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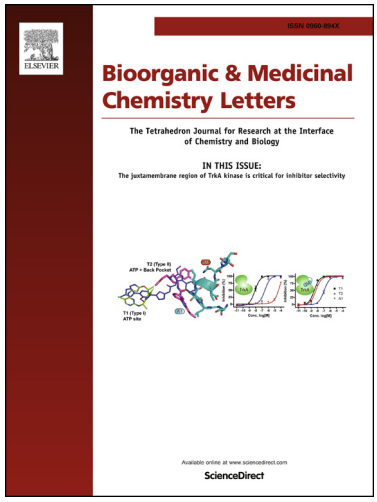

Quinoline-azetidinone hybrids: Synthesis and in vitro antiproliferation activity against Hep G2 and Hep 3B human cell lines

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PII: S0960-894X(17)30165-8
DOI: http://dx.doi.org/10.1016/j.bmcl.2017.02.043
Reference: BMCL 24715

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 2 October 2016
Revised Date: 31 January 2017
Accepted Date: 15 February 2017

Please cite this article as: Alegaon, S.G., Parchure, P., Araujo, L.D., Salve, P.S., Alagawadi, K.R., Jalalpure, S.S., Kumbar, V.M., Quinoline-azetidinone hybrids: Synthesis and in vitro antiproliferation activity against Hep G2 and Hep 3B human cell lines, *Bioorganic & Medicinal Chemistry Letters* (2017), doi: http://dx.doi.org/10.1016/j.bmcl.2017.02.043

Quinoline-azetidinone hybrids: Synthesis and in vitro antiproliferation activity against Hep G2 and Hep 3B human cell lines

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Abstract

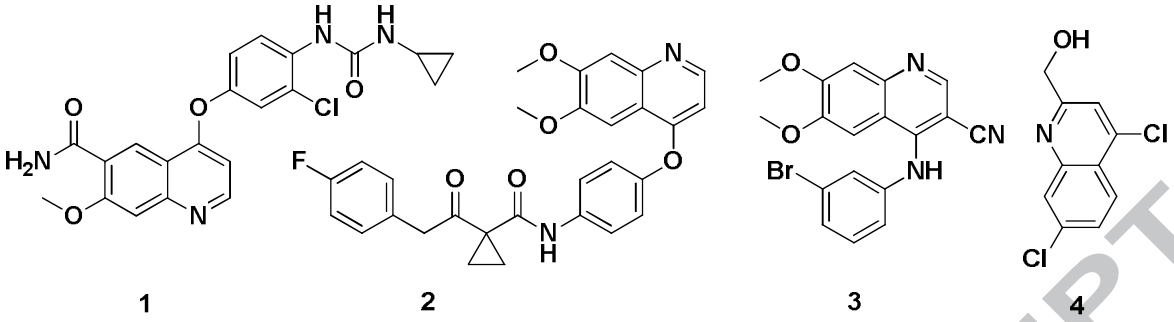
In search of new heterocyclic anticancer agents, a new quinoline-azetidinone hybrid template have been designed, synthesized and screened for their cytotoxic activity against human cancer cell lines such as Hep G2, and Hep 3B by the MTT assay and results were compared with paclitaxel, 5-fluorouracil and doxorubicin. Interestingly, some of the compounds were found significantly active against both cell lines. The compound **6f (**IC_{50} = 0.04 ± 0.01 µM**)** exhibited potent antiproliferation activity against Hep G2 cell line, and **6j** compound **(**IC_{50} = 0.66 ± 0.01 µM**)** demonstrated potent antiproliferation activity against Hep 3B cell line and provide to be more potent as cytotoxic agents than standard drugs. Morphological changes suggest the induction of apoptosis and describe the mechanism of action of these hybrid antitumor agents.

Keywords: Quinoline, Azetidinone, antiproliferation, Drug likeness.

Cancer is a prevalent and second leading cause of death worldwide.[1] It is responsible for increase in the mortality rate and hence it has become a life threatening disease affecting to all the people at all ages in both developing and developed countries.[2] Cancer is a disease which is characterized by uncontrolled cell growth and proliferation;[3] which is due to mutation or mis-regulation of cell cycle regulatory genes and proteins.[4] Altered apoptosis is one of the central steps in cancer development. It has become an attractive approach for anticancer therapeutics which can overcome the resistance to apoptosis by directly activating the normal cell death machinery.[5] Many efforts have been taken to fight against cancer which includes successful treatment of certain tumor types and hence it still continues to be challenge owing to their aggressiveness, the mechanism of malignant cell metastasis, chemoresistance and lack of selectivity of drugs. Therefore, the development of novel anticancer agents is required to synthesize which would be safe and more effective.[6]

Literature data has been established that nitrogen containing heterocyclic compounds plays an important role in designing new class of structural entities for medicinal applications.[7] The skeleton of quinoline is one of the key building elements for a large number of natural and synthetic heterocycles. The derivatives of quinoline that belong to this class have shown wide applications as drugs and pharmaceuticals. Quinoline scaffolds have demonstrated that they have high ability to draw out an anti-proliferative and antitumor activity based upon the reports on natural, synthetic and semisynthetic biologically active molecules. They act through different mechanism of action such as growth inhibitors by cell cycle arrest, apoptosis, inhibition of angiogenesis, and disruption of cell migration and modulation of nuclear receptor responsiveness. The two examples of cytotoxic quinolines with antitumor activity are natural alkaloid camptothecin and its semisynthetic analogtopotecan which act through inhibition of the DNA enzyme topoisomerase-I. According to literature survey, it has been proven that quinoline derivatives have an excellent class of broad spectrum anticancer activity against variety of cancer cell lines such as breast cancer, hepatocellular carcinoma, and acute myelogenous leukemia.[8, 9] Certain drugs based on quinoline moiety such as bosutinib, lenvatinib and cabozantinib have been established as one of the most effective class of anticancer agents in clinical use with broad application in the treatment of several types of cancer.[9]

The derivatives of quinolines also have varieties of biological activities like anti-microbial,[10] antitubercular,[11-15] antimalarial,[16-18] antibiotic,[19] antihypertensive,[20] anti-inflammatory,[21] tyrokinase PDGF-RTK inhibition[22] and anti-HIV properties.[23,24] Few examples of quinoline derivatives include Quinine (antipyretic, antimalarial, analgesic and anti-inflammatory properties), Chloroquine (antimalarial), Amidaquine (antimalarial and anti-inflammatory properties), Camptothecin (DNA enzyme topoisomerase II) and Saquinavir (anti-HIV drug), Ciprofloxacin and Moxifloxacin (anti-TB drugs), which are actively used in pharmacological field.[7] Azetidinone and their derivatives have attracted interest in organic medicinal chemistry, exhibiting biological and therapeutic properties, since the introduction of various monobactams into clinical applications. Chemical modifications of these four–membered heterocycles constantly result in compounds with a wide spectrum of pharmacological activities such as anticancer, anti-microbial, anti-tubercular, anti-inflammatory, anti-malarial, thrombin inhibitor, human leukocyte elastase inhibitor, human cytomegalovirus inhibitor, cysteine protease cathepsin K inhibitor, antidiabetic, and antiparkinsonian agents.[25] In addition, recently, Geesala et al. in 2016 have reported azetidinone derivatives are potential anti-cancer agents.[26] Based on the above findings, in an attempt to pursue new potent anticancer agents with potent inhibitory activity, a series of new quinoline-azetidinone compounds were synthesized using molecular hybridization technique (**Fig.1.**)[9, 26-28] and their inhibitory activity against two human cancer cell lines (Hep G2, Hep 3B) were evaluated. In addition, Molecular parameter and possible drug likeness were also illustrated.



