



Review

Chloroquine and its analogs: A new promise of an old drug for effective and safe cancer therapies

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ABSTRACT

Chloroquine (CQ), *N*'-(7-chloroquinolin-4-yl)-*N,N*-diethyl-pentane-1,4-diamine, is widely used as an effective and safe anti-malarial and anti-rheumatoid agent. CQ was discovered 1934 as "Resochin" by Andersag and co-workers at the Bayer laboratories. Ironically, CQ was initially ignored for a decade because it was considered too toxic to use in humans. CQ was "re-discovered" during World War II in the United States in the course of anti-malarial drug development. The US government-sponsored clinical trials during this period showed unequivocally that CQ has a significant therapeutic value as an anti-malarial drug. Consequently, CQ was introduced into clinical practice in 1947 for the prophylaxis treatment of malaria (*Plasmodium vivax*, *ovale* and *malariae*). CQ still remains the drug of choice for malaria chemotherapy because it is highly effective and well tolerated by humans. In addition, CQ is widely used as an anti-inflammatory agent for the treatment of rheumatoid arthritis, lupus erythematosus and amoebic hepatitis. More recently, CQ has been studied for its potential as an enhancing agent in cancer therapies. Accumulating lines of evidence now suggest that CQ can effectively sensitize cell-killing effects by ionizing radiation and chemotherapeutic agents in a cancer-specific manner. The lysosomotropic property of CQ appears to be important for the increase in efficacy and specificity. Although more studies are needed, CQ may be one of the most effective and safe sensitizers for cancer therapies. Taken together, it appears that the efficacy of conventional cancer therapies can be dramatically enhanced if used in combination with CQ and its analogs.

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1. Introduction

If we want to further improve cancer cure rates, we need to understand the detailed control mechanism how anti-cancer therapeutics function in the context of cellular and molecular control pathways in normal and cancer cells. Furthermore, the limitation of drug concentrations used for therapy is a serious problem, mainly due to side effects. Thus, there is an urgent need for developing more effective cancer modalities with minimal side effects. Since cancer is caused by a complex chain of events, combinational modalities may provide a better response to cancer therapy (Cheng et al., 2008; Rosales-Hernandez et al., 2009).

A study of 68 newly approved drugs estimated that developing a single effective cancer drug takes an average of 15 years and US \$800 million (DiMasi et al., 2003). One approach to overcome this enormous problem may be developing a new use of existing drugs. The renowned pharmacologist and Nobel laureate James Black said, “the most fruitful basis for the discovery of a new drug is to start with an old drug” (Chong and Sullivan, 2007). Since many existing drugs have been studied for their pharmacokinetics and safety profiles, and often have already been approved by regulatory agencies for human use, any newly identified use of them can be rapidly evaluated in phase II trials (DiMasi et al., 2003). Developing a known drug for another clinical purpose is termed “repurposing”. The efficacy and specificity of a known drug can also be improved by modifications of its chemical side chains and functional groups, which is termed “repositioning”.

Chloroquine is a well-known 4-aminoquinoline class of drug that is widely used for the prophylaxis treatment of malaria (Wiesner et al., 2003). Even after six decades of use, CQ still remains the drug of choice for malaria treatment because it is effective, low toxic to humans, and inexpensive (Breckenridge and Winstanley, 1997). Biochemical data suggest that this class of compounds enter acidic vacuoles of host cells, where they inhibit the growth of parasites by forming a complex with haematin (Dorn et al., 1995; Pandey et al., 2001; Sullivan et al., 1996). The anti-inflammatory property of CQ also makes it a useful agent for the treatment of rheumatoid arthritis (Augustijns et al., 1992; Titus, 1989), lupus erythematosus (Meinao et al., 1996) and amoebic hepatitis (Conan, 1948). Furthermore, CQ inhibits pro-inflammatory cytokine release into the blood stream, suggesting that it may have therapeutic benefits for chronic inflammation diseases as well as for relieving symptoms caused by bacteria-induced inflammation (Karres et al., 1998). Chloroquine is currently in clinical trials as an investigational anti-retroviral agent in humans with HIV-1/AIDS (Savarino et al., 2003, 2006) and as a potential anti-viral agent against chikungunya fever (Savarino et al., 2007). In addition, CQ has been studied for its potential in the enhancement of radiation therapy (Beierwaltes et al., 1968; Zhao et al., 2005), chemotherapy, and combinational therapy for cancer (Carew et al., 2006; Degtyarev et al., 2008; Hagihara et al., 2000; Hu et al., 2008). Our current review focuses on CQ and its analogs as enhancement agents for cancer therapies.

2. Discovery of CQ from quinine

In the 18th century, the first attempt of successful treatment of malaria was made by utilizing the bark of cinchona trees, which had been used for the treatment of fever since the beginning of the 17th century (Meshnick and Dobson, 2001). Subsequently, Pelletier and

Caventou isolated the cinchona alkaloid active ingredient quinine (Fig. 1) and cinchonine from the crude extracts of cinchona bark (Pelletier and Caventou, 1820). Quinine was then widely used as an anti-malarial agent, replacing the crude bark extracts for malarial treatment. Thus, malaria was the first human disease controlled by a pure chemical compound (Hofheinz and Merkli, 1984). The molecular structure of quinine was finally determined by Woodward in 1944 (Woodward and Doering, 1944). However, the synthetic route of quinine was too complex for commercial production for many years (Turner and Woodward, 1953).

Chemical synthesis of CQ was stemmed from a work by the Paul Ehrlich group (Guttmann and Ehrlich, 1891), who cured two malaria patients in 1891 with methylene blue, a synthetic dye (Fig. 1). Their approach was based on the previous observation that methylene blue was selectively taken up by the parasites causing malaria. This was the first synthetic drug used for the treatment of human malaria (Guttmann and Ehrlich, 1891). Subsequently, an analog of methylene blue was synthesized by replacing one methyl group with a basic side chain (i.e., diethyl amino isopentyl amino side chain), which significantly improved the anti-malarial activity. This positive result eventually led in 1925 to the synthesis of pamaquine, an 8-aminoquinoline derived drug (Fig. 1). This was the first drug capable of preventing relapse of *P. vivax* malaria. Amazingly, this drug is still being used today (Greenwood, 1995). Subsequently, the basic side chain of pamaquine was attached to several different heterocyclic ring systems, leading to the synthesis of the acridine derivative mepacrine (Schlitzer, 2007) (Fig. 1). By studying mepacrine structure, Andersag et al. (1942) discovered two 4-aminoquinoline, sontoquine and SN 3294 (Fig. 1), which showed an excellent anti-malarial activity (Schlitzer, 2007). The studies on these compounds then led to the discovery of Resochin, *N'*-(7-chloroquinolin-4-yl)-*N,N*-diethyl-pentane-1,4-diamine. This compound was ignored for a decade, since it was initially thought to be too toxic for clinical use. However, its toxicological property was re-examined and found that Resochin was safe for human subjects. As a result, it came into the market with a different brand name chloroquine (CQ) (Fig. 1). Chloroquine is one of the most successful anti-malarial drugs still being used for prophylaxis treatment (Andersag et al., 1942; Coatney et al., 1948; Loeb et al., 1946; Schlitzer, 2007; Stocks et al., 2001).

3. The property of CQ

Chloroquine is usually prepared as a diphosphate salt of *N'*-(7-chloroquinolin-4-yl)-*N,N*-diethyl-pentane-1,4-diamine, which is a diprotic weak base ($pK_{a1} = 8.1$, $pK_{a2} = 10.2$) that can exist in both charged (i.e., protonated) and uncharged (i.e., unprotonated) forms. An unprotonated CQ can diffuse freely and rapidly across the membranes of the cell and organelles. Once protonated, CQ may be “trapped” in the organelles such as the lysosomes since it can no longer freely diffuse out. This may be relevant to the increase in the plasma and organelle membrane volumes/surfaces (Borgonovo et al., 2002; Volkl et al., 1993; Zhao et al., 2005). The accumulation of CQ in the lysosomes due to its high lysosomotropic property results in the inhibition of the enzymes phospholipase A2, lysophospholipid acylhydrolase, and monoacylglycerol lipase activities. These enzymes release arachidonic acid from phospholipids of the cell membrane (Kunze et al., 1982; Nosal and Jancinova, 2002). Thus, the accumulation of CQ in the lysosomes

