# Antitumor Potential of Antimicrobials: An Anticipated Armour For Hepatocellular Carcinoma

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***Authors' contribution***

*This work was carried out in collaboration of all the authors who read and approved the final manuscript*

**ABSTRACT**:
Hepatocellular carcinoma also known as hepatoma is considered as the most common type of primary liver malignancy and is the leading cause of death worldwide. The incidence and mortality is still on the rise despite the drastic progress in early screening tools and new advancements in diagnosis and treatment. For the patients presenting with advanced disease, Sorafenib is the only approved drug, however the treatment outcome of metastatic cancer is still unsatisfactory with median overall survival below 15 months. Over the past few years great progress has been achieved in anticancer therapy, but development of resistance and unavoidable side effects have weakened these attainments. Keeping in view this stern condition, a number of drugs with novel antitumor mechanisms are under investigations including antimicrobials which have been shown to possess anti-inflammatory, immunomodulatory and cytotoxic effects. In this regard, both conventional and novel antimicrobials are being studied to explore their anticancer potential along with underlying mechanisms which may render them as effective anticancer drugs in near future. Moreover, the new approach of drug repurposing is also being encouraged especially in cancers in order to reduce cost and limit adverse effects. The purpose of this review is to provide comprehensive landscape of current information on anti-tumor evidence in support of certain compounds with well-known antimicrobial activities, against HCC based on relevant literature search on different HCC cell lines.

**KEY WORDS:**
Antimicrobials, Hepatocellular carcinoma, Anticancer effects

**ABBREVIATIONS:**
Hepatocellular carcinoma (HCC)
Hepatitus B virus (HBV)
Hepatitus C virus (HCV)
Vascular Endothelial Growth Factor Receptors (VEGFRs)
Platelet-derived growth factor receptors (PDGF-R)
Matrixmetalloproteinase-9(MMP-9)
Extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAP kinase)
Reactive oxygen species (ROS)
Signal Transducer and activator of transcription pathway (STAT)
Roxithromycin (RXM)
Transarterial chemoembolization (TACE)
Percutaneous ethanol injection (PEI)

Activator protein-1 (AP-1)
Poly (ADP-ribosepolymerase) (PARP)
Nuclear factor kappa b (NF-kB)

## INTRODUCTION:

Hepatocellular carcinoma (HCC) is one of the well-known cancers around the globe carrying the highest rate of incidence in the region of East Asia and Africa (Papatheodoridis et al., 2010). It stands as sixth most common malignancy worldwide (Ferenci et al., 2010) but due to poor prognosis it is third foremost cause of deaths occurring due to cancer around the globe (Njei et al., 2015)with adenocarcinoma of liver being the most common type (Zhu et al., 2016). Approximately 554,000 men and 228,000 women are being reported as new cases of this cancer every year (de Martel et al., 2015) out of which more than 70% of the cases are diagnosed in Asia and 55% of these cases occur in China alone (Yuen et al., 2009). Regarding Pakistan, a hospital-based registry stated that, hepatobiliary cancers are the most common malignancy in adult males and represent 10.7% of all cancers in our region (Bhatti et al., 2016).The incidence is found to be higher in men as compare to women with ratio of 2.4 (Venook et al., 2010). Cirrhosis is the single most etiological factors leading to the pathogenesis of HCC (70% to 90%) followed by HBV infection, HCV infection, smoking, heavy alcohol intake and metabolic syndrome (Trichopoulos et al., 2011) however, results are variable according to geographical differences. Clinically patients may present with jaundice, hepatic encephalopathy and ascites which are the main manifestations of ultimate decompensated liver cirrhosis or with hepatomegaly with hard and irregular borders or occasionally with shrunken liver or a mass. Patients may also present with non-cirrhotic malignancy with symptoms such as loss of weight, anorexia, generalized weakness and abdominal distension. Hepatoma has tendency to metastasize commonly to lung, bone and abdominal viscera via both lymphatic or hematogenous spread (Bialecki et al., 2005). Staging is crucial in predicting the prognosis and taking decisions about the treatment.(Duseja and hepatology, 2014)

