C. Sreedhar et al /J. Pharm. Sci. & Res. Vol. 15(5), 2023, 1080-1085

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Design, Synthesis and Biological Evaluation of Novel Quinoline-Based Hybrid Molecules as Antitumor Agents

Dr. C Sreedhar, Dr. Harsha K Tripathy, T Srinivasa Rao, K Manogna
Department of Pharmaceutical Chemistry, Karnataka College of Pharmacy, Bangalore-560064

Abstract:
Novel Quinoline based hybrid molecules were synthesised (a, b, c, d) from a mixture of 4, 7 di chloroquinoline (1 mmol) 4-hydroxy benzaldehyde (1 mmol). Compounds are characterized by spectroscopic methods (IR, H NMR, C13 NMR). Anti-cancer activity was evaluated on the liver and breast of mice for Ehrlich Ascites Carcinoma. Concentrations of pro-inflammatory cytokines tumor necrosis factor (TNF)-alpha, interferon (IFN)-g, interleukin (IL) 6, and as well as of TGF-b were determined by using antigen capture ELISA. The synthesised test molecules were tested for cytotoxic effect(KPB Series 1 to 4), IC50 – Value for KPB1, KPB3, KPB4 was found to be 46.12, 46.62, 46.29, 46.52 respectively. Highest concentration showed significant cell death and 100% demise in positive control i.e. DMSO.

Keywords: Quinoline derivatives, Hybrid Molecules,Antitumor activity

INTRODUCTION

Cancer is a complex disease and is measured as the most serious health problem all over the world. Despite modern advances in our understanding of the biological processes leading to the development of cancer, there is still a need for the development of more potent and effective anticancer agents for the complete eradication of the disease [1]. Multi-drug resistance and acute toxicity are the two major issues with most of the currently available chemotherapeutic agents [2,3]. Therefore, novel anticancer agents need to be developed that are more potent, safe and selective. In a quest for the discovery of more effective anticancer drugs, a large number of structurally diverse synthetic and natural products have been screened for their anticancer potential [4].

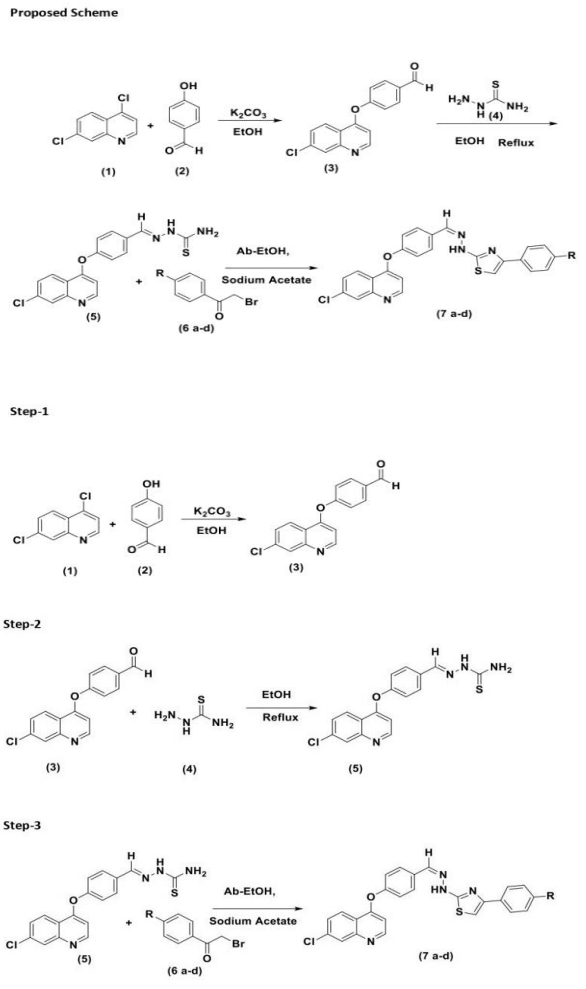
Numerous anticancer drugs are currently marketed for treating different types of cancer. However, owing to the increasing resistance, lack of target drug delivery, higher costs, and poor patient compliance, certain drugs are proving to be ineffective in curbing this gigantic disease. Our research project will shed some light on the newer possibilities of developing some alternative drugs for treating cancer. The proposed scheme may prove to be a guideline for synthesizing newer quinoline-based drugs as potential anticancer agents. The tumor is a solid mass of tissue that forms when abnormal cells group together. Tumors can affect bones, skin, tissue, organs, and glands. Many tumors are not cancer (they're benign). But they still may need treatment. Cancerous, or malignant, tumors can be life-threatening and require cancer treatment.