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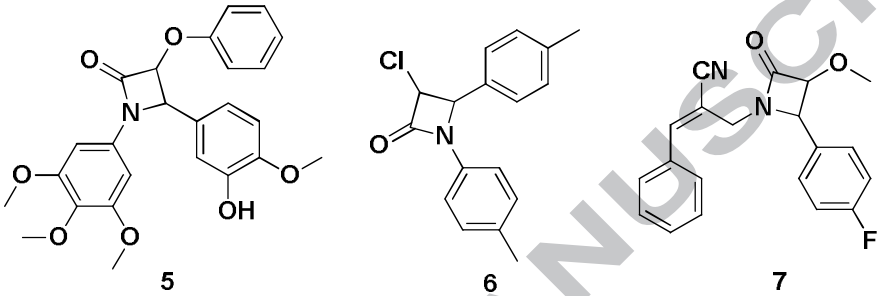
Structures of some reported bioactive quinolines as anticancer agents[9]



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Structures of some reported bioactive azetidinone as anticancer agents [26,27,28]

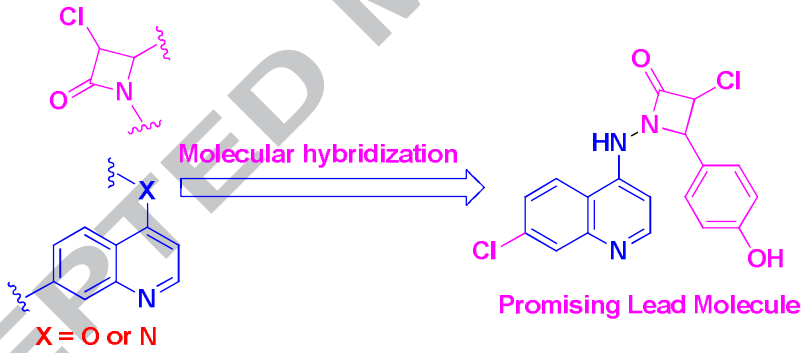
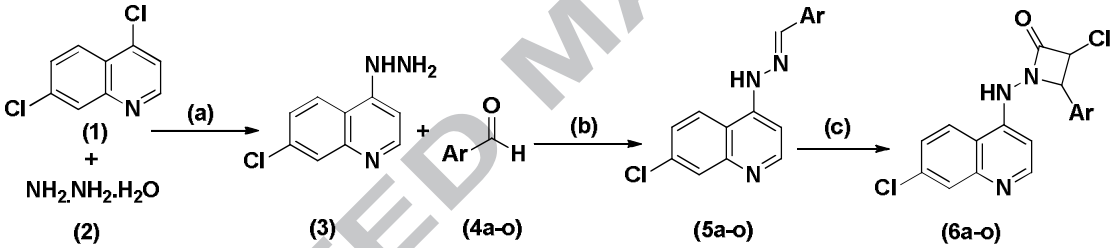


Figure 1. Design of quinoline-azetidinone compound using molecular hybridization technique

The reaction route employed for synthesis of the title compounds (6**a-o**) are illustrated in **Scheme 1.** The two step procedure was adopted for the synthesis of key intermediate 7-chloro-4-(2-(4-substitutedbenzylidene)hydrazinyl)quinoline **(5a-o)** compounds. The 7-chloro-4-hydrazinylquinoline (**3**) was synthesized by condensation of the 4, 7 dichloroquinoline with hydrazine hydrate.Reaction of the 7-

chloro-4-hydrazinylquinoline (**3**) with appropriate aryl/hetero aryl aldehydes in ethanol afforded the corresponding 7-chloro-4-(2-(4-substitutedbenzylidene) hydrazinyl) quinoline **(5a-o)** compounds. [29, 30] The prepared Schiff bases **(5a-o)** were reacted with chloroacetyl chloride and trimethylamine in dichloromethane and refluxed for 24 h. The reaction was monitored by TLC for completion. Mixture was filtered, washed with dichloromethane to obtain 3-chloro-1-((7-chloroquinolin-4-yl)amino)-4-(4-substituted phenyl)azetidin-2-one (**6a-o**) analogous.[31]The synthesized quinoline-azetidinone (**6a-o**) hybrids were characterized by IR, ^{1}H NMR, and mass spectral analysis. The IR spectra of quinoline-azetidinone (**6a-o**) compounds displayed absorption band at 1685 - 1710 cm^{-1} attributed to a carbonyl group of azetidinone, their proton NMR spectra showed characteristic new singles around δ 4.25 ppm and δ 5.73 ppm attributed to (CH of C3 and C4) azetidinone protons, which represents the formation of azetidinone ring system. The structures of the synthesized quinoline-azetidinone (**6a-o**) hybrids were further confirmed by mass spectra analysis.



Scheme 1. Reagents and conditions: (a) Reflux 8 h; (b) Dry ethanol, stirred at RT 24 h; (c) Chloroacetyl chloride, Dichloromethane, Triethylamine, reflux 24 h

The cytotoxicity activity of target compounds (**6a-o**) against Hep-G2 and Hep-3B (human hepatocellular) carcinoma cell lines was measured by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.[32] Paclitaxel, 5-fluorouracil and doxorubicin was selected as standard drug. The in vitro structural activity relationship of the target compounds was obtained from the analysis of biological data as summarized in **Table 1**.