Regardless of the etiology or symptomatology its median survival rate is not more than 1 year owing to many factors which include poor availability of early screening tools, late diagnosis and scarcity of therapeutic options (Frenette and Gish, 2012).At present few drugs are available for management of HCC beside surgical or radiological interventions which improve median survival rate for few months, amongst which sorafenib is the only FDA approved drug that is the inhibitor of Raf kinase (VEGFR-2/-3) and (PDGFR-β) (Cainap et al., 2015).These receptors upon ligand binding become activated resulting in proliferation and angiogenesis, with subsequent spread of tumor cells and peri neovascularization(Ikeda et al., 2017).It is the only approved drug which improves overall median survival in liver cancer patients (Zhu et al., 2016) . As mentioned by Intaraprasong et al in 2016, the median survival rate was improved from 7.9% to 10.7% in 602 patients suffering from HCC who received sorafenib in comparison with placebo group (Intaraprasong et al., 2016). However, Kim et al in 2011 compared two groups of advanced HCC patients, revealed no significance of sorafenib once the patient presents with advanced liver cancer and worst liver functions (Kim et al., 2011) moreover, the effects are transient and HCC follow its usual course of progression. Lastly certain adverse effects have also been reported such as fatigue, diarrhea, hypertension, non-characterized pruritis, skin dryness , flushing, (Autier et al., 2008). Zhu et al documented that approximately 28.9% patients show poor compliance due to serious adverse effects like hemorrhagic events and cardiac events such as stroke and myocardial infarction (Zhu et al., 2014).

Studies are being conducted to estimate the role of suratinib, brivanib, linifanib, sorafenib plus erlotinib, combination of molecular targeted drugs and cytotoxic agents such as sorafenib plus doxorubicin but unfortunately none of them is successful in exhibiting significant results in comparison with sorafenib alone to date. Newer agents like brivanib, everolimus, ramucirumab and tivantinib, have undergone investigation for advanced patients intractable to sorafenib but all of them reported insignificant results (Ikeda et al., 2017).In this context researches are not only being carried out on various natural biomolecules, herbs but also on renowned drugs which are already approved/recommended for other diseases, a phenomenon defined as drug repositioning which is searching for new uses of existing drugs (Shim and Liu, 2014). Several drugs like metformin and paracetamol demonstrated significant antiproliferative potential invitro studies (Dowling et al., 2011). Recently a cumulative number of studies have stressed the antitumor properties of antimicrobials. Therefore, keeping in view the severity of the

situation regarding bad prognosis and paucity of eventual treatment options for this devastating disease, this review is inscribed to highlight certain antimicrobials compounds both conventional and novel which have shown positive results attained from in vitro studies in impeding hepatocellular carcinoma cell lines proliferation and could be an ultimate treatment option in near future for the same.

## CONVENTIAL ANTIMICOBIALS:

## MACROLIDES (AZITHROMYCIN, CLARITHROMYCIN, ERYTHROMYCIN)

Macrolides are natural/synthetic antimicrobials that retard bacterial growth by inhibiting protein synthesis after binding to 50S ribosomal subunit. (Tenson et al., 2003).They have been evaluated for their anti-inflammatory (Kanai et al., 2004), immunomodulatory (Altenburg et al., 2011) and anticancer properties (Williams, 2001). For later effects macrolides are believed to inhibit the over expression of matrixmetalloproteinase-9 which is considered to play role in tumorigenesis of hepatoma via downregulation of apoptotic proteins. Macrolides such as azithromycin, clarithromycin, erythromycin have been assessed for their inhibitory effect on some HepG2 cell line and chemically induced hepatocarcinogenesis model in rats. Among macrolides, clarithromycin evidenced significant reduction in MMP-9 serum levels and marked reduction in serum TNF-alpha after 17 weeks of treatment with either clarithromycin or azithromycin when compare to control group. Moreover, cytotoxic analysis revealed that HepG2 treated with clarithromycin showed cytotoxicity of 24%, 23%, 28% and 29% at concentrations of 5, 12.5, 25 and 50 µgm/ml respectively, while azithromycin at the concentration of 50µgm/ml showed 29% (Abdel-Hamid et al., 2017). Bcl-xl and bcl-2 which are antiapoptotic members of Bcl-2 family exhibited significant reduced expression while Bax which is proapoptotic member showed increased expression after being treated with clarithromycin and azithromycin. (Abdel-Hamid et al., 2017).