METHODOLOGY:

General Procedure for the synthesis of (3): To a mixture of 4, 7 di chloroquinoline (1 mmol) 4-hydroxy benzaldehyde (1 mmol) and DMF, add DMF, potassium carbonate (6 mmol) is added, then refluxed the reaction mixture for 16 hrs. After the completion of the reaction poured the contents of the reaction mixture to crushed ice, stirred, filtered and recrystallized with ethanol to obtain cream-colored product.

General Procedure for the synthesis of (5): To a mixture of 3 and thiosemicarbazide or phenyl thiosemicarbazide in (1 mmol) was added to a RBF containing absolute ethanol, catalytical amount of PTSA and refluxed for 24 hrs. Cooled, filtered, and washed with diethyl ether. Recrystallized with ethanol to get white powder.

General Procedure for the synthesis of (7): To a mixture of 5 and substituted phenacyl bromides (1 mmol), sodium acetate (1.5mmol) in RBF containing absolute ethanol. Refluxed the contents for 24 hrs. After the completion of the reaction, cool the contents, filter, and washed with water to remove excess of sodium acetate. Finally recrystallized with ethanol.



***In vitro* and *in vivo* anticancer activity of novel quinoline-based hybrid molecules**

Model 1:

To Study the *in vitro* anticancer by estimating the following;

- Establishing a stable culture of the Breast cancer cell line (MCF-7 cell line)
- Cell proliferation Assays - Performed MTT assay
- Comparison of % cell growth inhibition against various synthesized test molecules

Model 2:

To Study the *in vivo* anticancer by estimating the following;

- Evaluation of the *in-vivo* anticancer activity of test molecules against Ehrlich Ascites Carcinoma induced mammary tumor in mice model.
- To compare the effectiveness of Test molecules against standard anticancer drug 5-FU
- The anti-tumour activity was screened with respect to the following parameter:
  - Body weight
  - Tumor Size
  - Effect on Survival time
  - % Increase life span
  - Effect on Hematological Parameters (Hb%, RBC, WBC, Neutrophils and Lymphocytes)
  - NLR Ratio
  - Pro-Inflammatory cytokines (TGF-beta, IFN-gamma, TNF-alpha, IL-6)
  - Biopsy Study – Liver- Tumor

Parameters:

Effect on Mean survival time:

Animals will be inoculated with EAC cells (1 X 10^6cells/mouse) on day '0' and the median survival time (MST) of each group, consisting of 6 mice were noted.[3]

$$MST = \frac{\text{(day of first death + day of last death)}}{2}$$

Percentage increase life span (% ILS):

The effect of the drugs on tumour growth was monitored by recording the mortality daily for a period of 6 weeks and a percentage increase in life span (%ILS) was calculated.

% ILS = [(Mean survival of treated group/ Mean survival of Disease control group)-1] x 100

Tumour size

Tumour size was measured from the 11^{th} day of tumour induction. The measurement was carried out every 5^{th} day for a period of 20 days.

Body weight

Body weights of the experimental mice were recorded both in the treated and control group at the beginning of the experiment (day 0) and sequentially on every 5th day during the treatment period.[5]

Effect on haematological parameters

At the end of the experimental period, 6 mice of each group from whom Blood was collected by Retro-orbital route the next day after an over-night fastand used for the estimation Hemoglobin (Hb%) content, red blood cell count (RBC) and white blood cell count (WBC), Neutrophils, Lymphocytes, NLR.