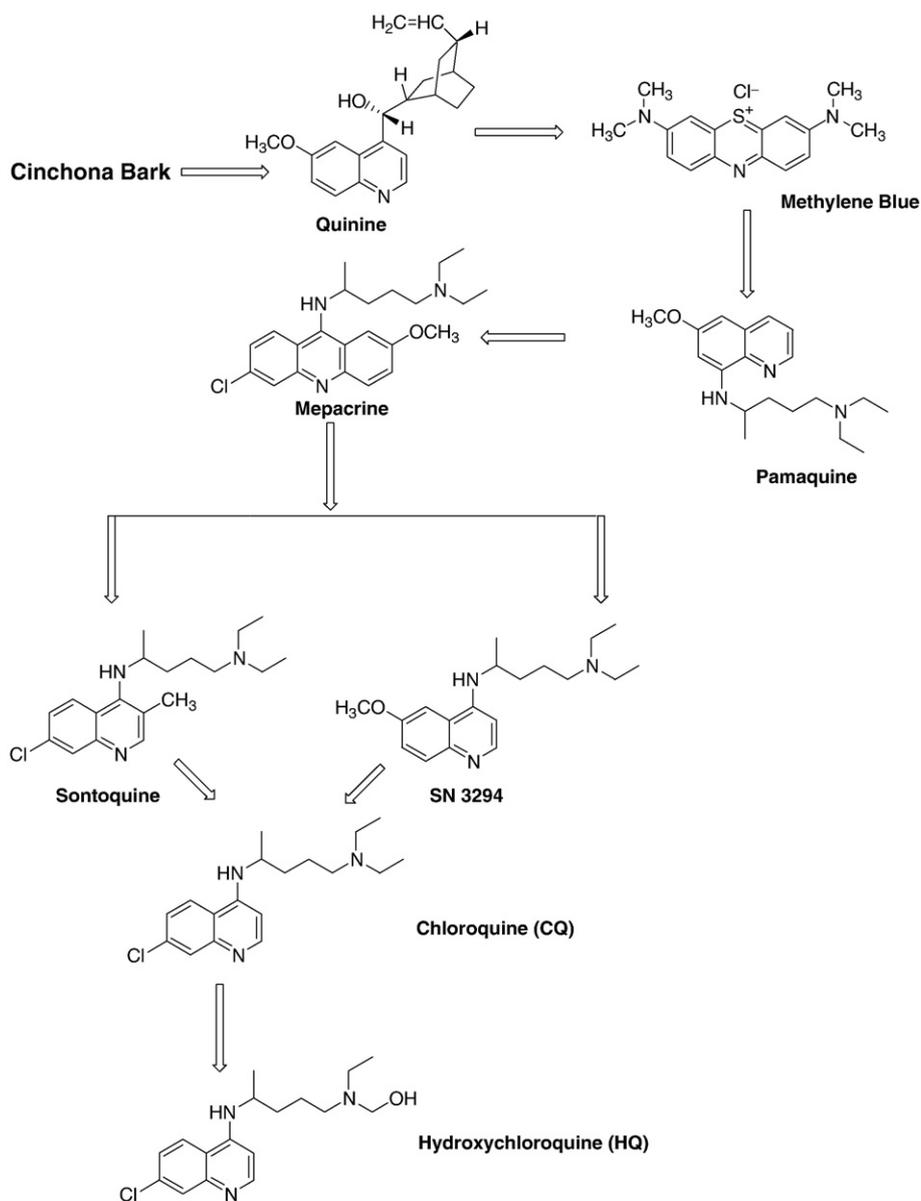


Fig. 1. Development of CQ from Quinine.

hampers proteolytic processes and metabolism of neoglycolipids, which results in the alterations of several cellular signal pathways (Hostetler et al., 1985; Law et al., 1984; Yin et al., 2003).

Data from physicochemical studies suggest that CQ forms a complex with DNA (Sternglanz et al., 1969; Waring, 1970), resulting in defects in DNA synthesis and repair (Field et al., 1978; Haworth et al., 1983; Michael and Williams, 1974; Sartorius and Schneider, 1997). Chatterjee and colleagues suggested that the extended *N*-diethylamino lateral side chain of CQ may be responsible for DNA intercalation (Chatterjee et al., 1998). Molecular modeling and docking studies by Snyder suggested that CQ has an intercalation potency factor (IPF) of 2 and a docking value of 72. Thus, it was concluded that the ability of forming a H-bond between the *N*-diethylamino lateral side chain of CQ and a DNA base may contribute to the DNA intercalative activity of the quinoline nucleus. Although the exact pharmacological mechanisms of CQ and its analogs in enhancing cancer therapeutics are still under investigation, several facts relating to the drug action are now widely accepted. Based on these observations, several hypotheses have been raised, which will be discussed in the following sections.

3.1. CQ-mediated enhancement of cancer therapy

It has been suggested that variations in pH could be exploited for a selective targeting for solid tumors, as tumors often develop an acidic extracellular environment (Boyer and Tannock, 1992; Newell et al., 1993; Vaupel et al., 1989). The acidity of solid tumors is approximately 0.5 pH units lower than that of surrounding normal tissues, although the intracellular pH of tumor cells is similar to that of normal cells (Boyer and Tannock, 1992; Vaupel et al., 1989). Jensen and colleagues reported that a combination of two therapeutics (i.e., weak DNA intercalating CQ and etoposide) resulted in selective cytotoxicity to the cells in acidic environments while those in a normal microenvironment were protected (Jensen et al., 1994). The epipodophyllotoxins etoposide and teniposide are widely prescribed for the treatment of small cell lung cancer. These drugs are used in maximally tolerated doses, and their toxicity precludes significant dose increments. These topoisomerase II inhibitors cause extensive DNA fragmentation (Bork et al., 1991), which can be antagonized by the DNA intercalating aclarubicin (Sehested et al., 1993). The authors further demonstrated that the intercalating agent CQ

could also prevent topoisomerase II inhibitors-mediated DNA breaks and, thereby, reduced normal cell cytotoxicity by etoposide while their efficacy for cancer-cell killing was not compromised. The authors postulated that this reduced cytotoxicity on normal cells was due to the weak base of CQ that does not allow it entering normal cells if the extracellular fluid is acidic as is the case in most solid tumors. Therefore, the authors proposed that such a pH-dependent drug interaction can be useful in targeting topoisomerase II inhibitors to solid tumors. Consistent with this hypothesis, CQ accumulation was decreased by 5-fold in the OC-NYH human small cell lung cancer cell line and the L1210 murine leukemia cells by lowering extracellular pH from neutral (pH = 7.4) to acidic (pH = 6.0). No protection effect by CQ was observed at pH 6.5, whereas etoposide cytotoxicity was almost completely antagonized at pH, 7.4 with a 460-fold protection. This exploitation of antagonistic extracellular trapping of CQ by acidic pH is a novel approach to increase efficacy and specificity of tumor cell killing.

Similarly, Lee and Tannock demonstrated that raising endosomal pH with CQ, omeprazole or bafilomycin A could decrease sequestration of anti-cancer drugs such as doxorubicin and mitoxantrone in endosomes, thereby increasing their cytotoxicity on EMT-6 and MCF7 cells (Lee and Tannock, 2006). Anti-cancer drugs with neutral pH (i.e., doxorubicin and mitoxantrone) are membrane permeable in their neutral form, but are relatively impermeable when protonated. Inside cells, these drugs relatively freely enter acidic cellular compartments such as endosomes where they become protonated and then sequestered inside the organelle (Hurwitz et al., 1997; Mayer et al., 1986; Schindler et al., 1996). This sequestration decreases the availability of anti-cancer drugs for their cellular target (e.g., DNA in the nucleus). Depending on the rate of recycling of endosomes to fuse with the plasma membrane and release their contents extracellularly, it may also lead to less drugs being available extracellularly for penetration to more distant cells. This may limit the penetration of anti-cancer drugs through solid tumor tissues (Phillips et al., 1998; Tannock et al., 2002). To overcome this problem, Lee and Tannock (2006) inhibited sequestration of doxorubicin in endosomes by increasing endosomal pH with CQ, omeprazole or bafilomycin A. The authors found that CQ and omeprazole but not bafilomycin A decreased the net uptake of doxorubicin into tumor cells by sequestration in endosomes. None of the three agents significantly affected the uptake of mitoxantrone. Clonogenic assays showed that omeprazole and bafilomycin A increased the cytotoxicity of the anti-cancer drugs in cultured cells, but CQ had minimal effects on cytotoxicity despite a reduced uptake of doxorubicin. Omeprazole increased the penetration of anti-cancer drugs through multicellular layers of tumor tissue. The authors concluded that modifiers of endosomal pH can increase therapeutic effectiveness of drugs with basic pH by increasing their toxicity and/or tissue penetration in solid tumors (Lee and Tannock, 2006).