## CIPROFLOXACIN:

Flouroquinolones commonly known as DNA gyrase inhibitors, are broad spectrum antibacterial which act by inhibiting topoisomerase II and IV that are involved in bacterial DNA synthesis and replication.(Blondeau, 2004)
Amongst flouroquinolones, ciprofloxacin has now been recently studied for its antiproliferative activity (Mondal et al., 2004) after attaining its tested results against different cancer cell lines including bladder, prostate, colorectal etc. In this context a study has been conducted on hepatocellular carcinoma cell line HepG2 and Bel-7402 where it has shown antiproliferative effects at the concentration of >100µM for Bel-7402 and >270µM for HepG2. Furthermore it also induced apoptosis in cisplatin induced hepatocellular carcinoma via upregulated Fas gene expression. (Fu et al., 2013). Beside this one more study done on two different cancer cell lines including colon cancer cell lines (CC-531, SW-403 and HT-29), and HCC cell line (HepG2). This study revealed that ciprofloxacin causes significant inhibition of mitochondrial DNA synthesis by inducing mitochondrial injury with (Mondal et al., 2004)subsequent apoptosis of HCC cell line but there were insignificant antiproliferative effects on the same. (Herold et al., 2002).

## TIGECYCLINE

Tigecycline is glycycline antibiotic related to tetracyclines, is a protein synthesis inhibitor which prevents protein synthesis by blocking entry of transfer RNA after binding to 30S ribosome (Greer, 2006). The molecular mechanisms behind its anticancer properties assumed to be inhibition of mitochondrial translation, deactivation of β-catenin/Wnt pathway, suppression of mTOR and autophagy inhibition. In a study conducted on liver cancer cell lines (HepG2 and HuH6) it showed significant inhibition of proliferation with IC50 of 5 µM. It was also noted that it inhibited mitochondrial translation which was tested by analyzing the expression of cytochrome c oxidase 1 and 2 (Cox-1, Cox-2) which belong to mitochondrial respiratory complex IV. It also induced oxidative damage in HCC cells by damaging DNA, proteins and lipids. (Tan et al., 2017)

## CHLOROQUINE:

Chloroquine is a 4-aminoquinilone antimalarial agent that exerts its antimalarial effect by preventing the polymerization of toxic heme released during proteolysis of hemoglobin in the Parasitic digestive vacuole (Sullivan et al., 1996). In recent years its role in combination with chemotherapeutic agent has been under investigation as it has been recognized as an autophagy inhibitor (Kimura et al., 2013) that has been assessed in a study conducted by Mei et al in combination with tetrandrine which is a multipurpose medicinal herb, an autophagy inducer at low and tumor apoptotic at higher dose. The suggested underlying mechanism by which it produces autophagy is based on cellular stress that it exerts by causing mitochondrial dysfunction resulting in ROS production which activated ERK/MAP kinase pathway ultimately leading to autophagy. This study reported that in the presence of autophagy inducer like teranidine, chloroquine can exert its anticancer effects more competently by inhibiting ongoing autophagy

In the same study, chloroquine and tetrandine at 20 µM at 5 µM respectively showed synergistic antitumor activity in human hepatoma cell lines (Huh7 and FHCC98), via activation of apoptotic related proteins and caspases thus induced apoptosis. (Mei et al., 2015)