Assessment of Proinflammatory Cytokines

Plasma was normalized for protein content by Lowry et al. Method. Concentrations of pro-inflammatory cytokines tumor necrosis factor (TNF)-alpha, interferon (IFN)-g, interleukin (IL) 6, and as well as of TGF-b were determined by using commercial kits.

At the end of the treatment, blood samples were collected by cardiac puncture under mild pentobarbital anesthesia.

The Plasma were used for the estimation of the cytokines viz, IFN-g, IL-6, TGF beta and TNF-α was done using antigen capture ELISA.

The ELISA well plates have been coated with 100µl of IFN-g, IL-6, TGF beta and TNF-α primary antibodies (2.5µg/ml) in carbonate buffer (Na_2HPO_4 and NaH_2PO_4, pH 9.6). Primary antibodies of IFN-g, IL-6, TGF beta and TNF-α were used. The plates have been incubated overnight at 4^0C to facilitate proper adsorption of antibodies on to the substrate. After 12-14 h of incubation, the plates have been washed thrice with washing buffer i.e. NaCl and Tween 20 in phosphate buffer, pH 7.4 and blocked with 250µl of blocking buffer i.e. BSA in phosphate buffer, pH 7.4 per well.

After followed by incubation at 37^0C for 1h, added standard cytokines for the construction of calibration curve. Remaining wells had been coated with 100µl of diluted cell lysate. The concentration range used for the standards was – 25, 12.5, 6.25, 3.125, 1.562, 0.781, 0.39 and 0.195ng/ml.

The standard cytokines were used, Incubated for 1hr and washed thrice. Anti-cytokine antibodies such as; anti IFN-g, IL-6, TGF beta and then anti TNF-α monoclonal antibodies were diluted 1:1000 and added 100 µl per well to the strips containing the respective antigens and incubated at 37^0C for 1h. After incubation, 100 µl of HRP-conjugate was added. Plate incubated at 37^0C for 1h. Then 100µl of freshly prepared substrate i.e. TMB in DMSO containing $H_2O_2$was added to all wells. After incubation in dark 37^0C for 15mins, colour has changed, the reaction has been terminated by adding 50µl of 2.5N H_2SO_4 per well and the A_{450nm} was measured by using ELISA reader.

A standard calibration graph was plotted (Abs Vs Conc.) and the concentrations of unknown samples have been determined from the graph.