3.2. The regulation of cell differentiation and apoptosis by CQ and its analogs

Quinidine is a quinoline alkaloid that is derived from the bark of the cinchona tree. Quinidine is used therapeutically to treat cardiac arrhythmia and malaria (Kaufman and Ruveda, 2005). *In vitro* studies showed that 25 μ M quinidine caused moderate growth arrest and morphologic differentiation on the MCF7 human breast cancer cells (Wang et al., 1998; Woodfork et al., 1995; Zhou et al., 2000). The authors found that quinidine promoted cell cycle arrest in G1/G0; caused accumulation of lipid droplets on the membrane and enlargement of the cytoplasm; and resulted in cell differentiation (Wang et al., 1998; Zhou et al., 2000). The arrest of MCF7 cells at G1/G0 phase was peaked between 24 and 48 h post-quinidine at 90 μ M. Wang et al. (1998) suggested that the G1/G0 arrest was due to the blockage of the potassium channel by quinidine. However, it was also found that quinidine-mediated growth arrest coincided with changes in the level of proteins involved in the cell cycle control (Melkoulmian et al., 2002; Zhou et al., 2000). In particular, cells treated with 90 μ M

quinidine showed an increase in the protein levels of p21^{WAF1}, p53, and p27^{Kip1}, and a decrease in cyclin D1, phosphorylated pRb and Myc. Quinidine also raised the level of acetylated histone H4, a response that is correlated with cellular differentiation in breast tumor cells (Munster et al., 2001; Zhou et al., 2000). The differentiation of MCF7 cells in response to quinidine was manifested by 48–72 h. However, cells underwent apoptosis when they were exposed to quinidine for a longer period (72–96 h), suggesting that quinidine can cause a decrease in proliferation by cell cycle arrest, cell differentiation, and programmed cell death (Wang et al., 1998). It was found that cell transition into the quiescent G0 phase is prerequisite for differentiation. All three aforementioned quinoline anti-malarial agents (i.e., CQ, quinine, and quinidine) were observed to induce differentiation in MCF7 cells (Table 1).

It has been suggested that the tertiary amino alkyl side-chain of CQ occupies the minor groove of DNA, which could interfere with the association of DNA binding proteins and enzymes (Michael and Williams, 1974; O'Brien et al., 1966). This may be relevant to the fact that CQ inhibits the activities of other DNA-intercalating agents topoisomerases I and II (Solary et al., 1994; Sorensen et al., 1997; Snyder, 2000). Like quinidine, CQ also induces p53 and p21^{WAF1} proteins, and eventually apoptosis in MCF7 cells. However, the activity of CQ in induction of apoptosis was less pronounced than quinidine. Contrarily, quinine, a stereoisomer of quinidine, did not induce p53, p21^{WAF1} or apoptosis. Not surprisingly from this result, quinine was not an effective DNA intercalating agent, although CQ, quinidine, and quinine all arrested MCF7 cells in G1/G0. Zhou et al. (2002) also found that the intercalation of DNA by CQ and quinidine, but not by quinine, could cause DNA damage in a cell-free system.

Strobl and colleagues screened a panel of anti-malarial and -proliferative quinolines shown in the National Cancer Institute database to identify new effective agents that can induce differentiation in breast cancer cells (Martirosyan et al., 2006). They found that five anti-proliferative quinoline compounds (Fig. 2) induced differentiation at the doses that can cause growth inhibition (i.e., 3–14 μ M) in the MCF7 cell line (Table 1). The cells undergoing differentiation by quinolines showed accumulation of lipid droplets. In addition, these quinoline compounds resulted in the loss of Ki67 antigen expression (i.e., a cell proliferation marker), withdrawing cells into G0, and a decrease in E2F1 (i.e., a critical regulator for the G1-S transition).

Five CQ analogs from the National Service Centre (NSC) are found to have 3–10 times more potent in killing cells than CQ and quinidine. NSC3852 and NSC86371 can cause DNA damage, facilitate differentiation, and induce apoptosis in MCF7 cells by inhibiting histone deacetylase (HDAC) (Table 1). It is known that E2F1 is suppressed by the quinoline compounds that facilitate differentiation, but not by those

Table 1
Differentiation activity.

Compound	Oil Red O (μ M) ^a	MTS IC50 (μ M) ^b	Ki67 Index (μ M) ^c	HDAC activity (% control) ^d
CQ	1	33	6.6	91 \pm 6
Quinidine	10	113	5	100 \pm 13
Quinine	30	40	4.1	102 \pm 14
NSC3852	10	10	7.2	69 \pm 6
NSC69603	1	14	6	111 \pm 7
NSC305819	1	7	5.8	90 \pm 17
NSC10010	3.5	4	8.3	94 \pm 8
NSC86371	1	6	6	65 \pm 5

^a The lowest concentration compound required to produce an Oil Red O response.

^b IC₅₀ level for inhibition of mitochondrial metabolism of MTS following a 48 h exposure to each compound in anti-proliferative activity.

^c Ki67 index is a measure of the fraction of G₀ cells in a population where a Ki67 index >1 indicates more G₀ cells in the population. Data shown are the average of at least n = 3 experiments in cells exposed for 48 h to the MTS IC₅₀ level of each compound.

^d Inhibition of histone deacetylase (HDAC) activity *in vitro* by IC₅₀ levels of each compound is expressed as % of HDAC activity in solvent control groups.

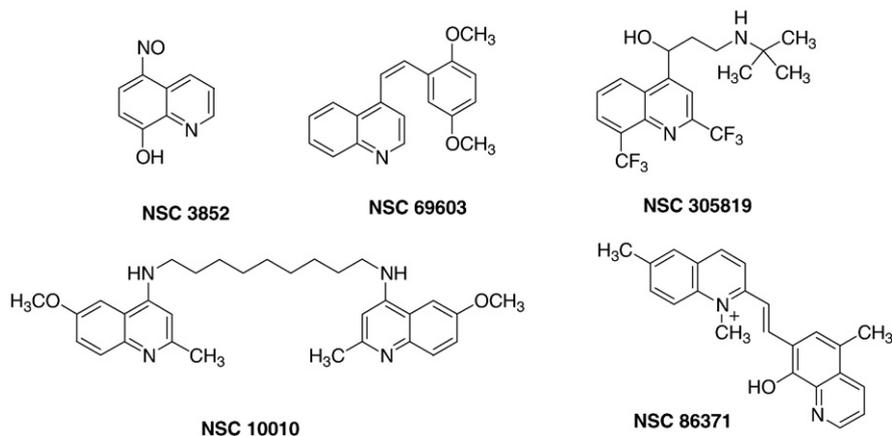


Fig. 2. Examples of active NSC compounds that induce cell differentiation in human breast cancer cells.

that do not facilitate differentiation. At an effective dose, NSC69603 caused the greatest decrease in E2F1 (90%), followed by quinidine and hydroxy quinoline (HQ) (Zhou et al., 2000). It is interesting to note that NSC69603 does not cause DNA damage, although it causes a great degree of differentiation. Most of the other highly active NSC compounds cause DNA damage and apoptosis (Martirosyan et al., 2006). NSC305819 is least toxic on MCF10A cells among the differentiation-inducing quinolines, and it may have tumor-specific activity. Martirosyan et al. (2006) argued that DNA damage and a decrease in E2F1 are two important factors for differentiation and anti-proliferative activities by quinoline-based drugs.

3.3. CQ induces cell death by necrosis and apoptosis in cancer cells

Fan and co-workers (Fan et al., 2006) showed that CQ inhibits cell growth and induces cell death by necrosis as well as by apoptosis in A549 lung cancer cells. The authors found that CQ at 0.25–32 μM induced vacuolation with increased volume of acidic compartments and exhibited overall anti-proliferation effects on A549 cells. In contrast, high doses of CQ (64–128 μM) induced apoptosis by 24 h, with only a low level of vacuolation. Interestingly, these authors also found that treatment of A549 cells with 32–64 μM CQ for 72 h (or 128 μM CQ for 48 h) induced cell death by necrosis. The authors repeated the experiment in the presence of D609, a specific inhibitor of phosphatidylcholine-specific phospholipase C (PC-PLC). The authors found that 50 μM D609 inhibited the effects of CQ at 32 μM (i.e., viability and vacuolation), but not CQ at 128 μM (i.e., apoptosis). This data suggests that CQ inhibits A549 lung cancer cell proliferation at low concentrations by increasing the vacuolation and antagonizing PC-PLC activity, while CQ induces apoptosis and necrosis at high doses (Fan et al., 2006).

