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ORIGINAL ARTICLE

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Single-dose pharmacokinetics of the DNA-binding bioreductive agent NLCQ-1 (NSC 709257) in CD2F$_1$ mice

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Abstract NLCQ-1 (NSC 709257) is a weak DNA-binding bioreductive antiproliferative agent, with potent in vitro antiproliferative activity against rodent and human tumor cell lines under aerobic and anaerobic conditions. Interest in this quinoline analog is based in part on its in vivo synergistic antitumor effect with radiotherapy or chemotherapy against mouse tumors and human xenografts. A sensitive, specific HPLC method was developed to measure NLCQ-1 in biological fluids. Calibration curves were linear in the range 10.4–667 ng/ml and the lower limit of quantitation was 10.4 ng/ml in plasma. NLCQ-1 was stable in organic solvents, buffered solutions and human plasma for 24 h at 37°C. NLCQ-1 was unstable in rodent and dog plasma when incubated for longer than 10 h. NLCQ-1 human plasma protein binding was high (about 99%), and included binding to both α_1-acid glycoprotein and serum albumin. The plasma elimination of NLCQ-1 in mice after a 10-mg/kg intravenous bolus dose was described by a two-compartment open model with $t_{1/2\beta}$, V_{ss}, and Cl_{TB} values of 41.3 min, 2.04 l/kg and 69.9 ml/min per kg, respectively. NLCQ-1 had high (85%) intraperitoneal and modest (28%) oral relative bioavailability. Little of the administered NLCQ-1 dose (6.4%) was excreted in 24-h urine. The mouse pharmacokinetic data suggested that oral administration may achieve plasma concentration and systemic exposure similar to those observed after intravenous administration of NLCQ-1.

Keywords DNA · Bioreductive compounds · Pharmacokinetics · HPLC · Mice

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Introduction

Poor oxygen delivery to tumors, due to poor vascular organization or tumor growth beyond the limits of oxygen diffusion in tissue, produces tumor hypoxia associated with poor prognosis and resistance to therapy (see reference 4 for review). Low oxygen partial pressures reduce the effect of DNA damage from radiation therapy by reducing the availability of oxygen free radicals. While specific mechanisms are unclear, tumor hypoxia is also associated with production of hypoxic stress proteins and loss of apoptotic potential [4]. These mechanisms may explain why certain anticancer drugs such as cyclophosphamide, 1,3-bis(2-chloroethyl)-1-nitrosourea and Adriamycin have substantially reduced activity in hypoxic tumors [15].

One strategy for treatment of malignancies has been to exploit compounds that are chemically or enzymatically reduced to reactive products (see reference 13 for review). Such compounds are preferentially activated to cytotoxic products at low oxygen concentrations present in hypoxic tumor cells, and have greater antiproliferative activity under anaerobic conditions. Among these bioreductive agents are mitomycin C, currently used in the clinic [1], and tirapazamine [5, 14], now in clinical development.

4-[3-(2-Nitroimidazolyl)-propylamino]-7-chloroquinoline hydrochloride (NLCQ-1, NSC 709257) is the most potent hypoxia-selective antiproliferative molecule among a series of substituted 2-nitroimidazole-linked quinoline analogs [6, 7, 10]. These compounds were developed to combine bioreductive activation via a 2-nitroimidazole moiety with the weak DNA-binding affinity of a substituted quinoline to enhance hypoxia selectivity in these cytotoxic molecules. NLCQ-1 has potent antiproliferative activity against several rodent (U79, EMT6, and SCCUII) and human (A549, OVCAR3) tumor cell lines under both aerobic and anaerobic conditions [10]. The hypoxic selectivity of NLCQ-1, defined as the ratio between anaerobic and aerobic

activity, increases from 5-fold to 388-fold as exposure time is increased from 1 to 4.5 h in those cell lines [10]. Similarly, NLCQ-1 potentiates the antiproliferative effect of radiotherapy or chemotherapy against mouse tumors and human xenografts in vivo [8, 9, 10, 11].