Table 1. *In vitro* anticancer activity (IC_{50}) of quinoline-azetidinone hybrid compounds (**6a-o**) expressed in µM

Comp	Ar	HEP G2[a]	HEP 3B[b]	VERO[c]	SI[d] HEP G2	SI[d] HEP 3B
6a	4-Cl-C_6H_4	0.85 ± 0.01	3.37 ± 0.02	44.39±1.0	52.22	13.17
6b	4-F-C_6H_4	0.76 ± 0.01	1.72 ± 0.01	48.21±1.1	63.43	28.02
6c	4-OCH_3-C_6H_4	0.71 ± 0.01	0.89 ± 0.02	51.13±1.2	72.01	57.44
6d	3,4-di-OCH_3-C_6H_3	0.36 ± 0.01	0.74 ± 0.02	36.33±0.9	100.91	49.09
6e	3,4,5-tri-OCH_3-C_6H_2	0.73 ± 0.02	1.10 ± 0.01	52.01±1.5	71.24	47.28
6f	4-OH-C_6H_4	0.04 ± 0.01	1.72 ± 0.01	52.33±1.3	>100	30.42
6g	4-NO_2-C_6H_4	0.86 ± 0.01	1.37 ± 0.01	49.13± 1.6	57.12	35.86
6h	2,3-di-Cl-C_6H_3	0.51 ± 0.01	1.14 ± 0.01	27.76±1.7	54.43	24.35
6i	2,4-di-Cl-C_6H_3	0.38 ± 0.01	0.89 ± 0.02	49.33±0.9	>100	55.42
6j	3,4-di-Cl-C_6H_3	0.51 ± 0.01	0.66 ± 0.01	54.63±0.8	>100	82.77
6k	2-Cl-C6H4	0.96 ± 0.02	0.96 ± 0.05	32.33±0.9	33.67	33.67
6l	3-Br-4-F-C_6H_3	0.65 ± 0.01	1.78 ± 0.05	46.66±1.8	71.78	26.21
6m	4-$N(CH_3)_2$-C_6H_4	0.31 ± 0.01	1.15 ± 0.01	41.33±1.3	>100	35.93
6n	4-CN-C_6H_4	0.54 ± 0.01	0.78 ± 0.01	38.33±1.2	70.98	49.14
6o	2-Thiophene	0.75 ± 0.01	3.06 ± 0.01	42.11±1.5	56.14	13.76
Pac[e]	-	0.3 ± 0.03	0.73 ± 0.05	-	-	-
5-FU[f]	-	2.10± 0.20	2.66 ± 0.01	-	-	-
Dox[g]	-	0.54 ± 0.02	1.15 ± 0.01	-	-	-

[a]HEP G2, human liver carcinoma cell line(Cat No- 11965-092); [b]HEP 3B, Human hepatoma cell line (Cat No- 11965-092);
[c]VERO, Monkey Kidney cell line (Cat No- 11095-080); [d]Selectivity indexs (SI) were calculated by IC_{50} values in normal cell line devided by IC_{50} values in cancer cell line; [e]Paclitaxel; [f]5-Fluorouracil; [g]Doxorubicin

The IC_{50} values are the average of the triplicate assay analysis. All quinoline-azetidinone hybrid compounds (**6a-o**) inhibited cell proliferation with IC_{50} values in range of 0.04 to 3.37 µM, As shown in **Table 1**, most of test compounds exhibited comparable activity when compared to standard drugs. It was observed that among the fifteen synthesized compounds, **6f** displayed much higher antiproliferation activity (IC_{50} = 0.04 µM) than standard paclitaxel (IC_{50} = 0.30 µM) against Hep-G2 cell line, compounds like **6m**, **6d**, and **6i**,showed good inhibitory activity against Hep-G2 cell line with IC_{50} values of 0.31 µM, 0.36 µM, and 0.38 µM respectively, which are comparable to paclitaxel and doxorubicin. Compounds **6h**, **6j**, and **6n** revealed moderate inhibitory activity against Hep-G2 cell line with IC_{50} values of 0.51 µM, 0.51 µM, and 0.54 µM respectively. **Table 1** also revealed that, compound **6j** exhibited better antiproliferation activity (IC_{50} = 0.66 µM) than standard drugs against Hep-3B

cell line. Compounds **6d**, **6c**, **6i**, and **6k** showed moderate antiproliferation effects (IC_{50} 0.74 – 0.96 µM), against Hep-3B cell line.

The significant activity of compound **6f** against Hep-G2 cell line can be attributed to the presence of a strong electron donating hydroxyl group at the para position of the phenyl ring attached to C-4 of azetidinone structure. Compound **6f** also showed activity against Hep-3B cell lines at IC_{50} = 1.72 µM. Any further changes in the substitution on the phenyl ring of the azetidinone skeleton resulted in different values of the biological activity. When two methoxy groups were attached at the meta and para position (compound **6d**), the activity was decreased to 0.36 µM against Hep-G2 cell line and 0.74 µM against Hep 3B cell line. When the methoxy group was attached only at the para position of the phenyl ring (compound **6c**) the activity reduced to 0.71 µM against Hep-G2 cell line and 0.89 µM against Hep 3B cell line. Further, compound with 3,4,5-trimethoxy (**6e**) substitution on the phenyl ring displayed lowered activity of 0.73 µM against Hep-G2 cell line and 1.10 µM against Hep-3B cell line. Replacing the substituents at para position by an electron withdrawing group has caused decrease in the anticancer activity as compared to the most potent compound **6f**. This can be justified by the fact that compounds bearing electron withdrawing groups like cyno (CN) (**6n**), fluoro (F) (**6b**), chloro (Cl) (**6a**) and nitro (NO_2) (**6g**) substituents at the para position of the phenyl ring have exhibited activity at IC_{50} values 0.54, 0.76, 0.85 and 0.86 µM respectively against Hep-G2 cell line and at 0.78, 1.72, 3.37 and 1.37 µM respectively against Hep-3B cell line. Compounds bearing chloro (Cl) group at different positions on the phenyl ring (compounds **6h-k**) have shown different activities. Compound **6k** with chloro (Cl) at ortho position of the phenyl ring showed activity at IC_{50} = 0.96 µM against both Hep-G2 and Hep-3B cell lines. Compound **6h** with two chloro (Cl) group attached at ortho and meta positions on the phenyl ring displayed activity at 0.51 ± 0.01 against Hep-G2 and 1.14 µM against Hep-3B cell lines. When two chloro (Cl) groups were further attached at ortho and para positions (compound **6i**) the activity decreased to 0.38 and 0.89 µM against Hep-G2 and Hep-3B cell lines respectively. The subsequent substitution of two chloro (Cl) groups at meta and para positions showed anticancer activity at 0.51 µM against Hep-G2 and at 0.66 µM against Hep-3B cell lines. Compound **6l** with two electron withdrawing substituents bromo (Br) at meta position and fluoro (F) at para position exhibited activity at 0.65 µM and 1.78 µM against Hep-G2 and Hep-3B cell lines respectively. Furthermore, inhibitory activities

decreased to 0.75 µM against Hep-G2 and 3.06 µM against Hep-3B cell lines when five membered heterocyclic ring like 2-thiophene was attached to the azetidinone structure instead of the phenyl group. Subsequently compounds **6d, 6f, 6i** and **6m** have remarkable activity against Hep G2 cell lines and **6c, 6d, 6e, 6i, 6j, 6k** and **6m** compounds showed significant activity against Hep 3B cell lines, these were selected to further investigation regarding their mechanism of action. To investigate whether or not compounds induce chromatin condensation and fragmentation, which are both recognized morphological features of apoptosis, Hep G2 cells were treated with the chosen compounds and stained with acridine orange (AO) and ethidium bromide (EB). Nuclear morphology and alterations in the size and the shape of cells were observed after 24 h treatment with IC_{50} concentrations of the compounds. The microscopic photographs are shown in **Fig. 2.**