Apart from these, Zheng and co-workers (Zheng et al., 2009) have recently demonstrated that CQ inhibits the proliferation of the CT26 mouse colon cancer cells *in vitro* and CT26 xenograft tumors *in vivo*, mainly by induction of apoptosis. This CQ-mediated anti-cancer effect was associated with decreases in p42/44 MAPK and Akt activities. The data shown by these authors provide clear evidence that CQ has significant therapeutic potential on human colon cancers (Zheng et al., 2009).

3.4. CQ-induced apoptosis: *in vivo* studies

Tremendous efforts have been made recently to understand the molecular mechanisms that regulate apoptosis and tumor cell proliferation (Evan and Vousden, 2001; Thompson, 1995). Apoptosis has been defined as programmed cell death associated with certain morphological and biochemical characteristics that can be distinguished from other forms of cell death (Kerr et al., 1972; Majno and Joris, 1995). The detection of apoptotic cells in patient biopsies is an important tool in assessing the prognosis of cancer and response of tumors to a therapy

(Goldsworthy et al., 1996; Thompson, 1995). Although apoptosis can be readily assessed *in vitro* with a variety of techniques, its detection in an *in vivo* setting poses more challenging, since apoptotic cells are rapidly removed by phagocytosis (Aderem and Underhill, 1999). Using Swiss albino mice as a model, Nyati and colleagues (Nyati et al., 2006) developed a method to enhance the detection of apoptotic cells *in vivo* by pretreating cells with CQ, as it is known to inhibit the activity of macrophage. The authors found that this CQ-based novel technique increased significantly the ability of apoptotic cell detection. This method was further optimized by combining with modern imaging techniques (Nyati et al., 2006).

3.5. Induction of apoptosis by CQ in the Bcap-37 cells

Jiang and colleagues examined CQ effects on several human breast cancer cell lines including Bcap-37. The authors found that CQ treatment resulted in a decrease in the viability of Bcap-37 cells in a concentration- and time-dependent manner, which was correlated with the number of cells arrested at G2/M phase. The authors suggested that estrogen receptor and estrogen dependency of the cancer cells appeared to be the most prominent factor influencing their response to CQ (Jiang et al., 2008). Other factors may also affect the outcome of CQ treatment in breast cancer cells, including p53 and caspase-3 status, and the level of c-Myc (Loehberg et al., 2007; McNeil et al., 2006; Yang et al., 2007). CQ-mediated cell cycle arrest was associated with a decrease in polo-like kinase 1 (Plk1), phosphorylated (i.e., activated) cell division cycle 25C (Cdc25C), phosphorylated extracellular signal regulated kinase 1/2 (ERK1/2), and phosphorylated Akt. CQ-treated Bcap-37 cells exhibited a marked decrease in the level of mitochondrial transmembrane potential ($\Delta\Psi\text{m}$), which was accompanied by the activation of caspase-3 and cleavage of poly(ADP-ribose) polymerase (PARP). It should be noted that phosphorylated Cdc25C can activate the Cdc2/cyclin B1 by dephosphorylating Cdc2 at Tyr 15, facilitating the initiation of mitotic events (Reagan-Shaw and Ahmad, 2005). These authors also found that the mechanism by which down-regulation of Plk1 induced mitotic arrest, followed by apoptosis via the Cdc25C/Cdc2/cyclin B1 feedback loop (Erikson et al., 2004). Exposure of Bcap-37 cells to CQ also induced spindle abnormality and down-regulated mitochondrial trans-membrane potential.

HQ has been shown to induce apoptosis via destabilization of lysosomal membranes, leading to mitochondrial membrane depolarization and activation of caspases (Boya et al., 2003). The intrinsic signal pathway of apoptosis has been recognized as a sequential event of the mitochondrial changes, involving the decrease of $\Delta\Psi\text{m}$ and the activation of the caspase cascade (Green and Reed, 1998; Zamzami et al., 1996). A key step in the mitochondria-dependent apoptotic pathway is the disruption of the mitochondrial membrane, leading to the loss of $\Delta\Psi\text{m}$ (Susin et al., 1999). Finally the CQ-mediated decrease in $\Delta\Psi\text{m}$ may

trigger apoptosis by 48 h post-CQ treatment. Taken together, CQ may be a potentially effective therapeutic agent against breast cancer.

3.6. CQ-induced autophagy and *Myc* expression

Cells that encounter a variety of stresses may undergo an evolutionarily conserved process of self-digestion termed autophagy. The importance of this intracellular damage response for pathophysiology has been established across multiple fields, including infectious disease, neurodegeneration, heart failure, and cancer (Cuervo, 2004, 2008). Autophagy is a catabolic process characterized by the appearance of autophagic vacuoles in the cytoplasm. This is mainly resulted from the self-digestion of cytoplasmic organelles and other constituents in the lysosomal compartments (Kondo et al., 2005). Although autophagy can kill cells when it becomes excessive, it is also thought to be a temporary survival mechanism under stress conditions. Thus, inhibiting autophagy may either promote or inhibit cell death, depending on the cell conditions and agents used to cause cell damage (Amaravadi et al., 2007; Kroemer and Jaattela, 2005; Levine and Yuan, 2005; Lockshin and Zakeri, 2004). Recent studies showed that inhibition of therapy-induced autophagy with CQ can enhance cell death in established tumors, leading to better tumor regression and delayed tumor (re)growth (Amaravadi et al., 2007).

Whether autophagy observed in tumor cells treated with chemotherapy or ionizing radiation represents a mechanism that allows tumor cells to survive or a mechanism that initiates a non-apoptotic form of programmed cell death remains controversial (Qu et al., 2003; Yu et al., 2004). To gain better insight into this issue, Amaravadi and colleagues (Amaravadi et al., 2007) examined the role of autophagy in a *Myc*-induced lymphoma cell line that was generated from *p53ER^{TAM}/p53ER^{TAM}* mice (i.e., ER knock-out mice). Induction of p53 in the *Myc-p53ER^{TAM}* tumors caused apoptosis, and tumor cells that survived from the p53-induced apoptosis displayed autophagy (Amaravadi et al., 2007). Thus, data from this study provided evidence that autophagy can be an adaptive mechanism that contributes to tumor cell survival and resistance to therapy-induced apoptosis. Impairment of autophagic vesicle clearance by the lysosomotropic CQ correlates with increase in apoptosis and tumor regression, and a delay in tumor recurrence. This appears to be due to a direct pro-survival effect of autophagy in tumor cells, since the inhibition of autophagy by either ATG5 shRNA or CQ enhanced tumor cell apoptosis and suppressed tumor cell recovery when p53 was induced. The ability of CQ to augment tumor cell death in response to p53 activation or alkylating drug therapy was correlated with its ability to hinder autophagy. A similar augmentation of tumor cell death in response to p53 activation or alkylating drug therapy was observed in the cells that autophagy is genetically suppressed by shATG5 (Beardsley et al., 2005). CQ treatment failed to further augment the death in cells lacking autophagy activities following p53 activation or alkylating drug therapy. The dose of 60 mg/kg/day used in the study strongly suggests that the inhibition of autophagy is likely the basis of the major anti-neoplastic effect by CQ. Taken together, inhibitors of autophagy enhance the efficacy of therapeutics by increasing cell death by apoptosis. These studies also identified that CQ and its analog HQ as inhibitors of autophagy that can be used *in vivo* to increase tumor-cell killing by conventional cancer therapeutics.

An animal study showed that a combination of CQ with tamoxifen or with the alkylating agent cyclophosphamide did not result in higher toxicity than treatment with tamoxifen or cyclophosphamide alone. Since glycolytic tumors are characteristically more acidic than surrounding normal tissues (Walenta et al., 2004), CQ may be preferentially accumulated in the tumor and showed greater efficacy in the inhibition of autophagy in the tumor than normal tissues (Jensen et al., 1994). The systemic administration of CQ at 60 mg/kg/day for tumor control is roughly equivalent to the dose used to treat malaria or rheumatoid arthritis. Thus, these data provide rationale for further investigation of combined modalities using CQ (and its derivatives) and systemic

chemotherapy and/or radiation to enhance therapeutic efficacy of existing cancer therapies. Also, these data suggest that the development of more specific autophagy inhibitors may be of clinical benefit if they can be utilized in combination with apoptosis-inducing agents.