The maximum tolerable single intraperitoneal (i.p.) dose of NLCQ-1 in Balb/c mice is 30 mg/kg. Antitumor activity and normal tissue toxicity in mice given a 10-mg/kg dose of NLCQ-1 compares favorably with those of tirapazamine [11]. A dose of 10 mg/kg per day NLCQ-1 can be administered i.p. to mice for four consecutive days, in combination with chemotherapy, without signs of toxicity [12]. In this report, we describe studies of the preclinical pharmacology of NLCQ-1 in mice.

Methods and materials

Materials

NLCQ-1 and NSC 13248 were provided by the Pharmaceutical Resources Branch, Division of Cancer Treatment, National Cancer Institute (Bethesda, Md.). Serum albumin fraction V (96–99%, catalog no. A-1653) and α_1-acid glycoprotein purified from Cohn fraction VI (99%, catalog no. G-9885) were obtained from Sigma Chemical Company (St. Louis, Mo.). All reagents were of analytical grade and all solvents were of HPLC grade.

HPLC assay

HPLC separations were achieved on a Jones Chromatography Genesis ODS (250×4.6 mm i.d., 5 μm) analytical column fitted with a Brownlee RP-18 (15×3.2 mm i.d., 7 μm) guard column eluted with a mobile phase consisting of methanol/10 m*M* potassium phosphate, 7.5 m*M* heptanesulfonic acid, 1% triethylamine, pH 3.5 (50:50, v/v). The flow rate, injection volume and detector wavelength were 1.0 ml/min, 50 μl and 330 nm, respectively.

NLCQ-1 was extracted from plasma (50–300 μl) by liquid extraction with diethyl ether (0.8–6 ml). After vigorous shaking for 10 min, the organic layer was separated by centrifugation (1000 *g* for 10 min), transferred to a conical centrifuge tube, evaporated to dryness under a gentle stream of nitrogen and reconstituted in mobile phase (125 μl). NLCQ-1 was extracted from plasma by solid-phase extraction through Varian Bond Elut C18 (1 ml, 100 mg sorbent) columns activated by washing with 2 ml each of MeOH and 0.1 *M* KH_2PO_4, pH 6.0. Plasma samples (300–500 μl) were added to the column after dilution with an equal volume of 0.1 *M* KH_2PO_4, pH 6.0. The adsorbed materials were washed with 1 ml 1.0 *M* acetic acid and 2 ml H_2O, the column was dried under vacuum (5 min) and NLCQ-1 and the internal standard were eluted with 2 ml 2% NH_4OH in ethyl ether.

Plasma samples containing 25–800 ng/ml of NLCQ-1 and 500 ng/ml of the internal standard NSC 13248 were prepared by addition of 23.8 μl of 0.312–10.0 μg/ml drug solutions and 23.8 μl of 6.26 μg/ml internal standard solution, respectively, to 250 μl mouse plasma prior to extraction.

NLCQ-1 solution stability

NLCQ-1 stability was investigated in buffered aqueous solutions (0.01 *N* HCl/pH 2.0, 0.05 *M* potassium phosphate/pH 4.0, 0.05 *M* potassium phosphate/pH 7.0, and 0.05 *M* glycine/pH 10.0), the HPLC mobile phase, and human, rodent and dog plasma and whole blood. Incubation solutions were prepared in silanized glass vials and maintained at 37°C in a shaking water bath. Aliquots were removed at the beginning of the incubation period and at selected times for a 48-h period. After centrifugation (10,000 rpm for 3 min) of the buffered and mobile phase solutions, 100 μl of the supernatant was mixed with 500 μl of mobile phase and chromatographed by reverse-phase HPLC as described above. Whole-blood samples (500 μl) were centrifuged to separate the plasma. After the plasma layer (250 μl) was transferred to a separate microcentrifuge tube and the red blood cells (RBC) (250 μl) were lysed by adding distilled water (250 μl), the samples were immediately frozen and stored at −20°C. Plasma incubation aliquots were immediately frozen and stored at −20°C. Prior to HPLC analysis, internal standard was added to the thawed plasma and RBC lysate samples which were then extracted with diethyl ether as described above.