## KETOCONAZOLE:

Ketoconazole is an azole antifungal having a broad spectrum antifungal activity against topical and systemic mycoses acts by damaging cell membrane through the depletion of ergosterol, a main sterol in cell membrane of fungi (Van Tyle and Therapy, 1984). Ketoconazole has been tested in human cancer cell line (Hep G2), where its cytotoxic potential is evident in different concentrations ranging from 0 to 50µM. In this study at 25µM it attenuated the cell viability to less than 30% after 72 h exposure while at 5µM it induced apoptosis in cancer cells through p53 pathway via bax protein induction and bcl-2 inhibition analyzed through western blotting.(Ho et al., 1998)

## INTERFERONS:

Interferons are family of cytokines that exhibit antiviral activities are known to possess antiproliferative and immunomodulatory effects (Medrano et al., 2017) acts through activation of Janus kinases leading to phosphorylation of STAT. Its subtypes including interferon alpha and beta are effective antiproliferative agents in some solid tumors like malignant melanoma and renal cell carcinoma. For the same reason they were tested in hepatocellular cancer cell lines HepG2, Huh7 and JHH4 and it was found that as compare to alpha, interferon beta significantly showed antiproliferative activity in HepG2 cell line after 48 hour treatment. The mechanism involved was increased S-phase ratio and decrease in G2/M phase ratio when compared to the controls. When apoptotic related markers were tested, interferon beta induced apoptosis by exhibiting enhanced expression of Fas cell surface antigen in Hep G2 and Huh7 cell lines along with increased mean fluorescence of intracellular active caspase3. (Murata et al., 2006)

## INTERFERON-α2b:

Interferon- α2b is a cytokine that is known for its antiviral activity against hep B and C. Apart from its antiviral activity it also possesses antiproliferative, immunoregulatory and antiangiogenic activities. Its recent role has been implicated in suppression of hepatocellular carcinogenesis associated with chronic hepatitis where its anticancer effects were tested in thirteen HCC cancer cell lines. In this study it inhibited proliferation in most of the cell lines and also showed characteristic apoptosis like cytoplasmic shrinkage, chromatin condensation, nuclear fragmentation.(Yano et al., 2006)

## NOVEL ANTIMICROBIAL COMPOUNDS:

## SIROLIMUS:

Sirolimus is a macrolide with renowned immunosuppressant properties. A number of studies have been conducted to discover its antifungal and antineoplastic role. For its anticancer effects the exact

mechanism is not known yet but study conducted on human hepatoma cell lines displayed direct inhibition of cancer cells proliferation (Price et al., 1992) while study conducted by Schumacher et al reported increase in cell cycle arrest and apoptosis as suggested mechanism.(Schumacher et al., 2005)

**ASCOCHLORIN:**

Ascochlorin, a prenyl-phenol compound, isolated from the fungus *Ascochyta viciae* was formerly found to exhibit antiviral and antifungal activity but later on it was found to have antimicrobial , antihyperlipidemic, antihypertensive, antidiabetic and tumor suppressor activities.(Hong et al., 2005)Multiple studies suggest that its anticancer activity is thought to be associated with activation of tumor suppressor gene p53, inhibition of mitochondrial cytochrome bc1 complex and activator protein-1 (AP-1) ultimately reducing matrix metalloproteinase-9 (MMP9) functioning (Cho et al., 2018, Hong et al., 2005). In one study done by Dai et al proved its antitumorigenic potential on HCC cell lines (HepG2, Hep3B and Huh7) and orthotopic mouse model where it inhibited the STAT3 activation and ultimately phosphorylation which is known to be persistently activated in most of the HCC and is linked with poor prognosis. MTT analysis showed decreased viability of HCC cells in its presence. Moreover, it also decreases migratory and metastatic potential of HCC cells. Finally it also affects the apoptotic process by increasing expression of pro-apoptotic proteins (Bak and cleaved-Bid) and decreasing the expression of Bcl-2, Mcl-1, surviving and XIAP.(Dai et al., 2015).