3.7. Autophagy and the effect of CQ on the human Burkitt lymphoma model

Kastan and colleagues demonstrated that CQ activates the stress-responsive Atm-p53 tumor-suppressor pathway, preferentially enhancing the death of *Myc* oncogene-overexpressing primary mouse B cells and mouse embryonic fibroblast (MEF) cells (Maclean et al., 2008). Furthermore, CQ could impair *Myc*-induced lymphomagenesis in a transgenic mouse model of human Burkitt lymphoma (Maclean et al., 2008). The authors found that CQ activated the p53-dependent pathway and increased cell death when using a dose similar to that used to control malaria. CQ substantially increased apoptosis in the cells overexpressing *Myc*, largely due to partial permeabilization of lysosomes and the decrease in protein degradation by autophagy (Amaravadi et al., 2007; Maclean et al., 2008). Some studies also suggested that CQ increases cell death by blocking fusion of autophagosomes with lysosomes (Paludan et al., 2005; Shacka et al., 2006), while others postulated that accumulation of CQ in the lysosomes inhibits the acid-dependent degradation of autophagosomes (Amaravadi et al., 2007; Glaumann and Ahlberg, 1987; Poole and Ohkuma, 1981). The latter phenomenon may result in the accumulation of autophagic vesicles that cannot be cleared.

Kastan and colleagues showed that CQ was effective in killing *Myc*-overexpressing cells even in the presence of caspase inhibitors or overexpression of anti-apoptotic Bcl-2 or Bcl-XL (Bissonnette et al., 1992; Fanidi et al., 1992). Furthermore, CQ was also effective in killing MEF cells lacking pro-apoptotic *Bax* and *Bak* (Wei et al., 2001). The death of *Bax/Bak* deficient cells by CQ may result from the fact that these cells retain a normal lysosomal membrane permeabilization despite failing to undergo mitochondrial membrane permeabilization (Boya et al., 2003). Data from these studies suggest that CQ facilitates apoptotic cell death in *Myc* overexpressing cells via lysosomal alterations. However, CQ-induced lysosomal alterations can also lead to a p53-dependent cell death in the absence of apoptosis or autophagy.

Interestingly, CQ can effectively reduce spontaneous tumor formation in mice lacking Atm, a finding that potentially has significant implications for patients with ataxia telangiectasia (AT), a cancer-prone disorder. Malignancies that arise in AT patients are particularly difficult to treat because of the hypersensitivity of AT patients to cytotoxic agents used to treat cancer. Together, CQ provides a novel opportunity for the treatment of lymphomas in AT patients.

3.8. Autophagy induced by CQ in ovarian cancer cells

Aplasia Ras homolog member I (ARHI; also known as DIRAS3), a maternally imprinted Ras-related tumor suppressor gene, is down regulated in more than 60% of ovarian cancers (Luo et al., 2001; Lu et al., 2006; Wang et al., 2003). ARHI was found to regulate autophagy and tumor dormancy in human ovarian cancer cells (Lu et al., 2008). It was previously shown that expression of ARHI in several human ovarian cancer cell lines induced autophagy by blocking the PI3K-mTOR signaling pathway and upregulating ATG4 (Lu et al., 2008). Furthermore, ARHI is required for spontaneous and rapamycin-induced autophagy in normal and malignant cells (Lu et al., 2006; Rosen et al., 2009). Although ARHI expression led to autophagic cell death when SKOV3 ovarian cancer cells were grown in culture, it did not lead to cell death in mice xenografts. However, a decrease in the level of ARHI resulted in rapid growth of xenograft tumors, suggesting that ARHI can suppress tumor proliferation *in vivo*. Inhibition of ARHI-induced autophagy with CQ dramatically reduced regrowth of xenograft tumors, suggesting that autophagy contributes to the survival of dormant tumor cells (Lu et al., 2008). Furthermore, autophagy-mediated cell death decreased when cultured ovarian cancer cells with high levels of ARHI were treated with

growth factors (IGF-1, M-CSF), angiogenic factors (VEGF, IL-8) and matrix proteins. Taken together, ARHI can not only induce autophagic cell death, but also promote tumor dormancy in the presence of those factors that promote survival *in vivo*. Since the inhibition of ARHI-induced autophagy by CQ prevented tumor dormancy, CQ may have a significant clinical potential in controlling ovarian cancer (Lu et al., 2008).

3.9. Induction of autophagy by CQ in CML cells

Hatake and colleagues observed that autophagy in the K562 chronic myelogenous leukemia (CML) cell line was associated with the treatment of 12-O-tetradecanoylphorbol-13-acetate (TPA) that can induce differentiation in the K562 cells into a megakaryocytic lineage (Mishima et al., 2008). These authors found by examination with a confocal microscope that the cells undergoing autophagy did not express the CD41 megakaryocyte marker, suggesting that the autophagy was independent of megakaryocytic differentiation (Mishima et al., 2008). The authors found that the cells underwent autophagic cell death only when substantial autophagic degradation had already occurred. Furthermore, the block of TPA-induced autophagy by CQ rapidly promoted cell death that was not autophagic cell death. This result suggests that autophagy is involved in both the cell survival system and autophagic cell death in K562.

To confirm that autophagy regulates the cell survival in K562 cells, imatinib was used to induce cell death in K562 cells (Fader et al., 2005, 2008). The addition of CQ in this experiment markedly increased the imatinib-induced cell death in K562 cells. Furthermore, two imatinib-resistant cell lines, BaF3/T315I and BaF3/E255K, also underwent cell death when treated them with imatinib in combination with CQ. From this data, the authors concluded that autophagy is closely co-related with cell survival mechanisms, and that the inhibition of autophagy accelerates TPA or imatinib induced cell death (Fader et al., 2008). They suggested that the autophagic self-defense system would active in the imatinib-resistant cells. Since the inhibition of autophagy by CQ appears to disrupt the defense system against imatinib, CQ can be a novel strategy for the treatment of CML in combination with other cancer drugs (Mishima et al., 2008).

4. Combined therapies with CQ and other cancer therapeutics

Combination of one or more therapeutic agents has been used to improve the outcome of cancer therapies (Chau and Cunningham, 2006; Faivre et al., 2006; Hennig et al., 2004; Hosoya et al., 1999). Although some of the combination therapies have been shown to improve overall patient survival, such treatments are often associated with an increase in toxicity and the potential for developing cross-resistance (Ocana et al., 2006; Thomsen and Kolesar, 2008). Thus, there is an urgent need for developing new approaches that are effective and less toxic to normal cells/tissues (Abrams et al., 1994; Bonadonna, 1996). There have been several approaches to address this problem, including increasing specificity and lowering toxicity by using a low dose of therapeutic agents (Hu et al., 2008; Hu et al., unpublished data). Unfortunately, however, these approaches usually are not very effective. To overcome this problem, several laboratories have been studying the utilization of sensitizers for radiation and chemotherapies (Hu et al., 2008; Hu et al., unpublished data; Zhao et al., 2005; Kim et al., 1973). Among numerous sensitizers examined, CQ appears to be particularly promising since it does not cause significant side effects, and can effectively sensitize cell-killing effects by genotoxic agents in a cancer-specific manner.

4.1. Chloroquine as a sensitizer for radiation therapy

Lieberman and colleagues showed in 1968 that CQ could preferentially enter human malignant melanoma cells (Beierwaltes et al., 1968; Lieberman et al., 1971). Kim et al. found that CQ could enhance the

radiation response of cultured tumor cells (Kim et al., 1973). Data from these studies suggested that the enhancement of radiation effects by CQ is due to the impairment of post-radiation recovery process. To gain a further understanding of how the lysosomotropic CQ can potentiate the effects of ionizing radiation, our laboratory investigated the effects of CQ on lysosomal and mitochondrial membrane stability, the subcellular localization of ceramide, plasma membrane permeability, and the mode of cell death in response to radiation (Zhao et al., 2005). We found that CQ accumulated in the lysosomes by which lysosomal volumes substantially increased, followed by destabilization of both the lysosomal and plasma membranes. Upon 7 Gy radiation, most ceramide was associated with the lysosomes in the cells treated with CQ but not in the CQ-untreated control. The increase of ceramide in the lysosomes of the CQ treated cells appeared to further destabilize the lysosomal and plasma membranes. Both CQ-treated and untreated cells had approximately the same rate of cell death by apoptosis after 7 Gy radiation ($P < 0.05$, ns). However, in contrast to the CQ-untreated control, the CQ-treated cells underwent massive cell death by necrosis at 24–48 h after radiation ($P < 0.05$). Taken together, our data supported the idea that the increase in cytotoxic effects by the combination of CQ and radiation is due to radiation-mediated apoptosis and CQ-mediated necrosis (Zhao et al., 2005). The apparent discrepancy between CQ mediated cell cycle arrest and radiosensitization may derive from the various factors such as different drug concentrations, the length of drug treatment, and genetic background of the cell lines used.