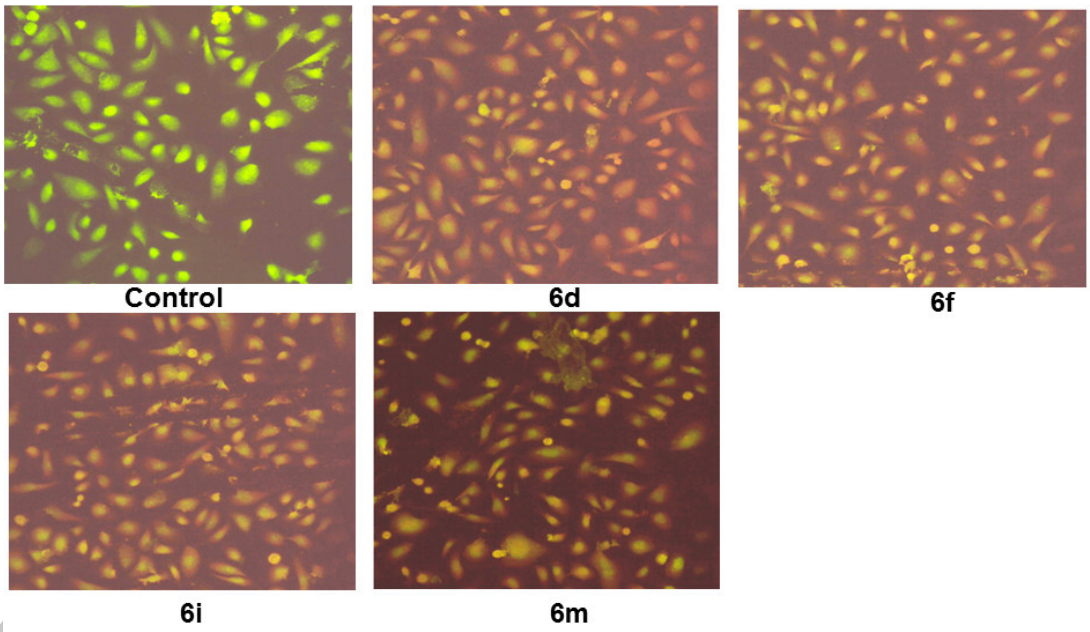


Figure. 2. Apoptosis in Hep G2 cells treatment with IC50 concentrations of **6d, 6f, 6f** and **6m** compounds

These results revealed that selected compounds can induce apoptosis in Hep G2 cells. Identification of deregulation of apoptosis is a possibility of all cancer cells and the molecules that are capable of inducing apoptosis in cancer cells are considered to be significant in anticancer agents. The selected compounds are taken for further study to assessment of nuclear morphology by fluorescence microscopy using cell-permeable nucleic acid stain, such as DAPI.[33] Nuclear fragmentation and nuclear condensation of Hep G2 cells in **Fig. 3**.

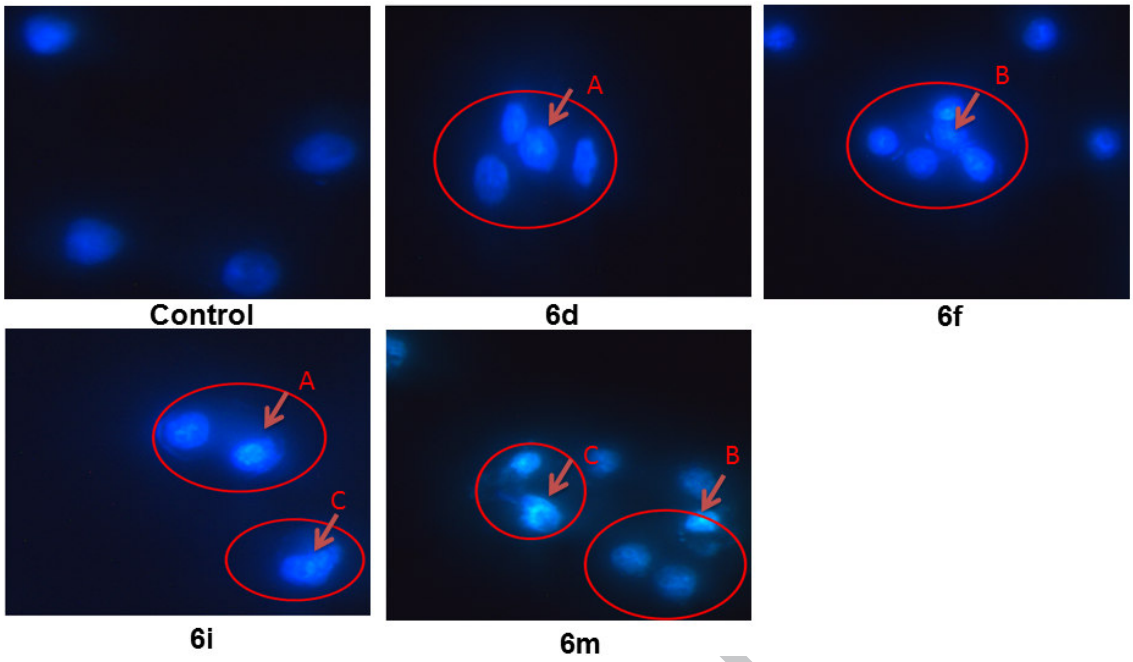


Figure. 3. Fluorescent microscopy pictures showing morphological changes in HEP G2 cells treated with compound. The cells were stained with DAPI and observed under fluorescent microscope. (A) Nuclear Condensation, (B) Blebbing and (C) Nuclear Fragmentation

Additionally, the *in vitro* cytotoxic activity of all tested compounds was evaluated against normal VERO cell line by MTT colorimetric assay in order to study estimate the safety of these agents. The cytotoxic activity results revealed that none of the tested compounds exhibited any significant toxicity effect on normal VERO cells. Selectivity indexs (SI) values were calculated by IC_{50} values in normal cell line divided by IC_{50} values in cancer cell line. The results revealed that most of the tested compounds were less toxic on VERO in comparison with tumor cells **(Table 1)**. The safety of **6d, 6f, 6i** and **6m** compounds were further evaluated against Chang Liver cell lines. The cytotoxicity results were summarized in **Table 2**. The data showed that selected **6d, 6f, 6i** and **6m** compounds were non-toxic towards Chang Liver cells.

