


Figure 4. Binding pose of the active compounds (A) **18b**, (B) **18f,** (C) **18h** and (D) **18j** in the active site of DNA–topo-I receptor (PDB: **1SC7**). Blue dotted lines represent the hydrogen bond with amino acid residue of the enzyme and DNA base pairs and (E) represents the validation of topo-I co-crystallised ligand interaction with Arg 364 and Leu 429.

3.0 Experimental section

3.1 Chemistry

The chemicals were used as received from local companies without further purification. Organic solvents were purified by distillation prior to use. Column chromatography was carried out using silica gel (60-120 mesh). Thin layer chromatography was performed on the pre-coated silica gel 60 F_{254} aluminium sheets. Melting points are determined in open capillary and are uncorrected. FT-IR spectra were recorded on Bruker Alpha FTIR spectrometer between 4000-400 cm^{-1} in solid state as KBr discs. The NMR spectra were recorded on 400 MHz Bruker Avance-III instrument and chemical shifts are given in parts per million. In the NMR data for

^{19}F decoupled ^{1}H NMR experiments, the data for the affected signals only are included. ^{19}F chemical shift values are of ^{1}H decoupled ^{19}F signals. Thermo Fischer elemental analyser was used for elemental analysis. ESI mass spectra were recorded on Waters' Xevo G2-XS QToF mass spectrometer at Zydus Research Centre, Ahmedabad. Compounds **9a/9b** [20,42], **12a-f** [22]**, 15** and **17** [23,24] were synthesized according to the procedures reported in the literature (see supporting information).

3.2 General Procedure for the Synthesis of 4-{ N'-arylidene-hydrazinyl}-6-bromo-2-methylquinoline.

The final target compounds were synthesized by following the general method [20,42]. Alkyloxy benzaldehydes **12/15/17** (0.46 mmol) and 6-bromo-2-methyl-quinolin-4-yl-hydrazine **9a** (0.46 mmol) were dissolved in ethanol (5 mL). A catalytic amount of concentrated sulphuric acid was added. The reaction mixture was heated at 80 °C for 4 h, and then kept at room temperature overnight. The resultant solid separated was filtered, washed with chilled ethanol and crystallized from ethanol to afford the pure final hydrazones **18**. Yield: 76-82%.

3.2.1 [6-Bromo-4-{N'-(4-ethyloxybenzylidene)hydrazinyl}-2-methylquinoline], 18a.

Yield = 0.072g, 82%; Yellow Solid; MP = 202 °C; IR (KBr) cm^{-1}: 3288, 2977, 1569, 1242, 846, 771; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.1 (t, 3H, terminal -CH_3), 2.73 (s, 3H, -CH_3), 4.12 (q, 2H, alkoxy-OCH_2), 7.06 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.55 (s, 1H, -N=CH-), 7.87 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.90 (d, 1H, *J* = 9.2 Hz, Ar-H), 8.01 (d, 1H, *J* = 9.2 Hz, Ar-H), 8.55 (s, 1H, Ar-H), 8.70 (d, 1H, *J* = 2 Hz, Ar-H), 12.22 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 15.5 (-CH_3), 20.6 (-CH_3), 63.9 (-OCH_2), 101.0, 115.7, 115.9, 119.4, 122.6, 125.7, 126.1, 130.05, 136.6, 137.71, 150.6, 151.16 155.1, 161.4; Anal. Calcd. for $C_{19}H_{18}BrN_3O$: C, 59.39; H, 4.72; N, 10.94; found C, 59.36; H, 4.78; N, 10.88; Mass (TOF MS ES+): m/z 384.07 $(M+H)^+$, 386.06 $(MH+2)^+$.

3.2.2 [6-Bromo-2-methyl-4-{N'-(4-propyloxybenzylidene)hydrazinyl}quinoline], 18c.

