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Molecular pharmacology and antitumor activity of palmarumycin based inhibitors of thioredoxin reductase.

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ABBREVIATIONS

Trx-1 thioredoxin-1

TrxR-1 thioredoxin reductase-1

NF-κB nuclear factor κB

Ask-1 apoptosis signal-regulating kinase 1

PTEN phosphatase and tensin homolog deleted on chromosome ten

HIF-1 hypoxia inducible factor-1

VEGF vascular endothelial growth factor

SECIS selenocysteine insertion sequence

ip intraperitoneal

iv intravenous

po oral

DTNB dinitrothiobenzoate

AST aspartate amino transferase

ALT amino alanine transferase

MTD maximum tolerated dose

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Abstract

The cytosolic thioredoxin (Trx) redox system comprising Trx-1 and the NADPH dependent thioredoxin reductase -1 reductase (TrxR-1) is an important regulator of cell growth and survival. Trx-1 is overexpressed in many human tumors where it is associated with increased cell proliferation, decreased apoptosis and decreased patient survival. We hypothesized that TrxR-1 provides a target to inhibit the activity of overexpressed Trx-1 for the development of novel anticancer agents. We found that the naphthoquinone spiroketal fungal metabolite palmarumycin CP1 is a potent inhibitor of TrxR-1, but attempts to exploit the activity of palmarumycin CP1 analogs as antitumor agents *in vivo* were hampered by their insolubility. We have therefore developed PX-916, a water soluble prodrug of a palmarumycin CP1 analog. PX-916 rapidly releases the parent compound at physiological pH and in plasma, but is stable at acid pH allowing its iv administration. PX-916 is a potent inhibitor of purified human TrxR-1 and of TrxR-1 activity in cells and tumor xenografts when administered to mice, and inhibits the down stream targets of Trx-1 signaling, HIF-1α and VEGF, in tumors. PX-916 showed excellent antitumor activity against several animal tumor models with some cures. Thus, the study demonstrates that water soluble inhibitors of TrxR-1 such as PX-916 can block Trx-1 signaling in tumors producing marked inhibition of tumor growth.

Keywords

palmarumycin analogs; thioredoxin reductase; antitumor

INTRODUCTION

The cytosolic thioredoxin (Trx) redox system is comprised of Trx-1 and thioredoxin reductase -1 reductase (TrxR-1), which catalyses the NADPH dependent reduction of Trx-1. TrxR-1 is an important regulator of cancer cell growth and survival (1,2). Trx-1 acting with peroxiredoxin-1 is an antioxidant that scavenges H_2O_2 (3). Trxs are also able to reduce buried oxidized thiol residues in proteins (4) and regulate the activity of redox sensitive transcription factors, including p53 (5), nuclear factor κB (NF-κB) (6), the glucocorticoid receptor (7), AP-1 (8), hypoxia inducible factor-1 (HIF-1) (9), Sp1 (10) and Nrf2 (11). Trx-1 also binds and inhibits the activity of the apoptosis inducing proteins, apoptosis signal-regulating kinase 1 (Ask-1) (12) and the tumor suppressor PTEN (phosphatase and tensin homolog deleted on chromosome ten) (13), thus, inhibiting apoptosis. Trx-1 is overexpressed in many human tumors (14-17) where it is associated with increased cell proliferation, decreased apoptosis (18) and poor patient survival (19,20). Trx-R, thus, provides a target to regulate the activity of overexpressed Trx-1 (21,22).

TrxR-1 is a selenocysteine containing flavoprotein with broad substrate specificity because of the ready accessibility of its C-terminal redox active site which contains an essential selenocysteine residue (21). There are 3 TrxR isoforms; the canonical cytoplasmic TrxR-1, a mitochondrial TrxR-2 and a testes specific TrxR/glutathione reductase. The cellular level of TrxR-1 is subject to complex regulation [reviewed in (23)]. The core promoter of the TrxR-1 gene contains a number transcription factor activation sites including those for the redox sensitive factors Oct-1 and Sp1, as well as others. Differential splicing and alternative transcription start sites result in multiple forms of the enzyme. Postranscriptional regulation involving a selenocysteine insertion sequence (SECIS) element in the 3-UTR directs selenocysteine incorporation which is necessary for enzyme activity and, thus, Se supplementation, can lead to increased TrxR-1 activity in cell culture and in Se deficient