Plasma protein binding

Protein binding was determined by ultrafiltration in Amicon Centrifree Micropartition devices. The ultrafiltrate was collected by centrifugation (1500 *g*) in a fixed-angle rotor for 30 min at 4°C after a 30-min incubation period at room temperature. Drug concentrations were measured in samples before (sample reservoir) and after (filtrate cup) centrifugation. The percentage of drug recovered and protein binding were calculated by the equations:

$$\text{Percentage Recovered} = \left[\frac{\text{Filtrate cup concentration}}{\text{Sample reservoir concentration}}\right] \times 100$$

$$\text{Protein Binding} = \left[1 - \frac{\text{Percentage Recovered (Plasma)}}{\text{Percentage Recovered (Ultrafiltrate)}}\right] \times 100$$

Specific binding to human α_1-acid glycoprotein and human serum albumin was determined using a modification of the method of Fuse et al. [2]. Human plasma was diluted 1:10 with PBS, pH 7.4. Human serum albumin (40 mg/ml) and human α_1-acid glycoprotein (1.0 mg/ml) were each dissolved in PBS, pH 7.4. Proteins were separated using ultracentrifugation (273,000 *g*) for 18 h at 4°C after a 10-min incubation of NLCQ-1 in the solution on ice. Ultracentrifuge tubes (polycarbonate) were silanized with Sigmacote (Sigma Chemical Company, St. Louis, Mo.) prior to the addition of sample. The control human plasma ultrafiltrate was prepared by centrifugation (2000 *g*) of human plasma with Amicon Centrifree micropartition devices. NLCQ-1 was dissolved in DMSO and added to the samples so as not to exceed 1% DMSO. The NLCQ-1 content was determined in the solution prior to ultracentrifugation and in an aliquot removed from the upper portion of the ultracentrifuge tube after ultracentrifugation. Protein binding was calculated using the above equations.

Pharmacokinetic studies

Non-tumor-bearing male $CD2F_1$ mice (21–32 g), supplied by the National Cancer Institute, were housed five per cage on commercially obtained pure wood shaving bedding in an on-site facility with light provided from 6:00 a.m. to 8:00 p.m. Food (Purina Rodent Chow) and tap water were provided ad libitum.

NLCQ-1 (2 mg/ml in a 5% dextrose solution) was administered i.p. to the mice (21–26 g) using a 0.5-ml tuberculin syringe fitted with a 27-gauge needle or orally using a 1-ml tuberculin syringe fitted with a ball-tipped feeding needle. NLCQ-1 (1.25 mg/ml in a DMSO/0.9% sodium chloride solution, 2:1, v/v) was administered intravenously (i.v.) to the mice (21–32 g) using a 100-μl Hamilton glass syringe fitted with a 30-gauge 0.5-inch needle.

Blood samples were collected by cardiac puncture using syringes containing 150 μl of an anticoagulant solution (10% heparin in a citrate/phosphate/dextrose solution) from mice anesthetized under ether vapors, transferred to silanized microcentrifuge tubes, and separated by centrifugation (10,000 rpm for 3 min). Plasma was transferred to silanized microcentrifuge tubes for storage at −20°C until analysis.

Urine was collected for a 24-h period from mice (20–24 g) given i.v. NLCQ-1. After drug administration, mice were placed in plastic metabolism cages (seven per cage) and urine was collected in Erlenmeyer flasks placed on dry ice at the base of each cage. Following the collection period, the cages were washed with 40 ml water. Aliquots of urine (50 µl) and wash solution (250 µl) were assayed for NLCQ-1.