Table 2. Cytotoxicity of compounds against Chang Liver cell line

Concentration in µM	Cytotoxicity			
	Cell Viability %			
	6d	6f	6i	6m
200	84.45±7.32	95.00±2.69	88.45±3.72	85.00±1.38
100	93.90±3.72	96.81±1.92	92.72±3.08	89.27±6.05
50	97.36±1.67	96.90±2.05	94.45±7.09	91.00±7.61
25	98.45±1.38	97.18±2.12	96.81±3.72	92.72±6.42
12.5	98.45±3.59	98.36±2.82	98.90±4.88	94.18±5.65
6.25	98.18±3.85	99.16±1.51	99.18±1.41	95.72±7.12

Moreover, in this work we also performed a computational (Lipinski's rule of five) study for prediction of physical-chemical properties[34, 35] of all derivatives the results are summarized in **Table 3**. Briefly, this simple rule is based upon the observation that most of medicinal agents have a molecular weight (MW) of 500 or less, a log *p* not more than 5, number of hydrogen bond donor atoms of 5 or less and number of hydrogen bond accepter atoms of 10 or less (N or O). Interestingly our molecular parameter results pointed that most of the compounds obey the Lipinski 'rule of 5'. Number of rotatable bonds is an important topological parameter for molecular flexibility and conformational modification for binding to the enzyme or receptors. The data shown that quinoline-azetidinone hybrid compounds showed a good number of rotatable (n-rotb) (2-6) demonstrating their conformational plasticity.[36] The hydrogen bonding is important molecular descriptors for describing permeability of drugs.[37] Most of the quinoline-azetidinone hybrid compounds displayed several hydrogen bond acceptors (4 to 7) and moderate hydrogen bond donors as shown in **Table. 3**. *milogP*, the logarithm of the octanol/water partition coefficient, provides a amount of the lipophilicity of a ligand. Lipophilicity is an important parameter of an active pharmaceutical ingredient as it influences a number of physiological properties like transport through cell membranes, rate of metabolism and interaction with receptor binding sites. The results revealed that most of the compounds exhibited low Log *p*,by which these compounds have good bioavailability.[38] All synthesized quinoline-azetidinone hybrid compounds displayed molecular weight below 500 g/mol.

Table 3. Molecular parameters of quinoline-azetidinone hybrids (**6a-o)**

Comp	n-rotb[a]	nON[b]	nOHNH[c]	miLog*p*[d]	MW[e]	Rule of 5 violation	Drug-likeness model score[f]	TPSA[g] (Å^2)	Molecular volume[h] (Å^3)	Absorption percent. % ABS[i]
Rule of 5	-	≤10	≤5	≤5	≤500	≤1	0.37	-	-	-
6a	3	4	1	4.72	392.67	0	0.36	45.23	302.88	93.39
6b	3	4	1	4.21	376.21	0	0.48	45.23	294.27	93.39
6c	4	5	1	4.10	388.25	0	0.53	54.46	314.89	90.21
6d	5	6	1	3.69	418.27	0	0.48	63.70	340.43	87.02
6e	6	7	1	3.68	448.30	0	0.18	72.93	356.98	83.83
6f	3	5	2	3.57	374.22	0	0.51	65.46	296.36	86.41
6g	4	7	1	4.00	403.22	0	-0.12	91.05	312.68	77.58
6h	3	4	1	5.31	427.11	1	0.45	45.23	316.41	93.39
6i	3	4	1	5.33	427.11	1	0.42	45.23	316.41	93.39
6j	3	4	1	5.33	427.11	1	0.42	45.23	316.41	93.39
6k	3	4	1	4.68	392.67	0	0.35	45.23	302.88	93.39
6l	3	4	1	4.95	455.1	0	0.22	45.23	312.16	93.39
6m	4	5	1	4.15	401.29	0	0.24	48.47	335.25	92.27
6n	3	5	1	3.80	383.23	0	0.25	69.02	306.20	85.18
6o	3	4	1	3.95	364.25	0	0.27	45.23	280.05	93.39
Pac[j]	1	4	2	-0.711	123	0	0.66	68.87	756.60	85.23
5-FU[k]	0	4	2	-0.59	130.08	0	-1.07	65.72	96.91	86.32
Dox[l]	5	12	7	0.57	543.52	3	1.02	206.08	459.18	37.90

[a]n-rotb, number of rotatable bonds ; [b]nON, number of hydrogen bond acceptors; [c]nOHNH, number of hydrogen bond donors; [d]miLogP, logarithm of compound partition coefficient between n-octanol and water; [e]MW, molecular weight; [f]Drug-likeness model score: Calculated using molsoft (http://molsoft.com/mprop/); [g] TPSA, topological polar surface area; [h]Molecular volume; [i]Absorption percent (%ABS) was calculated by using %ABS = 109-(0.345 X TPSA); [j]Paclitaxel; [k]5-Fluorouracil; [l]Doxorubicin

Finally, Molecular polar surface area (TPSA), volume and percentage of absorption (% ABS) for the quinoline-azetidinone hybrid compounds are presented in **Table. 3.**

In conclusions, new quinoline-azetidinone hybrids were synthesized and structures of newly synthesized compounds were confirmed by FTIR, NMR and Mass spectral studies. The majority of the compounds exhibited significant antiproliferation activity against Hep G2, and Hep 3B human cancer cell lines with IC_{50} values in the range 0.04 - 3.37 µM. The compound **6f (**IC_{50} = 0.04 ± 0.01 µM**)** exhibited potent antiproliferation activity against Hep G2 cell line, and **6j** compound **(**IC_{50} = 0.66 ± 0.01 µM**)** demonstrated potent antiproliferation activity against Hep 3B cell line and

provide to be more potent as cytotoxic agents than standard drugs. Morphological changes suggest the induction of apoptosis and describe the probable mechanism of action of these hybrid antitumor agents. The reported quinoline-azetidinone hybrids showed better safer profiles against normal cell lines. Synthesized analogues displayed suitable drug like properties and are expected to extant good bioavailability property. Altogether, these results suggest that some of these hybrid-compounds would serve as potential anticancer agents with modifications on pharmacophore, especially; **6f** hybrid-compound was promising subjects for further studies regarding cancer chemotherapy.

Acknowledgements

Authors are grateful to Principal, KLE University's College of Pharmacy, Belagavi, for providing necessary facilities for the research. Authors are also grateful to NMR Research center, IISC, Bangalore, India, for providing the spectral data.