animals (24). TrxR-1 is necessary for cell proliferation. A TrxR-1 knockout is embryonic lethal in mice and TrxR-1 deficient fibroblasts derived from the TrxR-1 (-/-) embryos do not proliferate *in vitro* (25). Furthermore cancer cell growth is inhibited by TrxR-1 antisense (26), TrxR-1 siRNA (27) and by a mutant redox inactive TrxR-1 (28). There are reports that levels of TrxR-1 are increased by EGF (29) and hypoxia (30) in cancer cells although tumors show only moderately increased levels of TrxR-1 (23,30).

We have identified the naphthoquinone spiroketal fungal metabolite palmarumycin CP1 as a potent inhibitor of TrxR-1 but attempts to exploit the activity of palmarumycin CP1 analogs as antitumor agents *in vivo* were hampered by their insolubility (31). We have recently reported the synthesis of a series of water soluble palmarumycin CP1 analog prodrugs (32). We now report the molecular pharmacology and antitumor activity of one of these prodrugs in cancer cells and in human tumor xenografts.

MATERIALS AND METHODS

Materials

The palmarumycin CP1 analogs PX-911, PX-916 and PX-960 (Figure 1) were synthesized as previously described (31,32). PX-916 was dissolved at 3 mg/ml in 5% ethanol in 10 mM sodium phosphate, pH 4.0, for intraperitoneal (ip) and oral (po) administration and in 5% ethanol, 0.9% NaCl, 10 mM sodium phosphate, pH 4.0,for intravenous (iv) administration and used within 30 min. Human placental TrxR-1, specific activity 33 μmol NADPH oxidized /min/mg protein at room temperature, was prepared as previously described (33). Human recombinant Trx-1 was prepared as previously described (34). Mouse monoclonal anti-human HIF-1α antibody was purchased from Transduction Labs, (Lexington, KY) and rabbit polyclonal anti-human VEGF from Santa Cruz Biotechnology (Santa Cruz, CA). Immunohistochemistry for tumor HIF-1α and VEGF was performed as previously described (35).

Cells

A-673 human rhabdomyosarcoma cells were provided by Dr Peter Houghton (St Jude Children's Hospital, Memphis, TN). SHP-77 human small cell lung cancer cells and MCF-7 human breast cancer cells were obtained from the American Tissue Type Collection (ATCC, Manassas, VA). All cells were tested to be mycoplasma free using a PCR ELISA kit (Roche Diagnostics Inc., Indianapolis, IN) and grown in 95% humidified air with 5% CO_2 at 37°C in McCoy's 5A medium supplemented with 10% fetal bovine serum. For studies of effects of the palmarumycin CP1 analogs on cellular TrxR activity MCF-7 human breast cancer cells were grown in medium containing 1 μM Se for 7 days which increased cellular TrxR activity by about 5 fold, as previously reported (36).

TrxR-1 Assay

TrxR-1 activity was measured as the NADPH dependent colorimetric reduction of dinitrothiobenzoate (DTNB) as previously described (37). TrxR-1, NADPH and palmarumycin CP1 analogs were incubated for 15 min before adding DTNB. TrxR activity in cell lysates was measured as the Trx-1 dependent reduction of insulin by NADPH as previously described (37). TrxR activity in tumor tissue homogenates was measured also as previously described (24)..

Antitumor Studies.

Approximately 10^7 A-673 rhabdomyosarcoma, SHP-77 small cell lung cancer or MCF-7 breast cancer cells in log cell growth were injected subcutaneously (sc) in 0.1 ml phosphate buffered saline into the flanks of female nude Beige mice (A-673 cells) or female severe combined

immunodeficient (*scid*) mice (SHP-77 and MCF-7 cells). Female mice that were to receive the estrogen-dependent MCF-7 breast cancer cell line were implanted sc in the back with a 90-day 17-ß-estradiol release pellet (Innovative Research of America, Sarasota, FL) a day before the tumor cells. The animals were weighed weekly and tumor diameters measured twice weekly at right angles (d_{short} and d_{long}) with electronic calipers and converted to volume by the formula volume = $(d_{short})^2 \times (d_{long}) \div 2$ (38). When the tumors reached volumes between 100 and 170 mm^3, the mice were stratified into groups of 8 animals having approximately equal mean tumor volumes and administration of PX-916 was started. When the tumor volume reached ≥2,000 mm^3 or became necrotic, the animals were euthanized. Tumor growth rate was calculated from the linear portion of the least squares regression of the cube root of the tumor volume. One-way analysis of variance using the General Linear Model (39) was used to test for the effect of treatment on tumor growth rate.