NLCQ-1 plasma concentration-time data were analyzed by non-compartmental methods using the program WinNonlin version 1.5 (Statistical Consultants, Lexington, Ky.). The area under the plasma concentration-time curve (AUC) was determined by trapezoidal approximation, using the mean concentration value ($n = 2$) for each time point. The terminal elimination rate constant (k_{el}) was calculated by linear least squares regression of the last three or four time points in the plasma concentration time profiles. Plasma clearance (Cl_p), half-life ($t_{1/2}$), and steady-state volume of distribution (V_{ss}) were calculated using equations described previously [3]. Bioavailability was determined by comparison of AUC values found after oral and i.p. administration with the AUC values found after i.v. administration.

Results

HPLC assay

Efficient chromatography of NLCQ-1 and the internal standard NSC 13248 (Fig. 1) was achieved on a Jones Chromatography Genesis ODS column as illustrated for a plasma extract in Fig. 2. NSC 13248 was selected as the internal standard for NLCQ-1 analysis based on structural similarity, efficient chromatography (little peak tailing) and extraction efficiency similar to NLCQ-1. Solid-phase extraction with the Bond Elut C18 column achieved higher recovery (> 90%) for NLCQ-1 and internal standard than did solvent extraction with ethyl ether (60%). Standard curves, weighted using the reciprocal of the NLCQ-1/internal standard ratio were linear over the range of 10.4–667 ng/ml ($r^2 > 0.99$) with a lower limit of detection of 10.4 ng/ml. Reproducibility was high with coefficients of variation of back-calculated standard values < 15%. Accuracy was excellent with a difference between the nominal values and back-calculated values of < 5% for all except the lowest standard, which itself was < 15%.

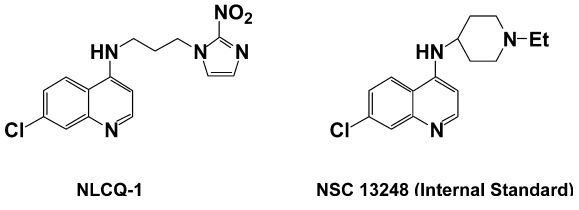

Fig. 1 Structure of NLCQ-1 and the internal standard NSC13248

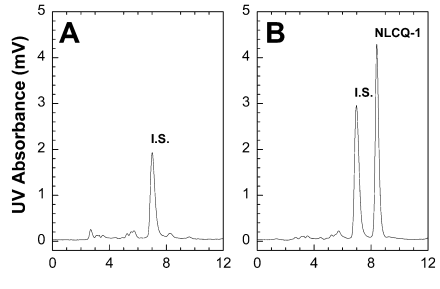

Fig. 2A, B HPLC chromatograms of mouse plasma containing the internal standard only (**A**) and containing 333 ng/ml NLCQ-1 and internal standard (**B**) following liquid extraction and elution from a Genesis ODS HPLC column with a mobile phase consisting of 50:50 methanol/10 m*M* potassium phosphate, 7.5 m*M* heptanesulfonic acid, 1% triethylamine, pH 3.5 (λ 330 nm, injection volume 50 µl)

NLCQ-1 stability

NLCQ-1 was stable in aqueous solutions at pH 2, 4, 7 and 10 with less than 5% decomposition after a 48-h incubation period (data not shown). NLCQ-1 and the internal standard were stable following plasma extraction and reconstitution in mobile phase and storage at ambient temperature in autosampler vials for 18 and 22 h, respectively (data not shown).

The NLCQ-1 peak area remained unchanged during a 24-h incubation in thawed human and fresh dog plasma. In contrast, the NLCQ-1 peak area remained unchanged for 7–10 h during incubation in fresh mouse and rat plasma, but was reduced to 13% and 56%, respectively, of the initial value at 24 h (Fig. 3). Losses of NLCQ-1 at the later time points were not reduced by the addition of ascorbic acid. The mechanism of drug degradation during the 10 to 24-h incubation interval is not known. NLCQ-1 was stable in human whole blood for 24 h (data not shown). A 1:2 distribution ratio between RBC and plasma was maintained throughout the 24-h incubation.