Yield = 0.071g, 78%; Yellow Solid; MP = 204 °C; IR (KBr) cm^{-1}: 3211, 2964, 1605, 1249, 1168, 913, 845; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 0.99 (t, 3H, terminal -CH_3), 1.75 (m, 2H, -CH_2-), 2.71 (s, 3H, -CH_3), 4.01 (t, 2H, alkoxy-OCH_2), 7.06 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.53 (s, 1H, -N=CH-), 7.81 (m, 3H, Ar-H), 8.11 (d, 1H, *J* = 9.2 Hz, Ar-H), 8.53 (s, 1H, Ar-H),

8.82 (s, 1H, Ar-H), 12.22 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 10.8 (-CH_3), 15.5 (-CH_3), 22.4 (-CH_2), 69.7 (-OCH_2), 101.0, 115.4, 115.8, 119.5, 122.2, 125.7, 126.1, 130.0, 136.7, 137.7, 150.7, 150.9, 155.0, 161.6 ; Anal. Calcd. for $C_{20}H_{20}BrN_3O$: C, 60.31; H, 5.06; N, 10.55; found C, 60.36; H, 5.01; N, 10.59; Mass (TOF MS ES+): m/z 398. 09 $(M+H)^+$, 400.08 $(MH+2)^+$.

3.2.3 [6-Bromo-4-{N'-(4-butyloxybenzylidene)hydrazinyl}-2-methylquinoline], 18e.

Yield = 0.075g, 80%; Yellow Solid; MP = 186 °C; IR (KBr) cm^{-1}: 3211, 2935, 1604, 1445, 1251, 772; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 0.95 (t, 3H, terminal -CH_3), 1.46 (m, 2H, -CH_2), 1.72 (m, 2H, -CH_2), 2.72 (s, 3H, -CH_3), 4.05 (t, 2H, alkoxy-OCH_2), 7.06 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.54 (s, 1H, -N=CH-), 7.82 (m, 3H, Ar-H), 8.11 (dd, 1H, *J* = 8.8 Hz, Ar-H), 8.54 (s, 1H, Ar-H), 8.83 (s, 1H, Ar-H), 12.23 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 14.1 (-CH_3), 15.5 (-CH_3), 19.1 (-CH_2), 31.0 (-CH_2), 67.9 (-OCH_2), 101.0, 115.3, 115.8, 119.5, 122.2, 125.6, 126.0, 130.0, 136.7, 137.7, 150.6, 150.9, 155.1, 161.6 ; Anal. Calcd. for $C_{21}H_{22}BrN_3O$: C, 61.17; H, 5.38; N, 10.19; found C, 61.15; H, 5.36; N, 10.16; Mass (TOF MS ES+): m/z 412.10 $(M+H)^+$, 414.10 $(MH+2)^+$.

3.2.4 [6-Bromo-2-methyl-4-{N'-4-(pentyloxybenzylidene)hydrazinyl}quinoline], 18g.

Yield = 0.066g, 68%; Yellow Solid; MP = 194 °C; IR (KBr) cm^{-1}: 3211, 2935, 1643, 1253, 899; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 0.90 (t, 3H, terminal -CH_3), 1.52 (m, 4H, -CH_2), 1.74 (m, 2H, -CH_2), 2.70 (s, 3H, -CH_3), 4.05 (t, 2H, alkoxy-OCH_2), 7.06 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.52 (s, 1H, -N=CH-), 7.81 (m, 3H, Ar-H), 8.08 (d, 1H, *J* = 8.4 Hz, Ar-H), 8.53 (s, 1H, Ar-H), 8.82 (s, 1H, Ar-H), 12.18 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 14.3 (-CH_3), 20.5 (-CH_3), 22.3 (-CH_2), 28.0 (-CH_2), 28.6 (-CH_2), 68.2 (-OCH_2), 100.9, 115.3, 115.7, 119.6, 122.1, 125.6, 126.0, 130.0, 136.7, 137.5, 150.7, 150.8, 154.8, 161.5; Anal. Calcd. for $C_{22}H_{24}BrN_3O$: C, 61.98; H, 5.67; N, 9.86; found C, 61.96; H, 5.69; N, 9.89; Mass (TOF MS ES+): m/z 426.08 $(M+H)^+$, 428.08 $(MH+2)^+$.

3.2.5 [6-Bromo-4-{N'-(4-hexyloxybenzylidene)hydrazinyl}-2-methylquinoline], 18i.