Toxicity Studies

PX-916 was administered iv daily at 25 mg/kg for 5 days to female *scid* mice. The mice were killed 24 hr after the last dose and changes in body weight from the start of the study, blood lymphocyte, neutrophil, red blood cell and platelet counts, and aspartate amino transferase (AST) and amino alanine transferase (ALT) were measured. In separate studies, the maximum tolerated dose (MTD) was determined as the dose that caused ≥4 g body weight loss or death of at least one of 3 animals.

Pharmacodynamic Studies

10^7 MCF-7 breast cancer cells were injected subcutaneously into the flanks of female *scid* mice previously implanted one day earlier with a 60 day 17-β-estradiol release pellet and tumors were allowed to grow to approximately 300 mm^3. Mice were administered a single iv dose of PX-916 25 mg/kg or vehicle alone. Mice were killed at various times and the tumors removed and immediately frozen in liquid N_2 for the TrxR assay. In a separate study, female scid mice with 300 mm^3 MCF-7 tumor xenografts were treated with PX-916 25 mg/kg iv or vehicle alone daily for 5 days. Twenty four hours after the last dose the tumors were remove and fixed for immunohistochemistry or frozen for the TrxR assay.

Pharmacokinetic Studies

Male C57Bl/6 mice were administered PX-916 iv at 25 mg/kg. The mice were killed at various times, blood was collected into heparinized tubes and plasma was prepared. Plasma (0.2 ml) was immediately mixed with an equal volume of 0.25 M sodium phosphate buffer at pH 4.0 and extracted for 1 hr by inversion with 4 ml ethyl acetate. After centrifugation, 3.8 ml of the organic layer was removed and evaporated under N_2 and the residue dried on a lyophilizer.

Chromatographic separation was achieved with a Waters Symmetry C-18 3.9 x 150 mm column (Waters, Milford, PA) with a mobile phase of 0.1% trifluoroactetic acid in 60% methanol, at a flow rate of l/min with detection at 254 nm. For the assay, the sample residue was dissolved in 100μl mobile phase and centrifuged at 15,000 g for 5 min at 4 °C. The limit of detection of the assay for all the compounds from 0.2 ml mouse plasma was 0.1 μg/ml.

RESULTS

Stability

PX-916 was stable as a stock solution in ethanol at room temperature with a half life of > 5 days. However, in 0.1 M sodium phosphate buffer, PX-916 showed pH dependent degradation with a half life at pH 4.0 of 37 hr, at pH 7.0 a half life of << 1 hr and at pH 10.0 a half life of n 1 hr (data not shown). Therefore, for *in vitro* studies PX-916 was used as a stock solution in ethanol, and for *in vivo* studies made fresh in pH 4.0 buffer vehicle.

Inhibition of TrxR-1

PX-916 was a potent inhibitor of purified human TrxR-1 with an IC_{50} of 0.28 μM, which is similar to that of palmarumycin CP1 (Table 1). However, unlike palmarumycin CP1 which is almost insoluble in aqueous media, PX-916 is soluble with a maximum solubility in water of around 10 mg/mL. Based upon the observation that PX-916 was rapidly converted to PX-960 in aqueous solution we measured the ability of PX-960 to inhibit purified human TrxR-1 and found it to be similar to that of PX-916 (Table 1). PX-916 was a selective inhibitor of human TrxR-1 compared to two other NADPH dependent reductases with an IC_{50} for human glutathione reductase of > 50 μM (> 200 fold selectivity for TrxR-1) and 29.2 μM for human cytochrome P-450 reductase (104 fold selectivity for TrxR).