Plasma protein binding

NLCQ-1 protein binding to human and mouse plasma proteins over the range 100–400 ng/ml of drug was 99% and 70%, respectively. High protein binding was

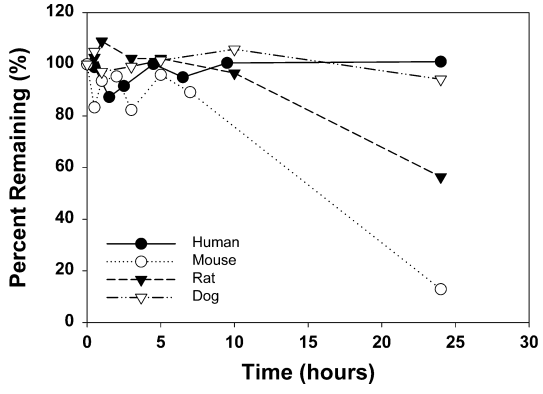

Fig. 3 Graph of NLCQ-1 peak area versus time during incubation of NLCQ-1 (400 ng/ml) in fresh mouse (○), rat (▼), dog (▽), and human (●) plasma for a 24-h period

observed when NLCQ-1 protein binding was further evaluated in dilute (1:10) human plasma, α_1-acid glycoprotein and human serum albumin (Table 1). Protein binding was highest (91%) with the α_1-acid glycoprotein, although protein binding was also high with dilute human plasma (67%) and human serum albumin (82%). Protein binding was reduced in the presence of a 25-fold greater concentration of NLCQ-1, most likely the result of saturation of protein binding sites.

NLCQ-1 pharmacokinetics in mice

NLCQ-1 was rapidly distributed and eliminated following i.v. administration (Fig. 4A). The NLCQ-1 peak plasma concentration achieved after injecting the 2.5-mg/kg dose was 1481 ng/ml (4.5 μ*M*). The NLCQ-1 half-life was 41 min and plasma concentrations of NLCQ-1 had fallen below 10 ng/ml (0.03 μ*M*) 90 min after i.v. injection. NLCQ-1 pharmacokinetic parameter estimates are summarized in Table 2. The plasma clearance and volume of distribution after i.v. administration were 69.9 ml/min per kg and 2.04 l/kg, respectively.

NLCQ-1 was rapidly absorbed and eliminated following i.p. administration (Fig. 4B). The peak plasma concentration of 8900 ng/ml (26.8 μ*M*) was achieved 5 min after injecting the 10-mg/kg dose. NLCQ-1 was not detected in plasma later than 2 h after i.p. administration. NLCQ-1 was also rapidly absorbed, but eliminated more slowly following oral administration (Fig. 4B). The peak plasma concentration of 461 ng/ml (1.4 μ*M*) was detected 15 min after administration of the 10-mg/kg dose. NLCQ-1 was not detected in plasma later than 4 h after oral administration. In comparison to the exposure after an i.v. dose, i.p. bioavailability was high (85%) and oral bioavailability was modest (28%). The apparent elimination half-life was dependent on administration route and was shortest after i.p. administration.

Table 1 Specific binding to human α_1-acid glycoprotein and serum albumin

Medium	Bound fraction (%)[a]	
	NLCQ-1 400 ng/ml	NLCQ-1 10,000 ng/ml
PBS, pH 7.4	9	14
	5	20
Plasma ultrafiltrate	0	5
	–	0
Dilute human plasma (1:10, v/v)	64	50
	71	32
α_1-Acid glycoprotein (1 mg/ml)	91	42
	92	31
Human serum albumin (40 mg/ml)	83	68
	82	71

[a]Each value represents one of two separate determinations for each condition (except for plasma ultrafiltrate at 400 ng/ml for which the result of only one experiment was available)