To further improve the CQ-mediated enhancement of cancer therapy, we generated a series of CQ analogs (Fig. 3, Compounds 1–10) by the reaction of 4-chloro-7-substituted-quinolines with the corresponding mono/dialkyl amines. Subsequently, the compounds were examined for their cytotoxic effects on two different human breast tumor cell lines, MCF7 and MDA-MB468. Although all compounds examined were quite effective on both cell lines, the compound *N'*-(7-chloro-quinolin-4-yl)-*N,N*-dimethyl-ethane-1,2-diamine emerged as the most active compound of the series. It was particularly potent against MDA-MB 468 cells when compared to CQ and amodiaquine. The compound butyl-(7-fluoro-quinolin-4-yl)-amine showed more potent effects on MCF7 cells when compared to CQ (Zhang et al., 2008).

4.2. Sensitization of Akt inhibitors-based tumor-cell killing by CQ and its analogs

We found that the cell-killing effects of Akt inhibitors on three breast cancer cell lines (MDA-MB468, MDA-MB231, MCF7) were substantially enhanced when combined with CQ or its analogs (Fig. 4, compounds 1–11) (Hu et al., 2008). Importantly, the CQ-mediated chemosensitization of cell killing by Akt inhibitors is cancer specific. In particular, when combined with 10 μ M CQ, 1,3-dihydro-1-(1-((4-(6-phenyl-1*H*-imidazo[4,5-*g*]quinoxalin-7-yl)phenyl)methyl)-4-piperidinyl)-2*H*-benzimidazol-2-one (an Akt1 and 2 inhibitor; Fig. 4, compound 8) killed cancer cells 10–120 times more effectively than 184B5 non-cancer breast cells. Thus, CQ is a very effective and cancer-specific chemosensitizer when used in combination with Akt inhibitors. The combination of the CQ analog 5 (Fig. 3) with a low concentration of PI3K-Akt inhibitor 10 (Fig. 4) was also a very promising anti-cancer modality. Overall, our data raise the possibility that CQ can significantly increase the therapeutic effects of PI3K-Akt inhibitors with a minimal side-effect on non-cancer cells (Hu et al., 2008).

4.3. The combination of CQ and the PI-103Akt inhibitor

Degtyarev et al. found that CQ is a superb sensitizer when used in combination with the Akt inhibitor 3-[4-(4-Morpholinyl)pyrido[3',2'4,5]furo[3,2-*d*]pyrimidin-2-yl]phenol hydrochloride (PI-103, Fig. 5), which can block the class I PI3K-mTOR pathway at nanomolar concentrations (Degtyarev et al., 2008). Since aberrant activation of

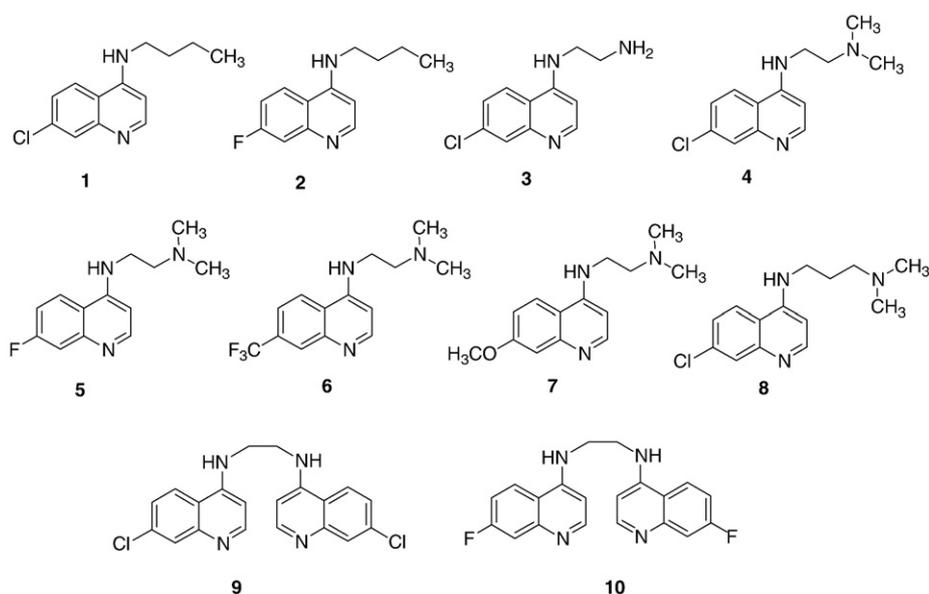


Fig. 3. Examples of CQ analogs that show activities on human breast cancer cells. The synthesis and their activities were previously reported by our group (Zhang et al., 2008).

the PI3K-Akt pathway has been widely implicated in the development and progress of many different tumors, this pathway is an attractive target of cancer therapeutics (Samuels and Ericson, 2006; Stambolic and Woodgett, 2006). Consistent with this expectation, Degtyarev and colleagues demonstrated that silencing Akt could suppress the growth of tumors in mice established from phosphatase and tensin homologue-null (PTEN) human cancer cells (Degtyarev et al., 2008). Akt knockdown with shRNA or inactivation with the small molecule inhibitor 3-[4-(4-Morpholinyl)pyrido[3',2'-4,5]furo[3,2-d]pyrimidin-2-yl]phenol hydrochloride (PI-103) markedly increased autophagy, although it did not significantly induce apoptosis (Fan et al., 2006). Addition of CQ to PI-103 caused further accumulation of abnormal autophagolysosomes and reactive oxygen species, leading to accelerated cell death *in vitro* and complete tumor remission *in vivo* (Fan et al., 2006). These authors found that the enhancement of cell death was also achieved when the Akt inhibitor was used in combination with the vacuolar H⁺-adenosine triphosphatase inhibitor bafilomycin A1 or with cathepsin inhibitors.

4.4. Chloroquine as an adjuvant therapeutic agent in glioblastoma multiforme

Chloroquine and quinacrine are shown to have an anti-mutagenic effect on cancer cells (Giampietri et al., 1980). These agents are also shown to have a strong potentiating effect on the anti-neoplastic action of carmustine on glioma cells *in vitro* and *in vivo* (Reyes et al., 2001). To examine the efficacy and toxicity of CQ-based combined modalities, Sotelo and colleagues conducted three different clinical trials (Briceno et al., 2003, 2007; Sotelo et al., 2006). The first trial was a prospective, controlled and randomized method, in which glioblastoma multiforme patients were treated with CQ in addition to conventional cancer therapy (i.e., surgery plus a standard course of radiotherapy and chemotherapy). Data from this study showed that the addition of CQ substantially increased the median survival: 33 ± 5 months for the patients in the experimental group versus 11 ± 2 months for the patients receiving only radiation and carmustine (control group) (Briceno et al., 2003).

The second clinical trial was randomized, double-blinded, placebo controlled trial method, in which the authors found that the median survival was 24 months for CQ-treated patients and 11 months for the CQ-untreated control (Sotelo et al., 2006). In the third clinical trial, 41 patients were treated with CQ and 82 patients were considered a control group. The authors found that survival time in patients treated with CQ was 25 ± 3.4 months, compared to 11.4 ± 1.3 months in the control group (Briceno et al., 2007). In all, the mean survival time of

the patients who received CQ ($n = 65$) was 27 months, and that of the CQ-untreated control group was 11 months ($n = 106$) (Briceno et al., 2003, 2007; Sotelo et al., 2006). The authors hypothesized that the CQ-mediated enhancement of the therapy may be due to the lysosomotropic and weak DNA intercalation property of CQ (Briceno et al., 2003, 2007; Sotelo et al., 2006). The molecular mechanism of CQ is not known in this case.

Chloroquine could effectively sensitize multidrug-resistant tumor cells in response to certain anti-neoplastic drugs such as vincristine (Inaba and Maruyama, 1988; Jensen et al., 1994). The addition of CQ to the culture medium of leukemic multidrug resistant cells also decreased resistance to vinblastine by 10 to 15-fold (Cho and Rando, 2000; Dalpke et al., 2001; Filippov et al., 1989; Jensen et al., 1994; Weber and Levitz, 2000; Zamora and Beck, 1986; Zamora et al., 1988).