In vitro activity

Cell growth inhibition of MCF-7 human breast cancer cells by palmarumycin CP1, PX-916 and PX-960 occurred at similar concentration of 1 to 3 μM (Table 1). When MCF-7 cells were exposed to 1 μM PX-916 there was a time dependent inhibition of cellular TrxR that was maximum at 24 hr (Figure 2A). The IC_{50} for inhibition of cellular TrxR by PX-916 was 0.25 μM and maximum inhibition occurred at 0.5 μM (Figure 2B). Thus, inhibition of purified human TrxR-1, MCF-7 cellular TrxR and cell growth inhibition of MCF-7 cells occurred at about the same concentration of PX-916.

In vivo inhibition of tumor TrxR and antitumor activity

A single iv dose of PX-916 of 25 mg/kg inhibited MCF-7 human tumor xenograft TrxR-1 up to 60% at 24 hr and the inhibition was maintained for at least 48 hr (Figure 2C). The growth of A-673 human rhabdomyosarcoma xenografts was decreased from (± SE, n = 6 mice) 153 ± 35 mm^3/day in the vehicle control group to 5 ± 3 mm^3/day 5 days after dosing with PX-916 at 30 mg/kg ip daily for 5 doses (97% inhibition, $p < 0.01$) (Figure 3A). PX-916 administered iv also showed good antitumor activity against the SHP-77 small cell lung cancer with a decrease in tumor growth rate 5 days after the end of dosing (± SE, n = 8 mice) from 150 ± 48 mm^3/day in the vehicle control group to 27 ±14 mm^3/day when administered at 25 mg/kg iv daily for 5 doses (82 % inhibition, $p < 0.05$) (Figure 3B). In this study 3/8 mice had no histologicaly detectable tumor when the study was terminated on day 42. When PX-916 was administered iv at 10 mg/kg iv daily for 8 doses tumor growth was decreased to only 91 ± 24 mm^3/day (39%, $p < 0.05$) by 5 days afer the end of dosing. The growth of MCF-7 human breast cancer xenografts was decreased 5 days after the end of dosing from 47 ± 10 mm^3/day in the vehicle control group to 22 ± 4 mm^3/day by PX-916 at 22.5 mg/kg iv daily for 5 doses (52 % inhbition, $p < 0.05$), to 22 ± 8 mm^3/day by PX-916 at 27.5 mg/kg iv every other day for 5 doses (52% inhibition, $p > 0.05$), and to 18.5 ± 8 mm^3/day (62% inhibition, $p < 0.05$) when administered orally at 27.5 mg/kg daily for 5 doses (Figure 3C).

Tumor HIF-1α and VEGF

Levels of the HIF-α transcription factor and its downstream target VEGF are increased by Trx-1 expression (9). We therefore examined the effect of PX-916 administration on tumor HIF-1α and VEGF levels (Figure 4A). Twenty four hours after the last dose of 5 daily doses of PX-916 of 25 mg/kg there was a decrease in MCF-7 xenograft staining for both HIF-1α and VEGF. At the same time levels of tumor TrxR activity were decreased by 75% (Figure 4B).

Toxicity

The MTD single iv dose of PX-916 to female *scid* mice was 50 mg/kg, and the mice tolerated 5 daily doses of PX-916 of 30 mg/kg iv. The major toxicities observed 24 hr after 5 daily doses of PX-916 of 25 mg/kg iv was neutropenia and thrombocytopenia, with no increase in plasma liver enzymes and no significant weight loss (Table 2). No other gross toxicities were observed.

Pharmacokinetics

When incubated with fresh mouse plasma at room temperature, PX-960 had a half life of 31 min at room temperature while PX-916 rapidly disappeared and was converted to PX-960 with a half life of < 2.0 min. Not surprisingly, when PX-916 was administered to mice at 25 mg/g iv it could not be detected in plasma 5 min after administration and only a very small peak of the parent compound PX-960 could be detected at 5 min, but not at 30 min. Two unidentified metabolite peaks could be seen at 5 min but these had disappeared by 30 min (data not shown).