References and notes

1. Ai, Y.; Liang, Y. J.; Liu, J. C.; He, H. W.; Chen, Y.; Tang, C.; Yang, G. Z.; Fu, L. J. *Eur. J. Med. Chem.* **2012**, *47*, 206.
2. Singh, K.; Verma, V.; Yadav, K.; Sreekanth, V. *Eur. J. Med. Chem.* **2014**, *87*, 150.
3. Shao, T.; Wang, J.; Chen, J. G.; Wang, X. M.; Li, H.; Li, Y. P.; Li, Y.; Yang, G. D.; Mei, Q. B.; Zhang, S. Q.; *Eur. J. Med. Chem.* **2014**, *75*, 96.
4. Sangani, C. B.; Makawana, J. A.; Zhang, X.; Teraiya, S. B.; Lin, L.; Zhu, H. *Eur. J. Med. Chem.* **2014**, *76*, 549.
5. Qin, Q. P.; Chen, Z. F.; Qin, J. L.; He, X. J.; Li, Y. L.; Liu, Y. C.; Huang, K. B.; Liang, H. *Eur. J. Med. Chem.* **2015**, *92*, 302.
6. Carvalho, E. D.; Elaine, F. F.; Marchetti, G. M.; Ramalho, T. C.; Alves, R. B.; *Eur. J. Med. Chem.* **2014**, *84*, 595.
7. Keri, R. S.; Patil, S. A. *Biomed. Pharmacother.* **2014**, *68*, 1161.
8. Arafa, R. K.; Hegazy, G. H.; Piazza, G. A.; Abadi, A. H. *Eur. J. Med. Chem.* **2013**, *63*, 826.
9. Afzal, O.; Kumar, S.; Haider, R.; Ali, R.; Kumar, R.; Jaggi, M.; Bawa, S. *Eur. J. Med. Chem.* **2015**, *97*, 871.
10. Desai, N. C.; Dodiya, A. M. *Arab. J. Chem.* **2014**, *7*, 906.
11. Murugesan, D.; Palaniappan, S.; Perumal, Y.; Arnab, C.; Valakunja, N.; Dharmarajan, S. *Bioorg. Med. Chem.* **2008**, *16*, 3408.
12. Annamaria, L.; Jialin, M.; Baojie, W.; Yuehong, W.; Scott, G. F.; Alan, P. K. *J. Med. Chem.* **2009**, *58*, 2109.
13. Sarva, J.; Yasuyoshi, I.; Baojie, W.; Scott, G. F.; Alan, P. K. *Chem. Med. Chem.* **2006**, *1*, 593.
14. Amit, N.; Vikramdeep, M.; Alpeshkumar, M.; Evans, C.; Rahul, J. *Bioorg. Med. Chem.* **2007**, *15*, 626.
15. Dinakaran, M.; Senthilkumar, P.; Yogeeswari, P.; China, A.; Nagaraja, V.; Dharmarajan, S. *Int. J. Antimicrob. Agents.* **2008**, *31*, 337.
16. LaMontagne, M. P.; Markovac, A. M. S.; Sami Khan, M. *J. Med. Chem.* **1982**, *25*, 964.
17. LaMontagne, M. P.; Blumbergs, P.; Strube, R. E. *J. Med. Chem.* **1982**, *25*, 1094.
18. Nasveld, P.; Kitchener, S. *Trans. R. Soc. Trop. Med. Hyg.* **2005**, *99*, 2.

19. Mahamoud, A.; Chevalier, J.; Davin-Regli, A.; Barbe, J.; Jean-Marie Pages, *Curr Drug Targets.* **2006**, *7*, 843.
20. Muruganantham, N.; Sivakumar, R.; Anbalagan, N.; Gunasekaran, V.; Leonard, J. T. *Biol. Pharm. Bull.* **2004**, *27*, 1683.
21. Leatham, P. A.; Bird, H. A.; Wright, V.; Seymour, D.; Gordon, A. *Eur. J. Rheumatol. Inflamm.* **1983**, *6*, 209.
22. Maguire, M. P.; Sheets, K. R.; McVety, K.; Spada, A. P.; Zilberstein, A. *J. Med. Chem.* **1994**, *37*, 2129.
23. Wilson, W. D.; Zhao, M.; Patterson, S. E.; Wydra, R. L.; Janda, L.; Strekowski, L. *Med. Chem.Res*. **1992**, *2*, 102.
24. Strekowski, L.; Mokrosz, J. L.; Honkan, V. A.; Czarny, A.; Cegla, M. T.; Patterson, S. E.; Wydra, R. L.; Schinazi, R. F. *J. Med. Chem.* **1991**, *34*, 1739.
25. Parul, D. M.; Sengar, N. P. S.; Pathak, A. K. *Eur. J. Med. Chem.* **2010**, *45*, 5541.
26. Geesal, R.; Jagadees, K. G.; Mahender, B.; Sridhar, B.; Jayathirtha, R. V.; Amitava, D. *Eur. J. Med. Chem.* **2016**,*124*, 544.
27. Adele, C.; Marina, S.; Isabel, M. G.; Simona, M.; Alessia, B.; Anna, C.; Maria, S. S.; Rosa, S.; Francesco, P.; Ortensia, I. P.; Carmela, C.; Emilia, M.; Ettore, N.; Carmela, S.; Pietro, C.; Vincenzo, P. *Bioorg & Med. Chem. Lett*. **2013**, *23*, 6401.
28. Thomas, F. G.; Shu, W.; Lisa, M. G.; Seema, M. N.; Jade, K. P.; Azizah, M. M.; Thomas, M. C.; Brendan, T.; Niamh, M. O.; Daniela, M. Z.; Mary, J. M. *J. Med. Chem.* **2016**, *59*, 90.
29. Andre L. P. C.; Marcelle, L. F.; Karla, C. P.; Laura, N. F. C.; Carlos, R. K.; Maria, G. M. O. H.; Maria C. S. L.; Flavio, A. F. M. B.; Marcus, V. N. S. *Bioorg & Med. Chem. Lett*. **2009**, *19*, 6272.
30. *Experimental: General procedure for synthesis of Synthesis of 7-chloro-4-hydrazinylquinoline* **(3):** The mixture of 4, 7 dichloroquinoline (25 mmol) and hydrazine hydrate (50 mL, excess amount) was refluxed for 8h. The reaction was monitored by TLC for completion. The reaction mixture was left to stand at 5 °C overnight. The resulted solid reaction mixture was then treated with dichloromethane (30 mL), filtered, washed with dichloromethane, dried and recrystallized from ethanol to yield 7-chloro-4-hydrazinylquinoline (**3**). Yield 83%; mp: 220-222 °C; FTIR (KBr, cm^{-1}): 3336 (NH_2), 1612 (C=N);