4.5. Vascular protective effects of CQ during brain tumor therapy

Youle and colleagues investigated the potential of CQ in utilizing the immunotoxin-based brain tumor therapy by selectively suppressing toxicity to the vasculature without compromising anti-tumor efficacy (Hagihara et al., 2000). The authors found that CQ has a vascular protective effect during brain tumor therapy with the Tf-CRM107 immunotoxin (Hagihara et al., 2000). Tf-CRM107 is a conjugate of a transferrin and a diphtheria toxin protein containing a point mutation, which can selectively kill tumor cells expressing a high level of transferrin receptor (Tf-R) (Greenfield et al., 1987; Johnson et al., 1988; Laske et al., 1994, 1997). Although approximately half of the patients exhibited a response, those who received a high dose of Tf-CRM107 developed small vessel thrombosis or petechial hemorrhage. Intracerebral injection of Tf-CRM107 into rats at a total dose > 0.025 mg caused brain damage that can be detected by MRI and histology (Laske et al., 1994). To improve the Tf-CRM107-based treatment, the authors explored a way to prevent damage to vasculature. It was previously shown that CQ could block the toxicity of diphtheria toxin (Leppla et al., 1980), raising the possibility that CQ could also reduce the toxicity caused by Tf-CRM107. Systemic administration of CQ indeed blocked the toxicity of Tf-CRM107 when infused intracerebrally in rats, allowing the increase of the maximum tolerated dose of Tf-CRM107 to 0.3 mg from 0.2 mg (Leppla et al., 1980). Taken together, CQ is a useful agent for the reduction of toxicity caused by Tf-CRM107 without compromising its efficacy (Hagihara et al., 2000). However, there is no clear evidence about molecular mechanism of CQ.

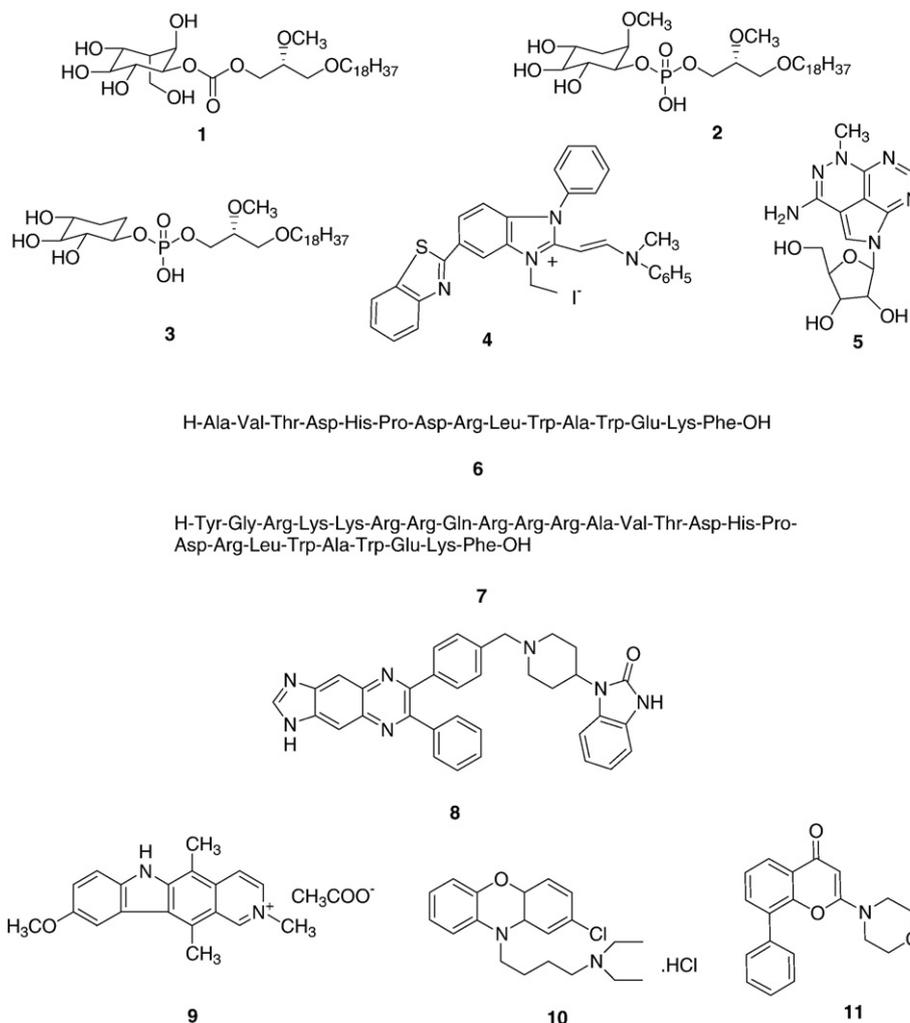


Fig. 4. The molecular structure of PI3K-Akt inhibitors. The combinational effects of these compounds with CQ on breast cancer cells were reported elsewhere [Hu et al., 2008](#). The inhibitors #1–11 (left to right) are as follows (the numbers in brackets are Calbiochem catalog numbers): **1** L-6-Hydroxymethyl-*chiro*-inositol 2-(R)-2-O-methyl-3-O-octadecylcarbonate (124005); **2** Phosphoric acid 2-methoxy-3-octadecyloxy-propyl ester 2,3,4-trihydroxy-6-methoxy-cyclohexyl ester (124008); **3** Phosphoric acid 2-methoxy-3-octadecyloxy-propyl ester 2,3,4-trihydroxy-cyclohexyl ester (124009); **4** 6-Benzothiazol-2-yl-1-ethyl-2-[2-(methyl-phenyl-amino)-vinyl]-3-phenyl-3H-benzimidazol-1-ium iodide (124011); **5** 2-(3-Amino-5-methyl-5H-1,4,5,6,8-pentaaza-acenaphthylen-1-yl)-5-hydroxymethyl-tetrahydro-furan-3,4-diol; **6** H-AVTDHPDRLWAWEKF-OH TCL110-24 (124013); **7** H-YGRKKRRQRRR-AVTDHPDRLWAWEKF-OH TAT-TCL110-24 (124014); **8** 1-[1-[4-(7-Phenyl-1H-imidazo[4,5-g]quinoxalin-6-yl)-benzyl]-piperidin-4-yl]-1,3-dihydrobenzimidazol-2-one (124018); **9** 9-Methoxy-2,5,11-trimethyl-6H-pyrido[4,3-b]carbazol-2-ium acetate (124019); **10** [4-(2-Chloro-4a,10a-dihydro-phenoxazin-10-yl)-butyl]-diethyl-amine hydrochloride (124020); **11** 2-Morpholin-4-yl-8-phenyl-chromen-4-one (LY294002).

4.6. Protective effects of CQ in mammary carcinogenesis

O'Malley and colleagues showed that a short exposure to CQ was preventative against N-methyl-N-nitrosourea (NMU)-induced mammary carcinogenesis in a rat ([Loehberg et al., 2007](#)). A similar protective effect by CQ was observed with cells treated with estrogen or progesterone ([Sivaraman et al., 1998, 2001](#); [Sivaraman and Medina, 2002](#)). In contrast, no protective effect by CQ was observed with the BALB/c p53-null mammary epithelial model, suggesting the CQ-mediated protective effect is p53 dependent. The authors subse-

quently found that cell cycle was arrested in G1 by the CQ-mediated activation of the tumor-suppressor p53 and p21, apparently in a DNA damage independent manner. The activation of p53 was through the phosphorylation (i.e., activation) of ATM protein kinase by CQ ([Pharoah et al., 1999](#); [Renwick et al., 2006](#)). As expected, mammary gland epithelial cells isolated from a p53-null mouse was not arrested at G1 by CQ, confirming the hypothesis that the CQ-mediated G1 arrest is p53-dependent. It should be noted that cells defects in cell cycle checkpoints are often vulnerable to cell damaging agents. Therefore, the activation of the tumor-suppressors ATM and p53 by CQ is highly significant for cancer therapy, since defects in p53 are frequently found in cancer ([Krajewski et al., 1999](#); [Pharoah et al., 1999](#); [Renwick et al., 2006](#)). This property of CQ can provide opportunities of selectively killing cancer cells.

4.7. The effect of CQ and suberoylanilide hydroxamic acid, a histone deacetylase inhibitor

[Carew et al. \(2006\)](#) hypothesized that targeting the autophagy pathway can enhance the anti-cancer activity of the suberoylanilide hydroxamic acid (SAHA) histone deacetylase inhibitor (HDAC). Since

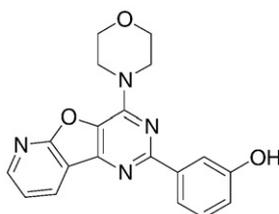


Fig. 5. The chemical structure of PI3K-Akt inhibitor PI-103.

the emergence of imatinib-resistant tumor is increasingly a problem, SAHA has been evaluated to control imatinib-resistant chronic myelogenous leukemia (CML) (O'Connor et al., 2006). CQ substantially potentiates the pro-apoptotic effects of SAHA on the imatinib-resistant Ba/F3 cells that are caused by the T315I (most resistant), E255K (partial resistant), or M351T (partial resistant) mutations. Importantly, primary cells from CML patients who had been clinically refractory to imatinib also displayed a markedly increased sensitivity to the combination of CQ and SAHA. This combined modality appeared to be specific to malignant cells and was effective even when p53 function was impaired. Since p53 defects are a major contributing factor in drug resistance, this finding is very promising (Lowe et al., 2004; Sax and El-Deiry, 2003). The increase in the level of ROS plays a critical role in cell killing by SAHA and other HDAC inhibitors (Dai et al., 2005; Rosato et al., 2003, 2006; Yu et al., 2003) Park et al. (2004) found that CQ alone can alter cellular redox status, and modestly but significantly increase the level of cellular superoxide in response to the treatment of autoimmune diseases. Importantly, the combination of CQ with SAHA could markedly increase the level of superoxide. The elevated level of ROS may be required for the maximal cell death by the combination of SAHA and CQ.