DISCUSSION

Trx-1 is overexpressed in many human tumors (14-17) where it is associated with increased cell proliferation, decreased apoptosis (18) and decreased patient survival (19,20). The redox activity of Trx-1 is necessary for its biological effects in stimulating cancer cell growth and inhibiting apoptosis (12,13,34). While TrxR-1 is necessary for the reduction of Trx-1 its levels are only moderately increased in tumors (23,30). We hypothesized that preventing the reduction of Trx-1 by inhibiting tumor TrxR-1 could be a critical point at which to block the redox dependent biological effects of Trx-1 leading to inhibition of tumor growth

We have previously reported that palmarumycin CP1 and some of its analogs are potent inhibitors of TrxR-1 (31). However, all prior compounds were very insoluble and could not be administered to animals. PX-916 was synthesized as a water soluble prodrug of the palmarumycin CP1 derivative PX-960 and was found to retain the ability to inhibit purified TrxR-1 with an IC_{50} of 0.28 μM. PX-916 also inhibited TrxR-1 activity in MCF-7 human breast cancer cells with an IC_{50} of 0.25 μM and was an inhibitor of MCF-7 cell growth with an IC_{50} of 3.1 μM. As with other reported inhibitors of TrxR-1 (40) PX-916 was an NADPH and time dependent, apparently irreversible inhibitor of TrxR-1, most likely reacting with the selenocysteine containing catalytic site. Two other NADPH dependent reductases, human glutathione reductase and cytochrome P-450 reductase, were not inhibited by PX-916 until concentrations at least 100 fold higher.

Stability studies showed that at physiological pH, PX-916 was converted to its parent PX-960 with a half life of 1 hr. It was much more stable at pH 4.0 with a half life of 37 hr and was formulated at this pH for iv administration. In mouse plasma the breakdown of PX-916 to PX-960 was rapid with a half life less than 2 min and no PX-916 could be detected in the plasma after iv administration of PX-916. Thus, the inhibition of TrxR-1 in tumor xenografts and the antitumor activity is likely to be almost completely due to the parent PX-960. PX-960 could be detected only transiently in mouse plasma after administration of PX-916 due to rapid metabolism, or more likely rapid distribution of the very lipophilic PX-960. When PX-916 was administered as a single dose of 25 mg/kg iv to mice with MCF-7 breast cancer xenografts, the tumor TrxR-1 activity was inhibited by up to 60% and remained inhibited for 48 hr. Repeated administration of PX-916 for 5 days gave 75% inhibition of tumor TrxR 24 hr after the last dose.

PX-916 administered ip or iv showed excellent antitumor activity against A-673 rhabdomyosarcoma, SHP-77 small cell lung cancer and MCF-7 breast cancer. In the case of SHP-77 complete tumor regressions were seen in some mice. The most active schedule was every other day administration, and tumor growth was inhibited as long as the drug was administered. Significant antitumor activity was not seen following oral administration at doses that gave iv antitumor activity. We have previously reported that Trx-1 acts by a redox mechanism to increase HIF-1α levels and VEGF formation, associated with an increase in tumor angiogenesis (9) and this effect was reversed by a Trx-1 inhibitor (41). MCF-7 tumor xenografts in mice treated with PX-916 showed a decrease in tumor HIF-1α and VEGF, presumably due to the inhibition of Trx-1 redox signaling. Inhibition of TrxR-1 might affect

other pathways in the cell. As previously noted, TrxR-1 is a relatively non specific enzyme and other potential natural substrates have been reported, including lipoic acid, ascorbyl free radicals and S-nitrosoglutathione (21,42). A recent study using siRNA to knock down TrxR-1 expression and microarray analysis showed surprisingly few changes in gene expression, only 8 of 20,000 genes on the array, possibly due to the fact that TrxR-1 activity was only decreased 43% by the treatment (27). The genes that did change, leukotriene B4 12 hydroxydehydrogenase (LTB4DH), ubiquitin D, differentiation enhancing factor (DDRF1), fibronectin1, apoliporotein 3, prosaposin, choline/ethanolamine phosphotransferase and interferon-α inducible protein, give little clue to the pathways affected by TrxR-1.

A number of anticancer agents have been reported to inhibit TrxR-1, including alkylating agents and platinum agents (40), the polyphenol curcumin (43), the porphyrin gadolinium macrocycle, motexafin gadolinium (22), and the quinol NSC 706704 (44). It remains to be determined whether concentrations of any of these compounds that inhibit tumor TrxR-1 can be obtained *in vivo*, and most of these agents have other mechanisms that are more likely than TrxR-1 inhibition to account for their antitumor activity.