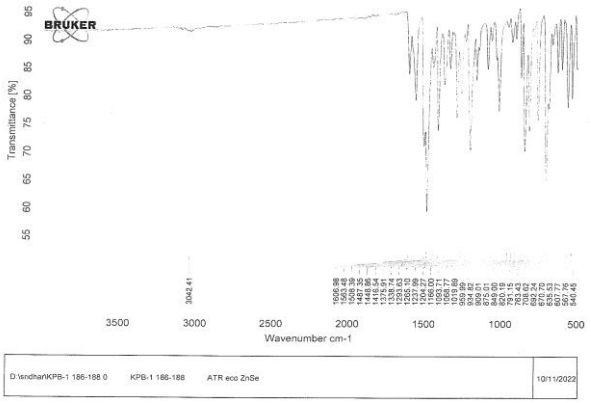
Histopathological studies

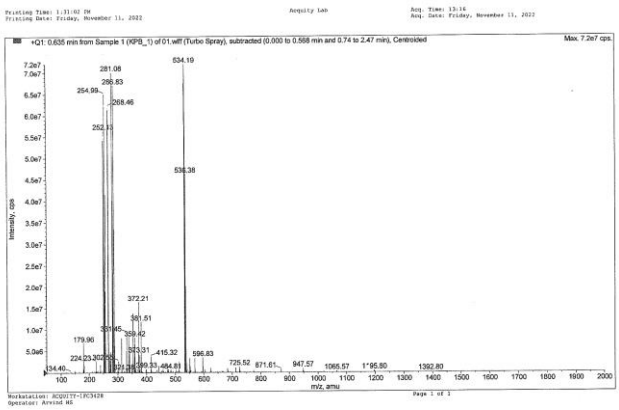
After collection of blood 1 mouse from each group was sacrificed by cervical dislocation and a portion of liver-Tumor biopsy were stored in container for 12 hours in 10% formalin (10 ml of formaldehyde in 90 ml of normal saline) solution and subjected to histopathological studies.

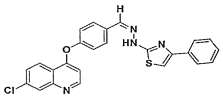
Statistical analysis

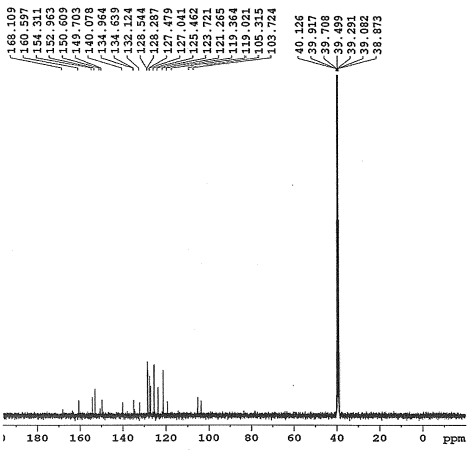
The results are expressed as mean ± S.D from n=6 mice in each group. The significance of difference among the groups was assessed using ANOVA followed by Tukey's test/Bonferroni post-tests compared between Normal control (Untreated) Vs all groups $p<0.05$ were considered significant.

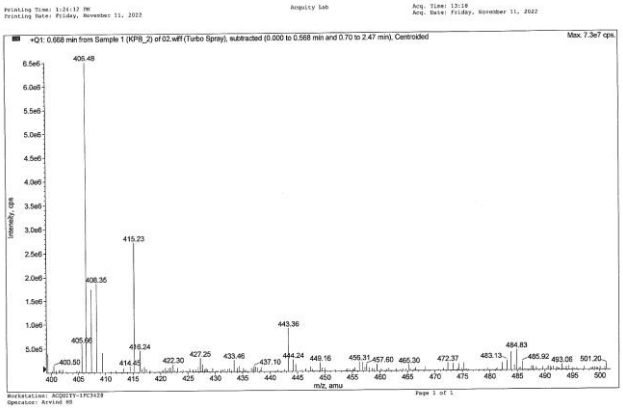
RESULTS:

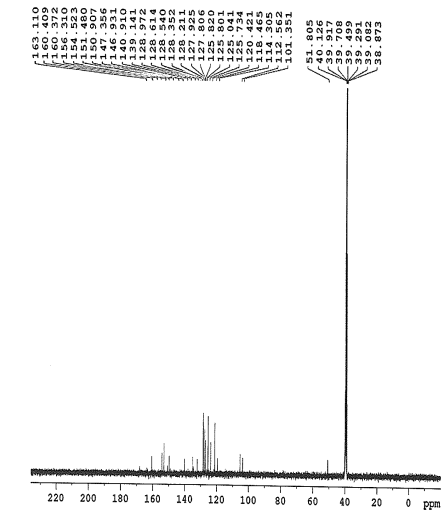










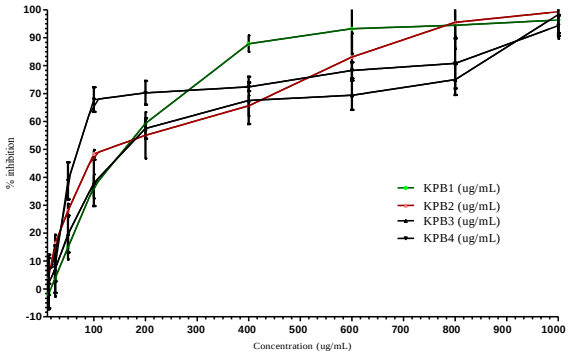


Cells/Cell lines used

Cells/Cell line	Tissue/cell type	Species	Obtained from
MCF-7 cell line	Breast Cancer	Human	NCCS, Pune
EAC cells	Ehrlich Ascites Carcinoma	Mammalian Cell	Amala Cancer Research Centre, Thrissur, Kerala, India.