The treatment of K562 and LAMA 84 CML cells with a combination of CQ and SAHA led to a dramatic decrease in the level of Trx. This down-regulation of the anti-oxidant Trx likely contributes to the synergistic increase in ROS generation observed in CML cells treated with the combination of CQ and SAHA (Carew et al., 2006). Furthermore, CQ treatment leads to a dramatic increase in the level of cathepsin D in K562 and LAMA 84 CML cells, of which response is further increased by SAHA. Data from knockdown studies in LAMA 84 CML cells showed that cathepsin D plays an essential role in potentiation of SAHA by CQ (Butler et al., 2005). Together, the combination of CQ with HDAC inhibitors is a promising new strategy to treat imatinib-refractory patients (Carew et al., 2006).

5. Miscellaneous

Accumulating lines of evidence now suggest that the development of weak DNA-intercalating bioreductive compounds is an effective strategy to ensure DNA affinity high enough to produce toxicity yet low enough to permit efficient extravascular diffusion and penetration to hypoxic tumor tissue (Brown, 2000). Based on this strategy, Papadopoulou et al. (2000) synthesized a CQ derivative, 4-[3-(2-nitro-1-imidazolyl)-propylamino]-7-chloroquinoline hydrochloride (NIPCQ,

NSC709257, Fig. 6). The NIPCQ/NSC09257 compound was selectively accumulating in hypoxic cells up to 388-fold within 4.5 h of exposure, probably due to its weak DNA-binding property under hypoxic conditions (Papadopoulou et al., 2000). An *in vitro* study suggested that cytochrome P450 and the b5 reductase play an important role in NIPCQ bioreductive activation (Papadopoulou et al., 1998, 2003). NIPCQ synergistically enhances the radiation effects on hypoxic cells *in vitro* and in xenografts (Papadopoulou et al., 2000; 2002a,b,c). It was also found that NIPCQ substantially enhances the anti-tumor effects of alkylating agents, 5-fluorouracil, and paclitaxel against murine tumors and xenografted human tumors. Importantly, this compound does not cause hypoxia-dependent retinal toxicity (Papadopoulou et al., 2002d). In addition, NIPCQ is stable in human plasma and desirable in many pharmacokinetic parameters such as bioavailability, plasma clearance and volume of distribution in animals (Papadopoulou et al., 2001). Because of these desirable properties of NIPCQ, it is about to enter a Phase I clinical trial.

Rajapakse et al. (Rajapakse et al., 2009) synthesized a series of Ruthenium complexes containing CQ, and screened their anti-cancer activity on the HCT-116 colon cancer cell line containing wild type p53 and the LS141 liposarcoma cell line containing defective p53. The authors found that the CQ analogs compounds **1** and **2** shown in Fig. 6 inhibited the growth of colon tumors independently of the p53 status. Especially, compound **1** showed (IC₅₀ 8 μM) greater sensitivity to killing liposarcoma tumor cells, which does not usually respond to currently available chemotherapeutic agents (Rajapakse et al., 2009).

CQ was previously shown to act as a reversal agent for multidrug resistant tumor cells in response to vinblastine (CEM/VLB100) and vincristine (K562/ADM) (Suzuki et al., 1997; Zamora and Beck, 1986; Zamora et al., 1988). Chibale et al. (2001) synthesized sulfonamide derivatives of CQ, and evaluated their activities against paclitaxel-sensitive (LCC-WT-human breast carcinoma) or paclitaxel-resistant (LCC6-MDR-MDR1 transfected) human breast cancer cells). All of the CQ derivatives showed at least 96% MDR reversal activities when co-administered with 5 μM paclitaxel. The authors found that the derivative **3** (Fig. 6) could reverse 99% MDR phenotype when used in combination with 1 μM paclitaxel (Chibale et al., 2001).

6. Future directions

The lysosomotropic CQ is accumulated in the lysosomes, raises intralysosomal pH, and interferes with autophagosome degradation in the lysosomes. This unique property of CQ may be important for the

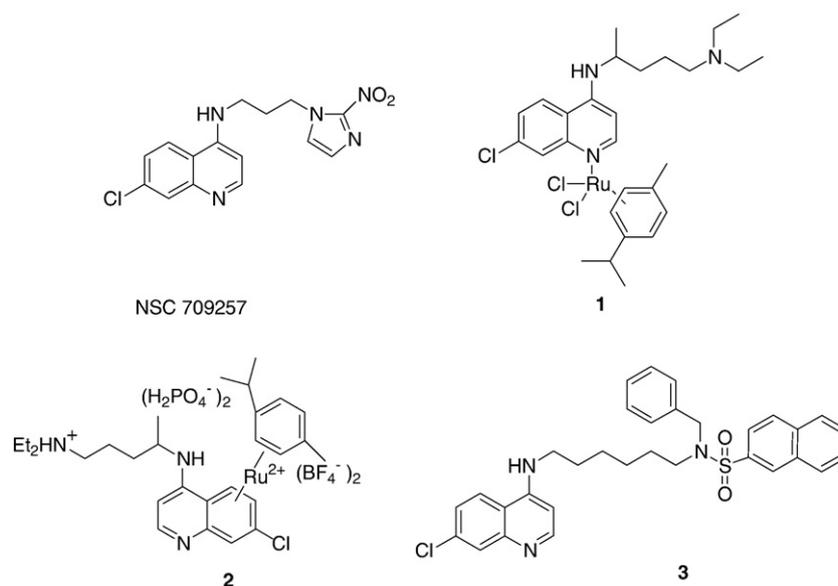


Fig. 6. Chemical structure of miscellaneous CQ analogs.

enhancement of cell killing by cancer therapeutic agents in a variety of different tumors and genetic backgrounds (Amaravadi et al., 2007; Carew et al., 2006; Maclean et al., 2008; Zhao et al., 2005). Together, published data suggest that combined modalities of CQ with other therapeutics are very promising for the increase in therapeutic efficacy and decrease in undesirable side effects. As shown in Table 2, several clinical trials using combinations of CQ and different therapeutic agents are currently being carried out (also see, the National Cancer Institute website, <http://clinicaltrials.gov>). The results of these clinical trials are likely to be informative in determining the directions of research on CQ-mediated cancer control. As we know more about the mode of CQ action in the normal and cancer cells, we can further refine combined modalities using low levels of specific signal inhibitors and CQ to increase efficacy and decrease side effects. We can also generate novel CQ analogs by modifying the lateral side chain with ring systems or functional groups that possess pharmacophoric feature of an anti-cancer effect. The latter case may be successfully done by a pharmacophore hybrid approach. Ultimately, we may be able to develop very effective modalities by combining a certain class of CQ derivatives and inhibitors directing specific cellular targets. This “customized” combinational approach will likely provide us a very powerful means to control many different cancers in future.

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Table 2
Clinical trials utilizing CQ and its analogs for cancer chemotherapy.

CQ or its analogs	Combination	Treatment	Study phase	Rationale and purpose
CQ	Carmustine + Radiation	Glioblastoma multiforme	III	Synergistic and selective cellkilling effect
HQ	Ixabepilone	Breast cancer	I, II	Synergistic and selective cellkilling effect
HQ	Bevacizumab + Carboplatin + Paclitaxel	Lung cancer	I, II	Synergistic and selective cellkilling effect
HQ	Docetaxel	Prostate cancer	II	Synergistic and selective cellkilling effect
HQ	Gefitinib	Non-Small Cell Lung cancer	I, II	Synergistic and selective cellkilling effect
HQ	Sunitinib malate	Cancer	I	Synergistic and selective cellkilling effect
HQ	Temozolomide	Unspecified adult solid tumor	I	Synergistic and selective cellkilling effect
HQ	Temozolomide + Radiation	Brain and central nervous system tumor	I, II	Synergistic and selective cellkilling effect
HQ	Bortezomib	Multiple myeloma and plasma cell neoplasm	I, II	Synergistic and selective cellkilling effect

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