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A novel approach towards design, synthesis and evaluation of some Schiff base analogues of 2-aminopyridine and 2-aminobezothiazole against hepatocellular carcinoma

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ABSTRACT

Hepatocellular carcinoma is the most common primary malignancy of the liver with poor prognosis. In this study novel, Schiff's bases of 2-aminopyridine (SSSC-26 to 31) and 2-aminobenzothiazole (SSSC-32 to 37) were designed, synthesised and evaluated for antioxidant potential using DPPH method, and anti-hepatocellular carcinoma property using diethylnitrosamine (DEN) induced hepatocellular carcinoma rat model. The in-silico pharmacokinetic, rule of five and toxicity studies reveals that all the leads have an excellent intrinsic quality and sufficient structural features necessary for an oral activity. Molecular docking studies of all compounds into the ligand binding pocket of checkpoint kinase1 and vascular endothelial growth factor receptor-2 was also performed using Schrodinger software suite *v*8.5, and which have shown good Glide scores. Further compounds were synthesised based on the docking score and ADMET profile. The 1,1-diphenyl2-picrylhydrazil (DPPH) scavenging study was carried out, and results showed that SSSC-29 (IC_{50}-63.60) and SSSC-33 (IC_{50}-60.32) were having good anti-oxidant potential in comparison with ascorbic acid (IC_{50}-55.27). SSSC-33 further evaluated for anti-cancer potential against diethylnitrosamine (200 mg/kg bw) induced hepatocellular carcinoma in rats. The biochemical, histopathological and morphological data showed that SSSC-33 can reverse the changes occurred in the cancerous liver significantly. All these findings suggested that SSSC-33-((benzo[d]thiazol-2-ylimino) methyl)phenol) could be a potential compound in combating the oxidative damage of hepatic cells occurred due to the development of hepatocellular carcinoma induced by a chemical carcinogen, DEN.

1. Introduction

Hepatocellular carcinoma (HCC), a primary malignancy of the liver, is the fifth most common cancer in men and seventh in women. Poor prognosis is the main reason for decreased cure rate of HCC and occurrence of approximately more than 50, 000 new cases of HCC worldwide every year [1]. Globally, death rate from all other common cancers (such as lung, breast, and prostate cancers) is declining, whereas mortality rate from liver cancer are increased by 2.8 and 3.4 percentage per year respectively in men and women [2]. Most cases of HCC develop in the liver having chronic damage with alcoholic liver disease, non-alcoholic fatty liver, hepatitis B (accounts for most 50% of all cases of HCC worldwide) and hepatitis C infection [3]. Multiple treatment options are available for HCC like surgical resection, locoregional ablation, cytotoxic chemotherapy (e.g., sorafenib) and liver transplantation but the later diagnosis of HCC is the major limitation for the success. At an advanced stage, sorafenib, a multi-kinase inhibitor, is the only US FDA-approved therapy [4]. In the study conducted by Xiao-Ke Guo et al., natural molecule like Gambogic acid (GA) and its derivatives have been proved as apoptotic agent against HepG2 cell lines [5].

Reactive oxygen species (ROS) includes superoxide anion radical ($O_2^{-\bullet}$), singlet oxygen (O), hydrogen peroxide (H_2O), and the highly reactive hydroxyl radical ($^{\cdot}OH$) play a significant role in carcinogenesis [6]. Oxidative stress occurs due to excess ROS production, depletion of antioxidants (like superoxide dismutase) or both and subsequent imbalance in the average level of ROS and antioxidants. Especially in HCC amount of ROS is more due to permanent damage of liver, a major metabolising organ. Compounds of having antioxidant property may have anti-HCC activity due to its free radical scavenging activity [7].

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Schiff bases or Azomethines, first reported by Hugo Schiff in 1864, broadly display range of biological activities such as anticancer, antifungal, antibacterial, antimalarial, antiviral, anti-pyretic, anti-inflammatory characteristics [8]. Schiff's bases with general formula RHC=N—R' (Imine group) are the class of compounds formed by the condensation between the primary amine and carbonyl compounds [9]. The lone pair of electrons present in sp^2 hybridised orbital of the nitrogen atom in the imine group responsible for its chemical and biological properties [10]. The chelating property of Schiff bases plays a significant role in its antioxidant activity, and this will be helpful in the development of compounds with anti-hepatocellular carcinoma.

In this work, some novel Schiff's bases based on 2-amino-pyridine and 2-aminobenzothiazole with various aldehydes are designed and evaluated theirs *in silico* toxicity and absorption, distribution, metabolism and excretion (ADME) properties using Protox and QikProp software respectively. Further, we docked the hits with checkpoint 1 (Chek1) and vascular endothelial growth factor receptor (VEGFR) using Schrodinger software suite *v*8.5 [11]. The leads having good docking score and reasonable ADMET property was selected for synthesis. The compounds were characterised by ^{1}H NMR, ^{13}C NMR, Mass spectral and elemental analysis. Compounds were then screened for its anti-oxidant property by 1,1-diphenyl-2-picrylhydrazil (DPPH) [12,13] method using ascorbic acid as standard. Subsequently evaluation of anti-HCC activity of the best compound by its anti-oxidant property and docking score was performed in DEN-induced HCC rat model [1].