While this work has focused on the antitumor consequences of the inhibition of TrxR there are a number of other diseases where TrxR is thought to play a pathophysiological, role such as diabetic neuropathy, rheumatoid arthritis, Sjogren's syndrome, AIDS and reperfusion injury (1,2,42). TrxR inhibitors may have therapeutic benefits in these conditions as well as in cancer.

In summary, we have shown that PX-916, a water soluble prodrug of a palmarumycin CP1 analog, rapidly releases the parent compound at physiological pH and in plasma, but is stable at acid pH allowing its iv administration. PX-916 is a potent inhibitor of purified human TrxR-1, and of TrxR activity in cells and tumor xenografts when administered to mice. PX-916 exhibited antitumor activity against several animal tumor models, with some cures, and blocked the expression of the down stream targets of Trx-1 signaling, HIF-1α and VEGF, in the tumors.

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Figure 1.
Compound structures.

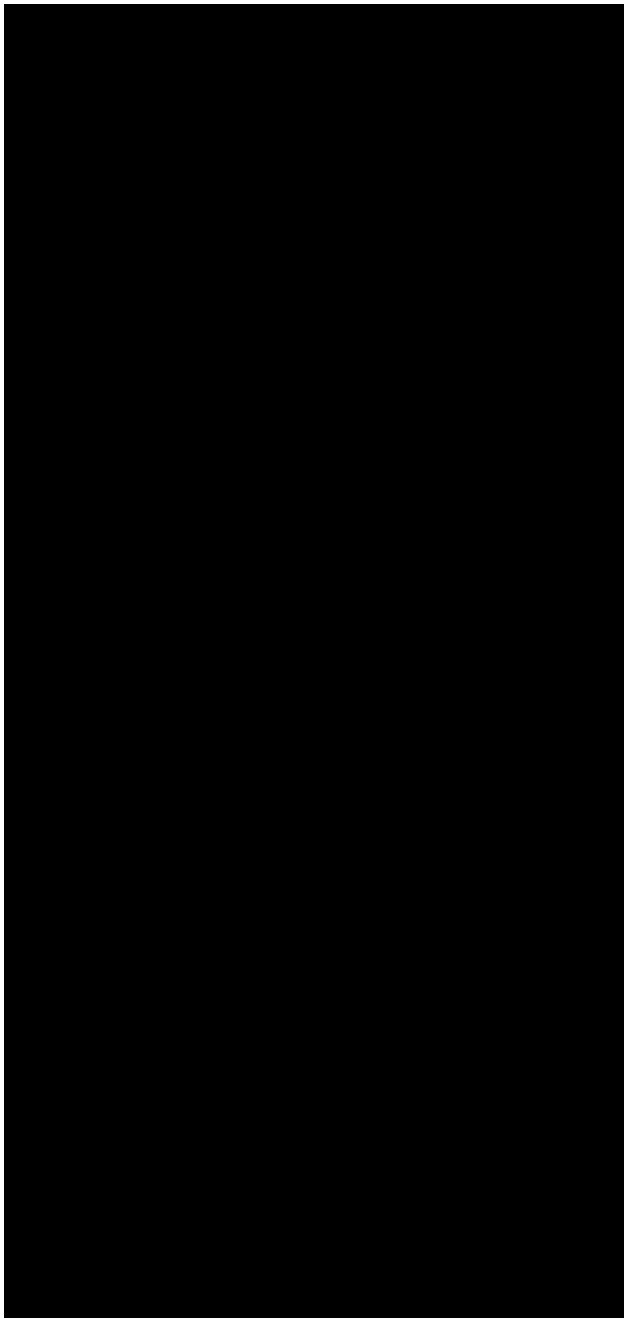

Figure 2.