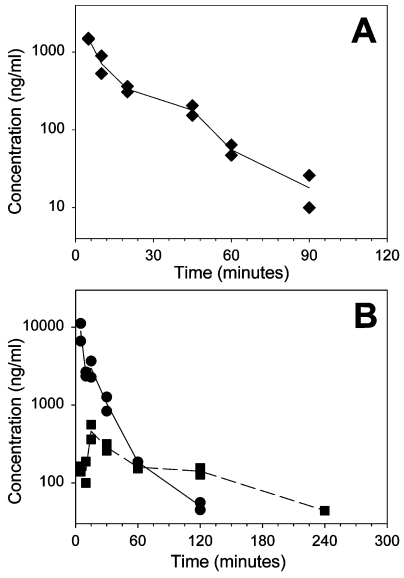

Fig. 4 **A** Plasma profile of NLCQ-1 following i.v. administration of a 2.5-mg/kg dose to male $CD2F_1$ mice. **B** Plasma profile of NLCQ-1 following i.p. or oral administration of a 10 mg/kg dose to male $CD2F_1$ mice. Symbols represent individual data: ◆ i.v., ● i.p., ■ oral. The lines represent mean data: *solid lines* i.v. and i.p., *dashed lines* oral

Table 2 NLCQ-1 pharmacokinetic parameters

Parameter	Route		
	Intravenous	Intraperitoneal	Oral
Dose (mg/kg)	2.5	10	10
AUC (ng/ml×min)	35,778	12,1774	40,469
$t_{1/2\beta}$ (min)	41.3	18.5	83.9
Bioavailability (%)	–	28	85
Cl (ml/min/kg)	69.9		
V_{ss} (l/kg)	2.04		

The 24-h urinary recovery of NLCQ-1 was 6.4% of the administered dose.

Discussion

The goal of this study was to characterize the pharmacokinetics of NLCQ-1 in mice administered at a dose associated with in vivo antitumor activity. Accordingly, a sensitive, specific HPLC method was developed for measurement of NLCQ-1 in biological fluids. Satisfactory chromatography of NLCQ-1 and NSC13248, a structurally related molecule used as the internal standard, was achieved with reverse-phase HPLC on a Genesis ODS column using the ion-pairing agent heptanesulfonic acid. Solid-phase extraction was the preferred method of NLCQ-1 extraction, although liquid extraction (used in early pharmacokinetic experiments) was also satisfactory.

While NLCQ-1 was stable in organic and buffered aqueous solutions as well as in thawed human and fresh

dog plasma, degradation was observed in rodent plasma after 7–10 h of incubation. While NLCQ-1 was stable in plasma and whole blood for the periods of time required to prepare samples for HPLC analysis, thawed samples should not be left at room temperature for prolonged periods. The mechanism of NLCQ-1 decomposition from 10–48 h is unknown. The modest (30%) partition of drug into RBCs is consistent with poor lipid permeability of a protonated drug. Both α_1-acid glycoprotein and serum albumin contributed to the high (about 99%) protein binding in human plasma.

The plasma elimination of NLCQ-1 in mice was described by a two-compartment open model with a terminal half-life of 41 min. The modest NLCQ-1 volume of distribution (twofold more than the average mouse body weight) was suggestive of limited tissue distribution. The high total clearance, threefold greater than liver blood flow, and low urinary recovery of the parent molecule suggest that metabolism is the principle route of drug clearance. NLCQ-1 plasma concentrations after i.p. administration of an active dose (10 mg/kg) remained above a concentration with demonstrated in vivo activity (3 μM) for approximately 30 min. The limited aqueous solubility (< 2 mg/ml) in the formulation used for the i.v. bolus dose study suggests that an alternative i.v. formulation may be required for subsequent studies unless continuous infusion regimens are appropriate. Our mouse data suggest that oral administration may achieve plasma concentration and systemic exposure similar to those observed after i.v. administration. Prolonged plasma elimination in mice after oral administration when compared to i.v. administration may allow intermittent dosing by this route.