Diethylnitrosamine (DEN) is a chemical carcinogen and DEN-induced HCC in the rat is considered an as well accepted model for the development of drugs against hepatocarcinogenesis. DEN was first hydroxylated to alpha-hydroxy nitrosamine in the presence of cytochrome P450- dependent enzymatic system and which is capable of alkylating DNA structure (Fig. 1). ROS are also generated during this enzymatic process and causes lipid peroxidation and adds up to the hepatocarcinogenesis [1]. DEN-induced HCC rat model to mimic the injury-fibrosis-malignancy cycle. Here, DEN was given to the rat in the period of hepatic cell proliferation, initiated by partial hepatectomy (PH) [14], which followed a necrotizing dose of carbon tetrachloride (CCl_4; hepatotoxin) [15,16]. Trichloromethyl radicals formed by the metabolism of CCl_4 by CYP 450 causes lipid peroxidation and membrane damage [17]. Phenobarbitone sodium (PB) (which increases the expression of CYP 450 enzyme and leads to the enhanced effect of DEN and CCl_4) was also given during induction period for the successful hepatocarcinogenesis.

2. Material and methods

2.1. General information

Docking calculation was carried out on HP Intel® Core 2 due ® processor E3-1200v2 family with 16GB RAMS, 1TB Hard disk, NVIDIA Quadro 2000, Linux. Chem Office (level: Ultra, version: 3.5) was used for drawing and energy minimization of structures. QikProp module of Schrödinger was employed for Absorption, Distribution, Metabolism and Excretion (ADME) prediction and docking studies. ProTox, a web server was the toxicity determining tool used for Lethal Dose, 50% (LD50) calculation in the rat.

All the chemical used for the synthesis and *in vivo* study (DEN, CCl_4, and phenobarbitone sod.) were of analytical grade purchased from Sigma-Aldrich. The progress of the reaction was determined by thin layer chromatography (TLC) on silica gel plates in various solvents. UV-lamp (λ = 254–365 nm) was used for visualisation of TLC plate and iodine vapour or ninhydrin reagent as detecting agent. Melting points were determined on OptiMelt (Stanford Research Systems, California) and were uncorrected. ^{1}H NMR (400 MHz) was performed on Bruker Advance 300 instrument (Bruker Instruments Inc., USA) using DMSO-d_6 as solvent and tetramethylsilane (TMS) as an internal standard. Chemical shifts were expressed in parts per million (ppm). Mass spectra were recorded by WATERS-Q-T of Premier-HAB213 using the ESIMS Electrospray Ionization technique.

2.2. Molecular design

2.2.1. ADME prediction

The prediction of ADME (Absorption, Distribution, Metabolism and Excretion) properties are considered to be crucial in the development of a new drug since lots of drugs are being withdrawn from the market due to the inappropriate ADME properties. The

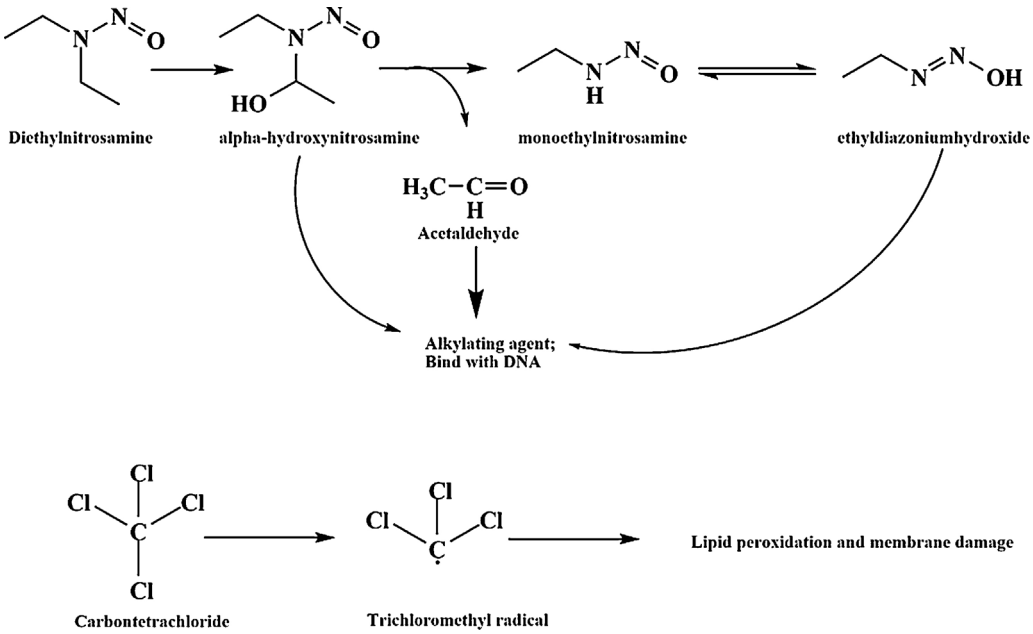

Fig. 1. Metabolism of DEN and CCl_4 in the presence of CYP 450 enzymatic system.