Yield = 0.072g, 72%; Yellow Solid; MP = 190 °C; IR (KBr) cm^{-1}: 3210, 2925, 1604, 1211, 772; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 0.86 (t, 3H, terminal-CH_3), 1.29 (d(b), 3H, -CH_2), 1.40 (t(b), 2H, -CH_2), 1.72 (m, 2H, -CH_2), 2.68 (s, 3H, -CH_3), 4.02 (t, 2H, alkoxy-OCH_2), 7.03 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.47 (s, 1H, -N=CH-), 7.80 (t(b), 3H, Ar-H), 7.06 (d, 1H, *J* = 8.8 Hz, Ar-H), 8.50 (s, 1H, Ar-H), 8.78 (s, 1H, Ar-H), 12.17 (s, 1H, -NH); ^{13}C NMR (100 MHz,

DMSO-d_6, δ ppm): 14.3 (-CH_3), 20.7 (-CH_3), 22.4 (-CH_2), 25.5 (-CH_2), 28.9 (-CH_2), 31.4 (-CH_2), 68.2 (-OCH_2), 101.0, 115.4, 115.9, 119.4, 122.5, 122.7, 125.6, 126.1, 130.0, 136.6, 137.7, 150.5, 150.7, 154.8, 161.5; Anal. Calcd. for $C_{23}H_{26}BrN_3O$: C, 62.73; H, 5.95; N, 9.54; found C, 62.75; H, 5.92; N, 9.59; Mass (TOF MS ES+): m/z 440.02 $(M+H)^+$.

3.2.6 [6-Bromo-2-methyl-4-{N'-(4-octyloxybenzylidene)hydrazinyl}quinoline], 18k.

Yield = 0.087g, 82%; Yellow Solid; MP = 186 °C; IR (KBr) cm^{-1}: 3242, 2925, 1602, 897; ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 0.84 (t, 3H, terminal -CH_3), 1.25 (d(b), 8H, -CH_2), 1.39 (d(b), 2H, -CH_2), 1.68-1.74 (m, 2H, -CH_2), 2.67 (s, 3H, -CH_3), 4.02 (t, 2H, alkoxy-OCH_2), 7.03 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.45 (s, 1H, -N=CH-), 7.79 (t(b), 3H, Ar-H), 8.05 (d, 1H, *J* = 8.8 Hz, Ar-H), 8.49 (s, 1H, Ar-H), 8.76 (s, 1H, Ar-H), 12.17 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 14.3 (-CH_3), 20.4 (-CH_3), 22.5 (-CH_2), 25.8 (-CH_2), 28.9 (-CH_2), 29.0 (-CH_2), 29.1 (-CH_2), 31.6 (-CH_2), 68.1 (-OCH_2), 100.9, 115.4, 115.9, 119.7, 122.3, 126.0, 130.0, 136.7, 137.6, 138.0, 150.9, 155.1, 156.5, 161.4 ; Anal. Calcd. for $C_{25}H_{30}ClN_3O$: C, 64.10; H, 6.46; N, 8.97; found C, 64.14; H, 6.49; N, 8.95; Mass (TOF MS ES+): m/z 468.16 $(M+H)^+$, 470.16 $(MH+2)^+$.

3.2.7 4-[N'-{3,4-Bis-octyloxybenzylidene}hydrazinyl]-6-bromo-2-methylquinoline, 18m.

Yield = 0.106g, 78%; Yellow Solid; MP = 184 °C; IR (KBr) cm^{-1}: 3212, 2923, 1607, 1270, 858; ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 0.85 (t, 6H, terminal -CH_3), 1.26 (d(b), 16H, -CH_2), 1.46 (d(b), 4H, -CH_2), 1.74 (m, 4H, -CH_2), 2.66 (s, 3H, -CH_3), 4.01 (t, 2H, alkoxy-OCH_2), 4.06 (t, 2H, alkoxy-OCH_2), 7.05 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.34 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.42 (s, 1H, Ar-H), 7.46 (s, 1H, -N=CH-), 7.77 (d, 1H, *J* = 9.2 Hz, Ar-H), 8.01 (d, 1H, *J* = 8.8 Hz, Ar-H), 8.45 (s, 1H, Qui-H), 8.76 (s, 1H, Qui-H), 11.97 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 14.3 (-CH_3), 20.6 (-CH_3), 22.5 (-CH_2), 26.0 (-CH_2), 26.1 (-CH_2), 29.2 (-CH_2), 29.3 (-CH_2), 31.7 (-CH_2), 69.1 (-OCH_2), 101.0, 111.8, 113.4, 115.4, 122.2, 123.4, 126.3, 131.3, 134.1, 137.3, 149.2, 151.1, 152.0, 155.0, 161.5; Anal. Calcd. for $C_{33}H_{46}BrN_3O_2$: C, 66.43; H, 7.77; N, 7.04; found C, 66.43; H, 7.77; N, 7.04; Mass (TOF MS ES+): m/z 596.29 $(M+H)^+$, 598.28 $(MH+2)^+$.