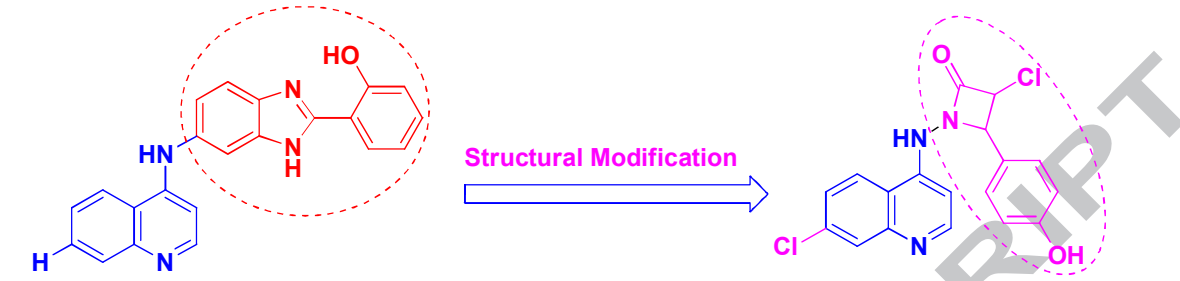
^{1}H-NMR (DMSO-d_6, 400 MHz) δ, ppm: 5.7 (bs, 3H, NH & NH_2), 6.86 (s, 1H, Ar-H), 7.38 (s, 1H, Ar-H), 7.74 (s, 1H, Ar-H), 8.16 (s, 1H, Ar-H), 8.36 (s, 1H, Ar-H). *General procedure for synthesis of 7-chloro-4-(2-(4-substituted benzylidene) hydrazinyl)quinolone* (**5**): A mixture of 7-chloro-4-hydrazinylquinoline **(3)** (15 mmol) and appropriate aryl or heteroaryl aldehydes **(4)** (18 mmol) in dry ethanol (100 mL) was stirred at room temperature for 24 h. The reaction was monitored by TLC for completion. The precipitate formed was filtered, washed with cold ether and dried to get 7-chloro-4-(2-(4-substituted benzylidene) hydrazinyl)quinolone.*7-chloro-4-(2-(4-chlorobenzylidene)hydrazinyl)quinoline* **(5a):** Yellow powder; Yield 74%; mp: 224°C; FTIR (KBr, cm^{-1}): 3238 (NH), 3172 (Ar-H), 1583 (C=N), 1575 (C=C); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ, ppm: 7.3 (s, 1H, CH=N), 7.51 (d, 2H, *J*=8.4 Hz, Ar-H), 7.55 (s, 1H, Ar-H), 7.80 (d, 2H, *J*=8.4 Hz, Ar-H), 7.82 (s, 1H, Ar-H), 8.35 (s, 1H, Ar-H), 8.38 (m, 2H, Ar-H), 11.37 (s, 1H, NH). *7-chloro-4-(2-(4-hydroxybenzylidene)hydrazinyl)quinoline* **(5f):** Yellow powder; Yield 57%; mp: 280°C; FTIR (KBr, cm^{-1}): 3647(OH), 3211(NH), 3120 (Ar-H), 1568 (C=N); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ, ppm: 6.83 (d, 2H, *J*=8.8 Hz, Ar-H), 7.25 (s, 1H, Ar-H), 7.49 (s, 1H, Ar-H), 7.62 (d, 2H, *J*=8.4 Hz, Ar-H), 7.8 (s, 1H,-CH=N), 8.38-8.30 (m, 3H, Ar-H), 9.83 (s, 1H, OH), 11.09 (s, 1H, NH). *7-chloro-4-(2-(4-nitrobenzylidene)hydrazinyl)quinoline (**5g**):* Yellow powder; Yield 57%; mp: 230°C; FTIR (KBr, cm^{-1}): 3591(NH), 3068 (Ar-H), 1566.20 (C=N), 1585(NO_2); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ, ppm: 7.84 (s, 1H, -CH=N), 8.0 (d, 2H, *J*=8.4 Hz, Ar-H), 8.47-8.26 (m, 7H, Ar-H), 11.6 (s, 1H, NH).

31. *General procedure for synthesis of 3-chloro-1-((7-chloroquinolin-4-yl)amino)-4-(4-substituted phenyl)azetidin-2-one* **(6)**. To a stirred solution of 7-chloro-4-(2-(4-substitutedbenzylidene)hydrazinyl)quinoline **(5)** (3.7 mmol) and triethylamine (9 mmol) in dichloromethane (25 mL), chloroacetyl chloride (9 mmol) in dichloromethane (25 mL) was added drowise at 0-5 °C. The reaction mixture was refluxed for 24 h. The reaction was monitored by TLC for completion. The precipitate formed was filtered, washed with dichloromethane and dried to get quinoline-azetidinone (**6**). *3-chloro-1-((7-chloroquinolin-4-yl)amino)-4-(4-chlorophenyl)azetidin-2-one* **(6a):** Lime-yellow powder; Yield 60%; mp: 328-330°C; FTIR (KBr, cm^{-1}): 3238 (NH), 3084 (Ar-H), 1705 (C=O);