Model 1: *In-Vitro* Anti-Cancer Study

Comparison of cytotoxic activity against various synthesized test molecules (KPB Series 1 to 4)

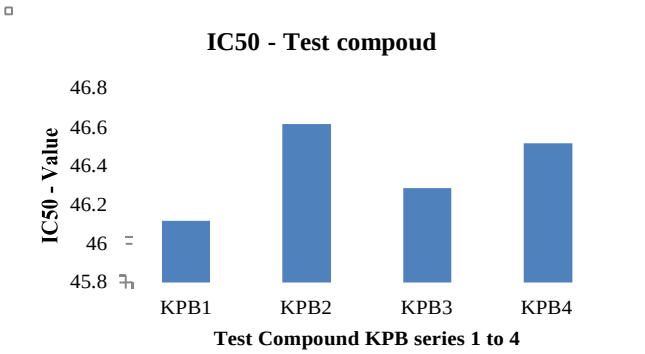


Value are expressed in Mean +/- SD (n=3)

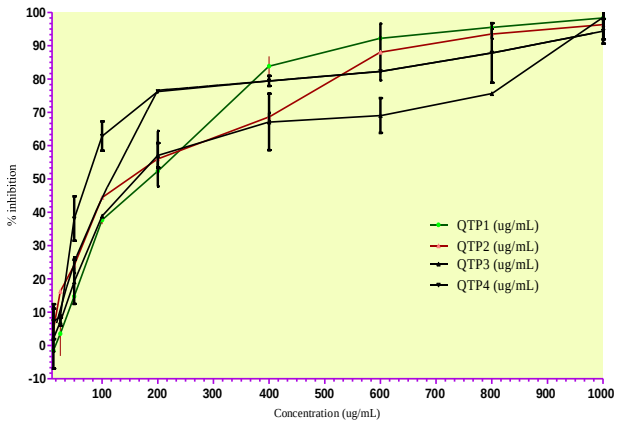
*DMSO (10%) Used as a positive control, where showing 100% Cytotoxic activity

Table 01: IC_{50} value for the compound series KPB 1 to 4

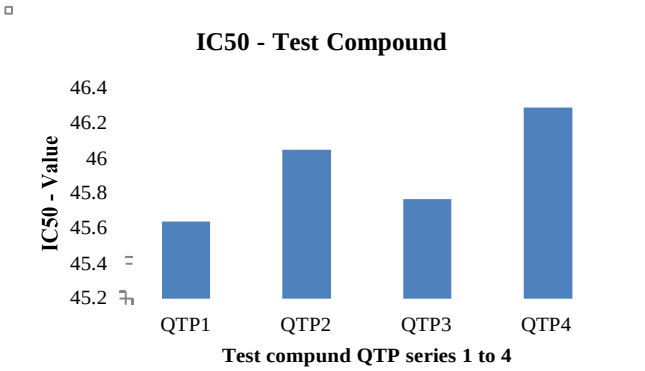
S. No.	Compound Series	IC_{50} - Value
1	KPB1	46.12
2	KPB2	46.62
3	KPB3	46.29
4	KPB4	46.52



Comparison of cytotoxic activity against various synthesized test molecules (QTP Series 1 to 4)



Value are expressed in Mean +/- SD (n=3)
*DMSO (10%) Used as a positive control, where showing 100% Cytotoxic activity



DISCUSSION:

Interpretation:

Overall the elevations were identical; it has been concluding that the differences between the elevations are not significant.