3.2.8 6-Bromo-2-methyl-4-[N'-{3,4,5-tris-octyloxybenzylidene}hydrazinyl]quinoline, 18o.

Yield = 0.122g, 74%; Yellow Solid; MP = 180 °C; IR (KBr) cm^{-1}: 3238, 2924, 1607, 1117, 722; ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 0.85 (t, 9H, terminal -CH_3), 1.27 (s(b), 26H, -CH_2), 1.55 (d(b), 6H, -CH_2), 1.73 (m, 4H, -CH_2), 2.73 (s, 3H, -CH_3), 3.91 (t, 6H, alkoxy-

OCH_2), 7.12 (s(b), 2H, Ar-H), 7.51 (s, 1H, -N=CH), 7.83 (d, 1H, *J* = 9.2 Hz, Ar-H), 7.11 (d, 1H, *J* = 9.2 Hz, Ar-H), 8.49 (s, 1H, Ar-H), 8.83 (s, 1H, Ar-H), 12.25 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 14.3 (-CH_3), 20.6 (-CH_3), 22.5 (-CH_2), 26.0 (-CH_2), 26.1 (-CH_2), 29.2 (-CH_2), 30.3 (-CH_2), 31.7 (-CH_2), 68.4 (-OCH_2), 101.2, 107.0, 119.7, 122.3, 122.3, 127.1, 129.2, 134.2, 137.6, 140.6, 150.9, 151.4, 153.4, 155.6; Anal. Calcd. for $C_{41}H_{62}ClN_3O_3$: C, 67.94; H, 8.62; N, 5.80; found C, 67.91; H, 8.65; N, 5.84; Mass (TOF MS ES+): m/z 724.34 $(M+H)^+$, 726.34 $(MH+2)^+$.

3.3 General Procedure for the Synthesis of 4-{ N'-arylidene-hydrazinyl}-6-chloro-2-methylquinoline.

The final target compounds were synthesized by following the general method [20,42]. Alkyloxy benzaldehydes **12/15/17** (0.46 mmol) and 6-chloro-2-methyl-quinolin-4-yl-hydrazine **9b** (0.46 mmol) were dissolved in ethanol (5 mL). A catalytic amount of concentrated sulphuric acid was added. The reaction mixture was heated at 80 °C for 4 h, and then kept at room temperature overnight. The resultant solid separated was filtered, washed with chilled ethanol and crystallized from ethanol to afford the pure final hydrazones **18**. Yield: 76-82%.

3.3.1 6-Chloro-4-{N'-(4-ethyloxybenzylidene)hydrazinyl}-2-methylquinoline], 18b.

Yield = 0.072g, 74%; Yellow Solid; MP = 210 °C; IR (KBr) cm^{-1}: 3288, 2977, 1606, 1211, 847; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.10 (t, 3H, terminal -CH_3), 2.73 (s, 3H, -CH_3), 4.12 (q, 2H, alkoxy-OCH_2), 7.05 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.53 (s, 1H, -N=CH-), 7.87 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.89 (d, 1H, *J* = 9.2 Hz, Ar-H), 8.00 (d, 1H, *J* = 8.8 Hz, Ar-H), 8.54 (s, 1H, Ar-H), 8.69 (s, 1H, Ar-H), 12.19 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 15.5 (-CH_3), 20.6 (-CH_3), 63.9 (-OCH_2), 101.1, 115.3, 115.5, 122.3, 122.7, 125.6, 126.1, 130.1, 131.3, 134.1, 150.7, 151.1, 155.2, 161.4; Anal. Calcd. for $C_{19}H_{18}ClN_3O$: C, 67.16; H, 5.34; N, 12.37; found C, 67.16; H, 5.34; N, 12.37; Mass (TOF MS ES+): m/z 340.12 $(M+H)^+$, 342.12 $(MH+2)^+$.

3.3.2 [6-Chloro-2-methyl-4-{N'-(4-propyloxybenzylidene)hydrazinyl}quinoline], 18d.