^{1}H-NMR (DMSO-d_6, 400 MHz) δ, ppm: 4.25 (s, 1H, CH), 5.74 (s, 1H, CH), 7.57 (d, 2H, *J*=8.4 Hz, Ar-H), 7.67 (s, 1H, Ar-H), 7.87 (s, 1H, Ar-H), 7.91(d, 2H, *J*=8.4 Hz, Ar-H), 8.10 (s, 1H, Ar-H), 8.68 (s, 1H, Ar-H), 8.80 (s, 1H, Ar-H), 13 (s, 1H, NH); LCMS *m*/*z* = 393 (M + 1). *3-chloro-1-((7-chloroquinolin-4-yl)amino)-4-(4-fluorophenyl)azetidin-2-one (**6b**):* Yellow powder; Yield 75%; mp: 320-322°C; FTIR (KBr, cm^{-1}): 4.25 (s, 1H, CH), 5.73 (s, 1H, CH), 6.78 (d, 2H, *J*=8.8 Hz, Ar-H), 7.51 (s, 1H, Ar-H), 7.67(d, 2H, *J*=8.8 Hz, Ar-H), 7.82 (s, 1H, Ar-H), 8.02 (s, 1H, Ar-H), 8.56 (s, 1H, Ar-H), 8.64 (s, 1H, Ar-H), 12.6 (s, 1H, NH); LCMS *m*/*z* = 375 (M + 1). *3-chloro-1-((7-chloroquinolin-4-yl)amino)-4-(3,4-dimethoxyphenyl)azetidin-2-one (**6d**):* Yellow powder; Yield 72%; mp: 302-304°C; FTIR (KBr, cm^{-1}): 3169 (NH), 3081 (Ar-H), 1708 (C=O), 1315 (OCH_3); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ, ppm: 3.83 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 4.25 (s, 1H, CH), 5.73 (s, 1H, CH), 7.07 (s, 1H, Ar-H), 7.38-7.36 (m, 1H, Ar-H), 7.49 (s, 1H, Ar-H), 7.61 (s, 1H, Ar-H), 7.88-7.85 (m, 1H, Ar-H), 8.05 (s, 1H, Ar-H), 8.64-8.62 (m, 1H, Ar-H), 8.70 (s, 1H, Ar-H), 12.8 (s, 1H, NH); LCMS *m*/*z* = 419 (M + 1); *3-chloro-1-((7-chloroquinolin-4-yl)amino)-4-(3,4,5-trimethoxyphenyl)azetidin-2-one (**6e**):* Yellow powder; Yield 70%; mp: 328-330°C; FTIR (KBr, cm^{-1}): 3210 (NH), 3084 (Ar-H), 1705 (C=O), 1334 (OCH_3); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ, ppm: 3.73 (s, 3H, OCH_3), 3.88 (s, 6H, OCH_3), 4.25 (s, 1H, CH), 5.7 (s, 1H, CH), 7.1(s, 2H, Ar-H), 7.65 (s, 1H, Ar-H), 7.86 (s, 1H, Ar-H), 8.09 (s, 1H, Ar-H), 8.64 (s, 1H, Ar-H), 8.75 (s, 1H, Ar-H), 10.93 (s, 1H, NH); LCMS *m*/*z* = 550 (M + 2). *3-chloro-1-((7-chloroquinolin-4-yl)amino)-4-(4-hydroxyphenyl)azetidin-2-one (**6f**):* Yellow powder; Yield 65%; mp: 340-342 °C; 3647 (OH), 3278 (NH), 3086 (Ar-H), 1703 (C=O); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ, ppm: 4.25 (s, 1H, CH), 5.73 (s, 1H, CH), 6.89 (d, 2H, *J*=8.8 Hz, Ar-H), 7.32 (s, 1H, Ar-H), 7.32 (s, 1H, Ar-H), 7.50 (s, 1H, Ar-H), 7.68 (d, 2H, *J*=8.8 Hz, Ar-H), 7.81 (m, 1H, Ar-H), 8.09 (s, 1H, Ar-H), 10.21 (s, 1H, OH), 12.9 (s, 1H, NH); LCMS *m*/*z* = 375 (M + 1). *3-chloro-1-((7-chloroquinolin-4-yl)amino)-4-(4-nitrophenyl)azetidin-2-one (**6g**):* Yellow powder; Yield 40%; mp: 320-322 °C; FTIR (KBr, cm^{-1}): 3157 (NH), 3078 (Ar-H), 1697 (C=O), 1585 (NO_2); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ, ppm: 4.25 (s, 1H, CH), 5.74 (s, 1H, CH), 7.32 (s, 1H, Ar-H), 7.90 (s, 1H, Ar-H), 8.10 (s, 1H, Ar-H), 8.16 (d, 2H, *J*=8.8 Hz, Ar-H), 8.33 (d, 2H, *J*=8.8 Hz, Ar-H), 7.75 (s, 1H, Ar-H), 8.57 (s, 1H, Ar-H), 13.02 (s, 1H, NH); LCMS *m*/*z* = 404 (M + 1).

*3-chloro-1-((7-chloroquinolin-4-yl)amino)-4-(3,4-dichlorophenyl)azetidin-2-one (**6j**):* Light yellow powder; Yield 81%; mp: 340-342 °C; FTIR (KBr, cm^{-1}):3170 (NH), 3054 (Ar-H), 1695 (C=O); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ, ppm: 4.26 (s, 1H, CH), 5.73 (s, 1H, CH), 7.80-7.71 (m, 3H, Ar-H), 8.10 (s, 1H, Ar-H), 8.17 (s, 1H, Ar-H), 8.68 (s, 1H, Ar-H), 8.75 (s, 1H, Ar-H), 8.80 (s, 1H, Ar-H),12.98 (s, 1H, NH); LCMS *m*/*z* = 427. *3-chloro-1-((7-chloroquinolin-4-yl)amino)-4-(2-chlorophenyl)azetidin-2-one (**6k**):* Yellow powder; Yield 50%; mp: 336-338 °C; FTIR (KBr, cm^{-1}): 3169 (NH), 3082 (Ar-H), 1710 (C=O); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ, ppm: 4.25 (s, 1H, CH), 5.74 (s, 1H, CH), 8.1-7.68-7.46 (m, 3H, Ar-H), 7.87 (s, 1H, Ar-H), 8.22-8.14 (m, 2H, Ar-H), 8.70 (s, 1H, Ar-H), 8.85 (s, 1H, Ar-H), 9.23 (s, 1H, Ar-H), 13.1 (s, 1H, NH); LCMS *m*/*z* = 393 (M + 1).

32. Mosmann, T. *J. Immunol. Methods.* **1983**, *65,* 55.
33. Ahmed, K.;. Srikanth ,Y.V.V.; Naseer Ahmed Khan, M.; Ashraf, M.; Kashi Reddy, M.; Farheen,S.; Tandeep, K.; Gousia, C.; Nitasha, S.; Irum, S.; Zahoor, A.W.; Arpita, S.; Parduman R.S.; Shashi, B.; Dilip, M. M.; Ajit, K.S. *Bioorg. Med. Chem.* **2011**, *19*, 7136.
34. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. *J. Adv. Drug. Deliv. Rev*. **2001**, *46*, 3.
35. Molinspiration Cheminformatics, Bratislava, Slovak Republic, Available from: http://www.molinspiration.com/services/properties.html.
36. Ahsan, M. J.; Samy, J. G.; Khalilullah, H.; Nomani, M. S.; Saraswat, P.; Gaur, R.; Singh, A. *Bioorg. Med. Chem. Lett*. **2011**, *21*, 7246.
37. Rajanarendar, E.; Rama Krishna, S.; Nagaraju, D.; Govardhan Reddy, K.; Kishore, B.; Reddy, Y. N. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 1630.
38. http://www.asteris-app.com/technical-info/core-properties/logp.htm (2016)



IC_{50} = 52.5 µM against HEP G2

IC_{50} = 0.04 µM against HEP G2

In search of new heterocyclic anticancer agents, a new quinoline-azetidinone hybrid template have been designed, synthesized and evaluated in vitro anticancer against Hep G2 and Hep 3B human cancer cell lines.

Highlights:

- Quinoline-azetidinone hybrids were synthesized
- Cytotoxic activity against Hep G2, and Hep 3B was reported
- Compound **6f** exhibited significant cytotoxic activity (IC_{50} = 0.04 µM) against Hep G2
- Compound **6j** exhibited better cytotoxic activity (IC_{50} = 0.66 µM) against Hep 3B