The cell growth inhibition % was determined and it was observed that the test items concentration of 0.7 to 50µg/ml didn't pretend inhibition. Compound producing 50% of cell growth inhibitions were shown at the dose of 50-100µg/ml. But in the highest concentration showed significant cell deathand 100% demise in positive control i.e. DMSO

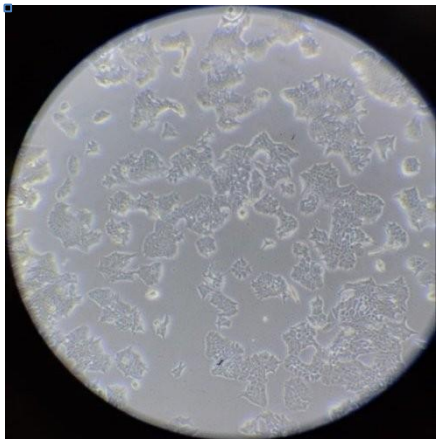


Fig 01: Normal Healthy cells – MCF-7 cell line treated with medium – Negative control (Appear as loosely attached 3 D clusters)

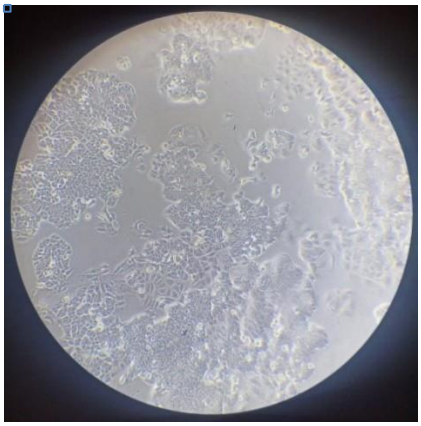


Fig 02: MCF-7 cell line with 100ug/ml of Test item (Appear as loosely attached 3 D clusters with some floating viable cells and dead cells)

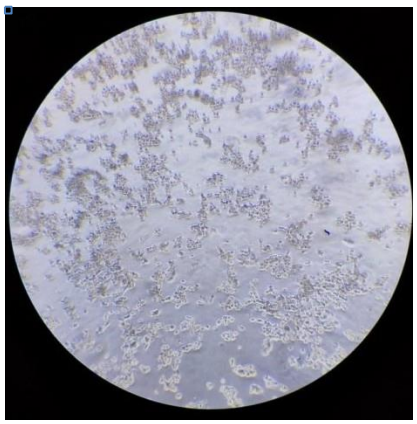


Fig 03: MCF-7 cell line with higher concentration of Test item (looks more than 90% cells inhibition)

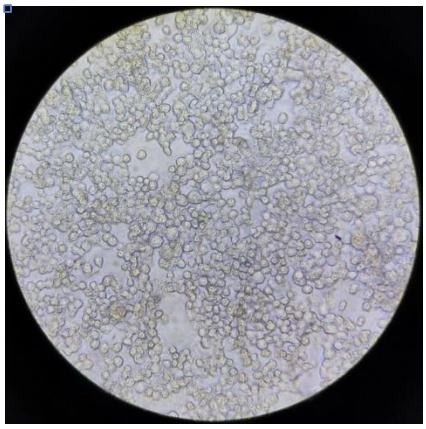
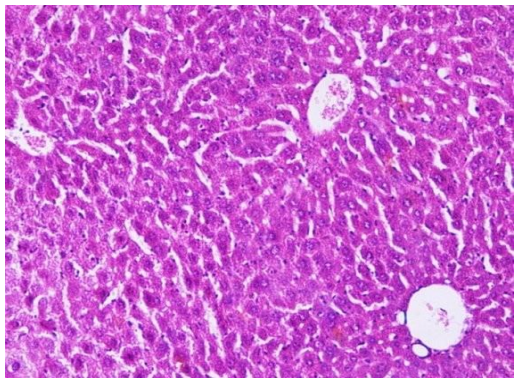