Yield = 0.075g, 74%; Yellow Solid; MP = 200 °C; IR (KBr) cm^{-1}: 3207, 2944, 1600, 1209, 772; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.10 (t, 3H, terminal -CH_3), 1.75 (m, 2H, -CH_2-

), 2.72 (s, 3H, -CH_3), 3.73 (t, 2H, alkoxy-OCH_2), 7.06 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.53 (s, 1H, -N=CH-), 7.88 (m, 3H, Ar-H), 8.00 (dd, 1H, *J* = 2.0 Hz, *J* = 8.8 Hz, Ar-H), 8.54 (s, 1H, Ar-H), 8.68 (d, 1H, *J* = 2.0 Hz, Ar-H), 12.21 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 10.8 (-CH_3), 15.5 (-CH_3), 22.4 (-CH_2), 69.7 (-OCH_2), 101.1, 115.4, 115.5, 122.3, 122.7, 125.6, 126.1, 130.1, 131.3, 134.2, 150.7, 151.1, 155.2, 161.6; Anal. Calcd. for $C_{20}H_{20}ClN_3O$: C, 67.89; H, 5.70; N, 11.88; found C, 67.83; H, 5.75; Cl, 11.90; Mass (TOF MS ES+): m/z 354.13 $(M+H)^+$, 356.13 $(MH+2)^+$.

3.3.3 [4-{N'-(4-Butyloxybenzylidene)hydrazinyl}-6-chloro-2-methylquinoline], 18f.

Yield = 0.078g, 76%; Yellow Solid; MP = 198 °C; IR (KBr) cm^{-1}: 3202, 2935, 1643, 1200, 772; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 0.94 (t, 3H, terminal -CH_3), 1.46 (m, 3H, -CH_2), 1.72 (m, 2H, -CH_2), 2.71 (s, 3H, -CH_3), 4.04 (t, 2H, alkoxy-OCH_2), 7.06 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.52 (s, 1H, -N=CH-), 7.85 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.88 (d, 2H, *J* = 9.2 Hz, Ar-H), 7.98 (d, 1H, *J* = 9.2 Hz, Ar-H), 8.53 (s, 1H, Ar-H), 8.68 (s, 1H, Ar-H), 12.17 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 14.1 (-CH_3), 15.5 (-CH_3), 19.1 (-CH_2), 31.1 (-CH_2), 67.9 (-OCH_2), 101.9, 115.4, 115.8, 119.5, 122.6, 125.6, 126.1, 130.0, 131.3, 133.9, 150.6, 150.9, 155.1, 161.6; Anal. Calcd. for $C_{21}H_{22}ClN_3O$: C, 68.56; H, 6.03; N, 11.42; found C, 68.51; H, 6.01; N, 11.44; Mass (TOF MS ES+): m/z 368.15 $(M+H)^+$, 370.15 $(MH+2)^+$.

3.3.4 [6-Chloro-2-methyl-4-{N'-(4-pentyloxybenzylidene)hydrazinyl}quinoline], 18h.

Yield = 0.079g, 72%; Yellow Solid; MP = 190 °C; IR (KBr) cm^{-1}: 3235, 2932, 1604, 1253, 772; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 0.90 (t, 3H, terminal -CH_3), 1.50 (m, 4H, -CH_2), 1.74 (m, 2H, -CH_2), 2.72 (s, 3H, -CH_3), 4.05 (t, 2H, alkoxy-OCH_2), 7.06 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.53 (s, 1H, -N=CH-), 7.88 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.88 (d, 1H, *J* = 8.8 Hz, Ar-H), 8.00 (d, 1H, *J* = 8.8 Hz, Ar-H), 8.54 (s, 1H, Ar-H), 8.69 (s, 1H, Ar-H), 12.20 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 14.3 (-CH_3), 20.7 (-CH_3), 22.3 (-CH_2), 28.1 (-CH_2), 28.7 (-CH_2), 68.2 (-OCH_2), 101.0, 115.3, 115.5, 122.5, 122.6, 126.1, 130.0, 131.2, 134.0, 137.7, 150.5, 151.0, 155.2, 161.5; Anal. Calcd. for $C_{22}H_{24}ClN_3O$: C, 69.19; H, 6.33; N, 11.00; found C, 69.16; H, 6.35; N, 11.04; Mass (TOF MS ES+): m/z 382.13 $(M+H)^+$, 384.13 $(MH+2)^+$.