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References

1. Bradner WT (2001) Mitomycin C: a clinical update. Cancer Treat Rev 27:35
2. Fuse E, Tanii H, Kurata N, Kobayashi H, Shimada Y, Tamura T, Sasaki Y, Tanigawara Y, Lush RD, Headlee D, Figg WD, Arbuck SG, Senderowicz AM, Sausville EA, Akinaga S, Kuwabara T, Kobayashi S (1998) Unpredicted clinical pharmacology of UCN-01 caused by specific binding to human alpha1-acid glycoprotein. Cancer Res 58:3248
3. Gibaldi M, Perrier D (1982) Pharmacokinetics, 2nd edn. Marcel Dekker, New York, p 409
4. Höckel M, Vaupel P (2001) Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. J Natl Cancer Inst 93:266
5. Lee DJ, Trotti A, Spencer S, Rostock R, Fisher C, von Roemeling R, Harvey E, Groves E (1998) Concurrent tirapazamine and radiotherapy for advanced head and neck carcinomas: a phase II study. Int J Radiat Oncol Biol Phys 42:811
6. Papadopoulou MV, Ji M, Bloomer WD (1996) THNLA-1 as radio/chemosensitizer of EMT-6 tumours in mice. Br J Cancer 74:S267
7. Papadopoulou MV, Ji M, Rao MK, Bloomer WD (1996) 9-[3-(2-Nitro-1-imidazolyl)propylamino]-cyclopenteno[b]quinoline hydrochloride (NLCPQ-1). A novel DNA-affinic bioreductive agent as cytotoxin and radiosensitizer. Oncol Res 8: 425
8. Papadopoulou MV, Ji M, Bloomer WD (1998) NLCQ-1, a novel hypoxic cytotoxin: potentiation of melphalan, *cis*DDP and cyclophosphamide in vivo. Int J Radiat Oncol Biol Phys 42:775
9. Papadopoulou MV, Ji M, Rao MK, Bloomer WD (2000) 4-[3-(2-Nitro-1-imidazolyl)propylamino]-7-chloroquinoline hydrochloride (NLCQ-1), a novel bioreductive agent as radiosensitizer in vitro and in vivo: comparison with tirapazamine. Oncol Res 12:325
10. Papadopoulou MV, Ji M, Rao MK, Bloomer WD (2000) 4-[3-2-Nitro-1-imidazolyl)propylamino]-7-chloroquinoline hydrochloride (NLCQ-1), a novel bioreductive compound as a hypoxia-selective cytotoxin. Oncol Res 12:185
11. Papadopoulou MV, Ji M, Bloomer WD (2001) Schedule-dependent potentiation of chemotherapeutic drugs by the bioreductive compounds NLCQ-1 and tirapazamine against EMT6 tumors in mice. Cancer Chemother Pharmacol 48:160
12. Papadopoulou MV, Ji M, Ji X, Bloomer WD (2002) Therapeutic advantage from combining 5-fluorouracil with the hypoxia-selective cytotoxin NLCQ-1 in vivo; comparison with tirapazamine. Cancer Chemother Pharmacol 50:291
13. Rauth AM, Melo T, Misra V (1998) Bioreductive therapies: an overview of drugs and their mechanisms of action. Int J Radiat Oncol Biol Phys 42:755
14. Senan S, Rampling R, Graham MA, Wilson P, Robin H Jr, Eckardt N, Lawson N, McDonald A, von Roemeling R, Workman P, Kaye SB (1997) Phase I and pharmacokinetic study of tirapazamine (SR 4233) administered every three weeks. Clin Cancer Res 3:31
15. Teicher BA, Holden SA, Al-Achi A, Herman TS (1990) Classification of antineoplastic treatments by their differential toxicity toward putative oxygenated and hypoxic tumor subpopulations in vivo in the FSaIIC murine fibrosarcoma. Cancer Res 50:3339