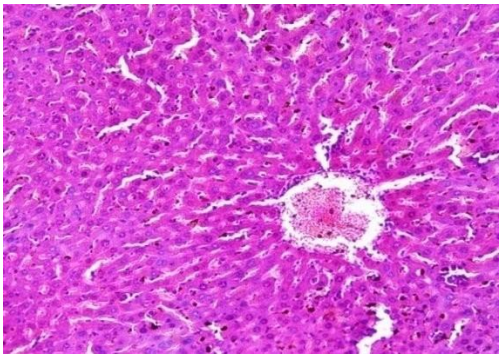
Fig 04: MCF-7 cell line treated with 10% DMSO (Positive control) (0% cell viable, 100% cell death)

Effect of Test compound series of KPB and QTP 1 to 4 onHistopathological studies: Liver - Tumor and Tumor free tissues



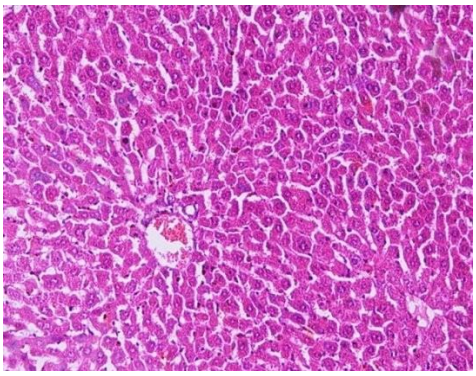
Normal vehicle control Liver

Section study shows Hepatocytes and central vein appear normal. Cords radiating from central vein can be observed prominently.



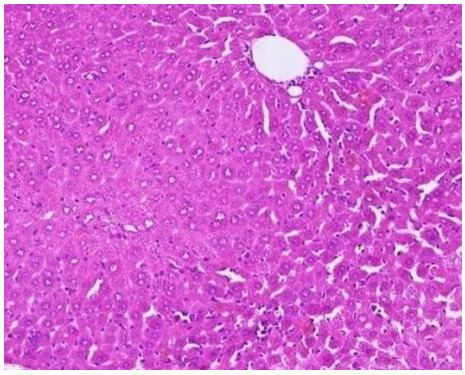
Effect on Liver of Disease Control - EAC Control

Section study shows Central vein appears damaged. Hepatocytes and sinusoids appear deranged. Perisinusoidal cells appear dilated at some regions. Micro vascular changes can be noted at blood vessels.



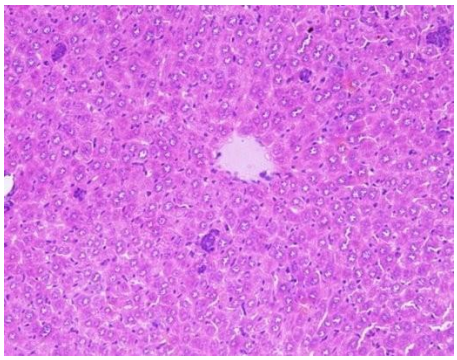
Effect of Std. drug - 5-fluorouracil on liver

Section studied shows Hepatocytes are polygonal and regular with prominent nuclei. Chords radiation from central vein can be seen. Sinusoidal structure appears as if returning to normal from pathological insult. Bile secreting cells can be seen.



Effect of Test molecule (KPB) on Liver

Section studied shows Portal vein, hepatic artery and duct appear normal making the portal triad complete. Sinusoids appear absent in major region. Hepatocytes appear of normal morphology.



Effect of Test molecule(QTP) on Liver

Perisinusoidal spaces are absent. Damage to blood vessels and internal haemorrhages can be seen. Hepatocytes appear of indistinct morphology.

Acknowledgement

This Research work was funded by the Advanced Research funding from Rajiv Gandhi University of Health Sciences, Karnataka, and Bangalore.

REFERENCES:

[1] AvendanoC,MenendezJC,MedicinalChemistryof AnticancerDrugs,Elsevier,2008.