3.3.5 [6-Chloro-4-{N'-(4-hexyloxybenzylidene)hydrazinyl}-2-methylquinoline], 18j.

Yield = 0.086g, 76%; Yellow Solid; MP = 188 °C; IR (KBr) cm^{-1}: 3242, 2928, 1604, 1252, 846; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 0.88 (t, 3H, terminal -CH_3), 1.38 (m, 6H, -CH_2), 1.73 (t(br), 2H, -CH_2), 2.72 (s, 3H, -CH_3), 4.05 (t, 2H, alkoxy-OCH_2), 7.06 (d, 2H, *J* = 8.4 Hz,

Ar-H), 7.53 (s, 1H, -N=CH- proton), 7.86 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.89 (d, 1H, *J* = 9.2 Hz, Ar-H), 7.99 (d, 1H, *J* = 8.8 Hz, Ar-H), 8.54 (s, 1H, Ar-H), 8.69 (s, 1H, Ar-H), 12.20 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 14.3 (-CH_3), 20.5 (-CH_3), 22.4 (-CH_2), 25.5 (-CH_2), 28.9 (-CH_2), 31.4 (-CH_2), 68.2 (-OCH_2), 100.9, 115.3, 122.2, 122.6, 125.6, 126.0, 130.0, 131.4, 137.3, 150.7, 150.9, 155.0, 161.5; Anal. Calcd. for $C_{22}H_{24}ClN_3O$: C, 72.71; H, 6.90; N, 11.06; found C, 72.74; H, 6.94; N, 11.09; Mass (TOF MS ES+): m/z 396.15 $(M+H)^+$, 398.14 $(MH+2)^+$.

3.3.6 [6-Chloro-2-methyl-4-{N'-(4-octyloxybenzylidene)hydrazinyl}quinoline], 18l.

Yield = 0.098g, 79%; Yellow Solid; MP = 182 °C; IR (KBr) cm^{-1}: 3244, 2926, 1604, 1250, 772; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 0.84 (t, 3H, terminal -CH_3), 1.25 (d(b), 8H, -CH_2), 1.39 (d(b), 2H, -CH_2), 1.70 (m, 2H, -CH_2), 2.69 (s, 3H, -CH_3), 4.02 (t, 2H, alkoxy-OCH_2), 7.02 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.46 (s, 1H, -N=CH-), 7.80 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.86 (d, 1H, *J* = 9.2 Hz, Ar-H), 7.97 (d, 1H, *J* = 8.8 Hz, Ar-H), 8.50 (s, 1H, Ar-H), 8.63 (s, 1H, Ar-H), 12.17 (s, 1H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 14.3 (-CH_3), 20.4 (-CH_3), 22.5 (-CH_2), 25.9 (-CH_2), 28.9 (-CH_2), 29.0 (-CH_2), 29.1 (-CH_2), 31.6 (-CH_2), 68.2 (-OCH_2), 100.9, 115.3, 115.8, 122.0, 122.5, 126.0, 130.0, 131.4, 134.1, 137.2, 150.7, 150.9, 154.9, 161.5; Anal. Calcd. for $C_{25}H_{30}ClN_3O$: C, 70.82; H, 7.13; N, 9.91; found C, 70.80; H, 7.16; N, 9.94; Mass (TOF MS ES+): m/z 424.21 $(M+H)^+$, 426.47 $(MH+2)^+$.

3.3.7 4-[N'-{3,4-Bis-octyloxybenzylidene}hydrazinyl]-6-chloro-2-methylquinoline, 18n.