**SIOMYCIN A:**

Siomycin A is a thiozole antibiotic active against various gram +ve and gram -ve bacteria. It interacts with 23S unit of ribosomal RNA resulting in cessation of translation. For its anticancer potential it has been shown to inhibit FoxM1 dependent transcription which is one of the most important gene upregulated in a number of solid tumors including HCC being involved in the process of mitosis.(Gartel, 2008).

**ROXITHROMYCIN:**

Roxithromycin is a macrolide having antibacterial and anti-inflammatory. As far as its anticancer role is concerned it inhibits angiogenesis, via impediment of VEGF production in human hepatoma cell line (HepG2) ultimately affected the tumor vascularity. In a study done by AOKI et al it was found that RXM was able to inhibit angiogenesis and growth of cancer cells at concentration of 50µM and more than 100 µM respectively.(AOKI et al., 2005). Another study conducted on rat model of hepatocarcinogenesis induced by diethylnitrosamine evidenced its role as potential antiproliferative cancer via inhibition of Nuclear factor kappa b (Nfkb) a transcriptional factor which play a critical role in cellular proliferation and apoptosis. Roxithromycin at the dose of (100mg/kg) inhibits oxidative stress, NF-kb, iNOS activity and reduces tumor formation(Ueno et al., 2005).

**RAPAMYCIN:**

Rapamycin is a macrolide that not only possess both antimicrobial and antifungal properties but also considered as an effective adjuvant in cancer therapy because of its immunomodulatory and antiproliferative abilities. It specifically targets mTOR resulting in cell cycle arrest at G1 phase and causes reduction in synthesis of translational processes involving cellular proliferation and growth.(Matsuda et al., 2011).

**FUCOIDANS:**

Fucoidans a sulphated polysaccharide isolated from brown algae and marine invertebrates has been shown to be effective antiviral, antiangiogenic, anticoagulant properties is currently being investigated for its anticancer role in different cancer cell lines. Its anticancer effects were tested in HCC carcinoma cell line (SMMC-7721) at different concentrations for up to 72 hrs, which resulted in inhibition of cell viability in concentration and time dependent manner. Moreover, the apoptotic cells proportion was significantly higher in fucoidan treated cells (14.5%-25.1%) as compare to the untreated cells (9.8%). The treated

group also showed downregulation of Bcl-2 (anti-apoptotic) and upregulation of Bax protein (pro apoptotic) in concentration dependent manner. Finally, it increase ROS-mediated mitochondrial oxidative damage leading to activation of caspase-3 and caspase-9 ultimately apoptosis.(Yang et al., 2013).

**PHYTOL:**

Phytol, an alkaloid and a precursor of vitamin E and K1 that carry a number of biological effects like anti-inflammatory, anti-microbial, antitumor effects. After its proven antiproliferative and pro-apoptotic potential in human lymphoid leukemia cells it was further studied in HCC cell lines (Huh7 and HepG2) where it induced apoptosis via cleaving PARP and caspase-3, and reduced expression of pro caspase-9 in these cell lines. It also showed enhanced expression of Bax protein and attenuated expression of Bcl-2, Mcl-1 and c-Myc anti-apoptotic proteins.(Kim et al., 2015) .

**CONCLUSION AND FUTURE RECOMMENDATION:**

HCC is known for its aggressiveness and treatment resistance, therefore the discovery and recognition of antimicrobials with anticancer properties is a new approach that may offer better prospect for the management of this cancer. Majority of the antimicrobials are found to possess cytotoxic effects and apoptotic properties in most of the HCC cell lines. Further efforts can be done to synthesize novel derivatives from these natural compounds or technique of nano conjugation for drug delivery can be employed for better targeting, and enhanced efficacy against HCC. Lastly most of the antimicrobials discussed above have been tested in vitro so preclinical in vivo studies followed by clinical trials, alone or in combination with standard chemotherapeutic agents are strongly recommended in order to revolutionize the available therapeutic options for HCC.

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