[2] Patrick GL, An Introduction to Medicinal Chemistry, Oxford UniversityPress,2013.

[3] Singla H, Kalra S, Kheterpal P, Kumar V, Munshi A, Role ofgenomicalterations in HER2 positive breast carcinoma: focus on susceptibilityandtrastuzumab-therapy,Curr. Cancer DrugTarget17 (2017)344–356.

[4] Kumar B, Sharma P, Synthesis and biological evaluation of pyrimidineridged combretastatin derivatives as potential anticancer agents and mechanisticstudies.BioorganicChemistry78 (2018)130–140.

[5] Alegaon SG, Parchure P, Araujo LD, Salve PS,Alagawadi KR, JalalpureS.et al.Quinoline-azetidinone hybrids: synthesis and *in vitro*antiproliferationactivity against Hep G2 and Hep 3B human cell lines.Bioorganic Med. Chem.Lett.2017;27:1566-71.

[6] Montoya A, Quiroga J, Abonia R, Nogueras M, Cobo J, Insuasty B.Synthesis and *in vitro* antitumor activity of a novel series of 2-pyrazolinederivatives bearing the 4-aryloxy-7-chloroquinoline fragment.Molecules.2014;19:18656-75.

[7] Tang Q, Zhai X, Tu Y, Wang P, Wang L, Wu C, et al. Synthesis andantiproliferative activity of 6,7-disubstituted-4-phenoxyquinoline derivativesbearing the 2-oxo-4-chloro-1,2-dihydroquinoline-3-carboxamide moiety.BioorganicMed. Chem.Lett. 2016; 26(7):1794-98.

[8] Krstulovic L, Stolic I, Jukic M, Opacak T, Starcevic K, Bajic M, *et al*.New quinoline-arylamidine hybrids: Synthesis, DNA/RNA binding andantitumoractivity.Eur. J. Med. Chem. 2017;137:196-210.

[9] Bingul M, Tan O, Gardner CR, Sutton SK, Arndt GM, Marshall GM,Synthesis, characterization and anti-cancer activity of hydrazide derivativesincorporatingaquinolinemoiety. Molecules.2016;21:916-35.

[10] Manohar CS, Manikandan A, Sridhar P, Sivakumar A, Kumar BS,Reddy SR. Drug repurposing of novel quinolineacetohydrazide derivatives aspotent COX-2 inhibitors and anti-cancer agents. J. Mol .Struct .2018;1154:437-44.

[11] He C, Preiss L, Wang B, Fu L, Wen H, Zhang X. Structuralsimplification of bedaquiline: The discovery of 3-(4-(N,Ndimethylaminomethyl) phenyl) quinoline-derived antitubercular leadcompounds.ChemMedChem. 2017; 12(2):106-19.

[12] Salve PS, Alegaon SG, Sriram D. Three-component, one-pot synthesisofanthranilamideschiffbases bearing4-aminoquinolinemoietyas *mycobacterium tuberculosis* gyrase inhibitors. Bioorganic Med. Chem. Lett.2017;27(8):1859-66.

[13] Rani A, Viljoen A, Kremer L, Kumar V. Microwave-assisted highlyefficient route to 4-aminoquinoline-phthalimide conjugates:synthesis and anti-tubercular evaluation. ChemMedChem .2017;2:10782-5.

[14] Bodke YD, Shankerrao S, Kenchappa R, Telkar S.Synthesis,antibacterial and antitubercular activity of novel schiff bases of 2-(1-Benzofuran-2-yl) quinoline-4-carboxylic acidderivatives.Russ.J.Gen.Chem.2017;87(8):1843-9.

[15] Somagond SM, Kamble RR, KattimaniPP, Shaikh KJ. Design, docking,and synthesis of quinoline-2H-1, 2, 4-triazol-3(4H)-ones as potent anticancerandantitubercularagents.ChemMedChem. 2018;3:2004-16.