Yield = 0.112g, 71%; Yellow Solid; MP = 182 °C; IR (KBr) cm^{-1}: 3242, 2919, 1604, 1239, 772; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 0.85 (t, 6H, terminal -CH_3), 1.30 (m, 16H, -CH_2), 1.45 (t(b), 4H, -CH_2), 1.74 (m, 4H, -CH_2), 2.72 (s, 3H, -CH_3), 4.03 (t, 2H, alkoxy-OCH_2), 4.06 (t, 2H, *J* = 6.4 Hz, -OCH_2), 7.06 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.37 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.50 (d(b), 2H, Ar-H), 7.89 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.99 (d, 1H, *J* = 9.2 Hz, Ar-H), 8.50 (s, 1H, Ar-H), 8.69 (s, 1H, Ar-H), 12.20 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 14.3 (-CH_3), 20.6 (-CH_3), 22.5 (-CH_2), 26.0 (-CH_2), 26.1 (-CH_2), 29.2 (-CH_2), 29.3 (-CH_2), 31.7 (-CH_2), 69.1 (-OCH_2), 101.0, 111.8, 113.4, 115.4, 122.2, 122.6, 126.3, 131.3, 134.1, 137.3, 149.2, 151.1, 152.0, 155.0 ; Anal. Calcd. for $C_{33}H_{46}ClN_3O_2$: C, 73.92; H, 8.65; N, 7.84; found C, 73.90; H, 8.61; N, 7.80; Mass (TOF MS ES+): m/z 552.33 $(M+H)^+$, 554.34 $(MH+2)^+$.

3.3.8 6-Chloro-2-methyl-4-[N'-{3,4,5-tris-octyloxybenzylidene}hydrazinyl]quinoline, 18p.

Yield = 0.141g, 72%; Yellow Solid; MP = 178 °C; IR (KBr) cm^{-1}: 3247, 2923, 1606, 1206, 880; ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 0.86 (t, 9H, terminal -CH_3), 1.27 (s(b), 26H, -CH_2), 1.46 (d(b), 6H, -CH_2), 1.70 (m, 4H, -CH_2), 2.73 (s, 3H, -CH_3), 3.91 (t, 2H, alkoxy-OCH_2), 4.05 (t, 4H, alkoxy-OCH_2), 7.01 (s(b), 2H, Ar-H), 7.49 (s, 1H, -N=CH-), 7.90 (d, 1H, *J* = 8.8 Hz, Ar-H), 8.00 (d, 1H, *J* = 9.2 Hz, Ar-H), 8.48 (s, 1H, Ar-H), 8.68 (s, 1H, Ar-H), 12.24 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 14.3 (-CH_3), 20.6 (-CH_3), 22.5 (-CH_2), 26.0 (-CH_2), 26.1 (-CH_2), 29.2 (-CH_2), 30.3 (-CH_2), 31.7 (-CH_2), 69.0 (-OCH_2), 101.0, 106.6, 115.5, 119.7, 122.3, 122.7, 128.8, 131.3, 134.2, 137.6, 140.4, 151.2, 153.4, 155.2; Anal. Calcd. for $C_{41}H_{62}BrN_3O_3$: C, 72.38; H, 9.18; N, 6.18; found C, 72.34; H, 9.14; N, 6.21; Mass (TOF MS ES+): m/z 680.45 $(M+H)^+$, 682.45 $(MH+2)^+$.

4. Conclusion

In summary, a series of new quinoline possessing hydrazones **18a-p** have been prepared and structure of the new compounds was elucidated by using various spectroanalytical techniques. Sixty human cancer cell lines (NCI60) were used to screen and evaluate their anti-proliferative activity. Among all the submitted and selected compounds (total 16) at single dose (10 μM) level, nine of the compounds (**18b**, **18d**, **18e**, **18f**, **18g**, **18h**, **18i**, **18j**, **18l**) showed prominent % inhibition of various cancer cell lines and further screened for five dilution assay. Five dilutions screening data revealed that, all nine compounds showed remarkable anti-proliferative activity with GI_{50} values ranging from 0.33-4.87 μM and LC_{50} values ranging from 4.67 μM to >100 μM. Among all compound, **18j** emerged as potent anticancer agent showed highest activity and selectivity for cancer cell lines. From the comparison of the mean values of GI_{50}, TGI and LC_{50} of compound **18j** with the clinically used anticancer agents, bendamustine and chlorambucil, clearly quinolyl hydrazones holds promise as potential anticancer agents. Added to this, all quinolyl hydrazones **18a-p** demonstrated outstanding antimicrobial activity (MIC 6.25-100 μg/mL) against the tested pathogenic strains. Furthermore, molecular docking studies were performed provided an understanding about the binding and interactions of the compounds into the active sites of the DNA-topoisomerase-I receptor. Finally, it is conceivable that, further derivatization and investigations of this class of compounds may be carried in a quest of new potential and selective anticancer agents.

Conflict of interest

Authors have no conflicts of interest**.**

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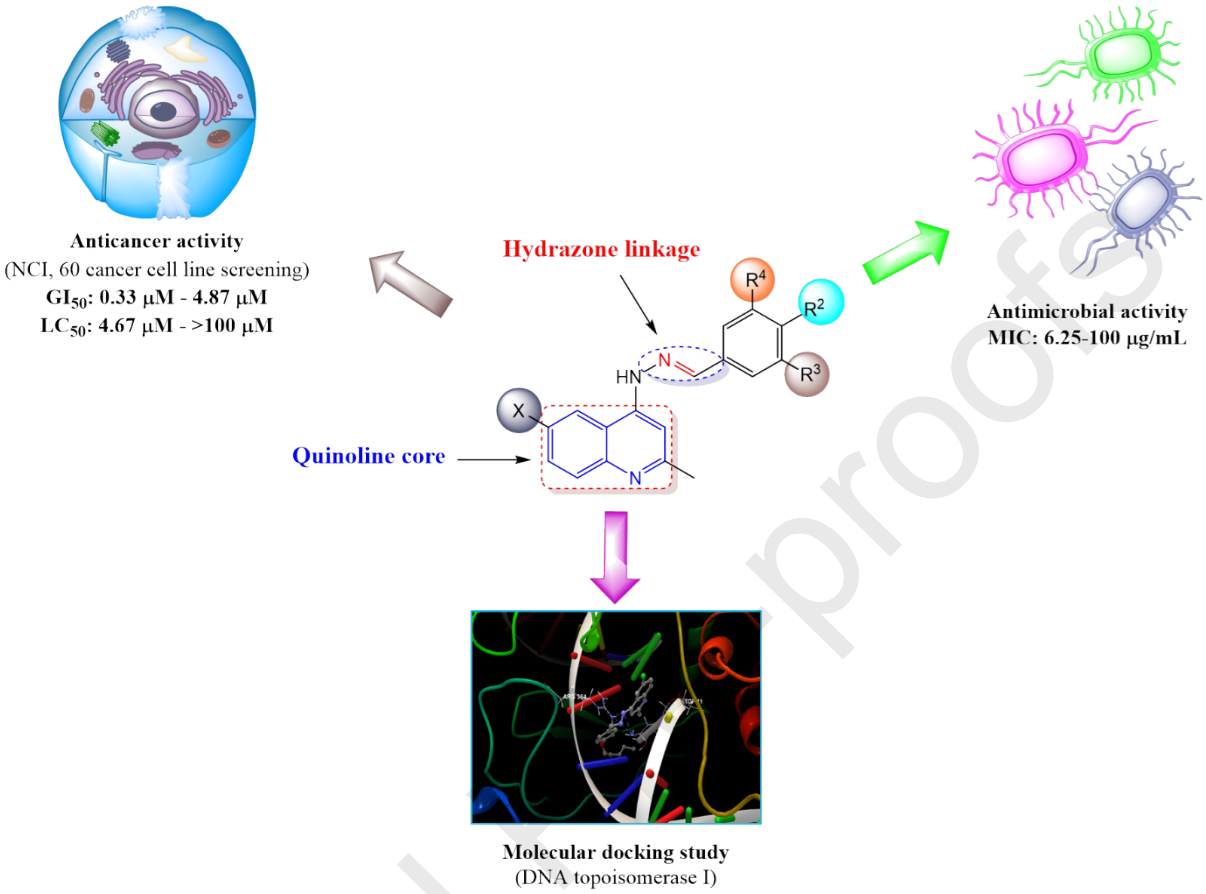
Graphical abstract



Highlights:

- A series of new quinolyl hydrazones **18a-p** have been prepared and characterized by IR, ^{1}H NMR, ^{13}C NMR, CHN analysis and MASS spectrometry.
- Among all compound **18j**, demonstrated highest ($\mathbf{GI_{50}}$ = 0.33 μM) anti-proliferative activity at single as well as five dose levels against panel of 60 cancer cell lines at NCI, USA.
- All the compounds showed promising activity against all four of bacterial and two fungal strains.
- Molecular docking studies were carried out into the active binding site of human DNA topoisomerase I (htopoI).

Conflict of Interest

Authors have no conflict of interest.