Inhibition of cellular and tumor TrxR by PX-916. MCF-7 human breast cancer cells grown in medium containing 1 μM Se for 7 days were treated with PX-916 and cellular TrxR activity was measured. **A**, Time course of the inhibition of TrxR on exposure to 1 μM PX-916. **B**, Concentration dependence of the inhibition of TrxR on exposure to various concentrations of PX-916 for 17 hr. Values are the mean of 3 determination and bars are SE. p =< 0.05, ** p = < 0.01 compared to control. **C**, MCF-7 human breast cancer xenografts were grown in female *scid* mice implanted with 17-β-estradiol 90 day slow release pellets until they were ~ 300 mm^3. The mice were administered a single dose of PX-916 of 25 mg/kg iv and tumors harvested

at various times. TrxR activity was measured in tumor homogenates. Values are the mean of 3 mice at each time point and bars are SE. ** $p < 0.01$ compared to pretreatment value.

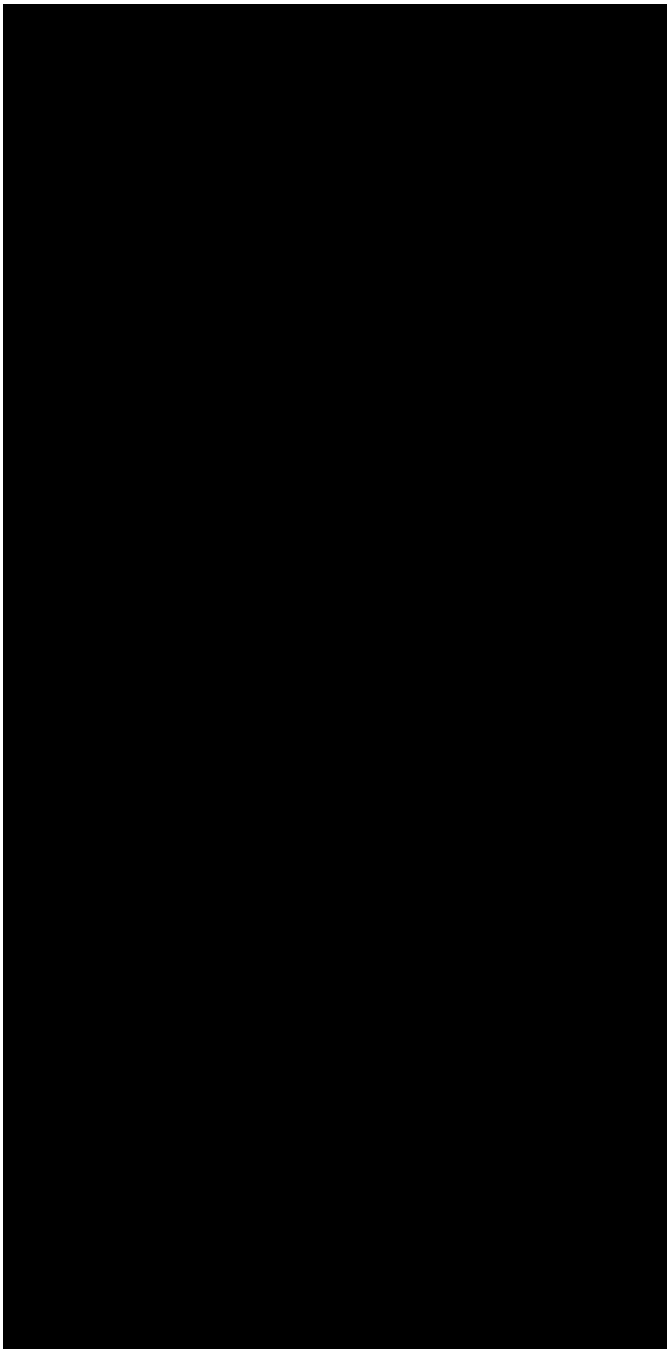

Figure 3.

Antitumor activity of PX-916. A, Female Beige nude mice were inoculated sc with 10^7 A-673 rhabdomyosarcoma cells. When tumors were 100 mm^3 on day 8 (arrow) dosing was begun with (○) vehicle alone; or (▲) **PX-916** 30 mg/kg ip daily for 5 doses. Values are the mean of 6 mice per group and bars are SE. **B**, Female *scid* mice were inoculated sc with 10^7 SHP-77 human small cell lung cancer cells. When tumors were 150 mm^3 on day 17 (arrow) dosing was begun with (○) vehicle alone; (■) PX-916 10 mg/kg iv daily for 8 doses; or (▲) PX-916 25 mg/kg iv daily for 5 doses. Values are the mean of 8 mice per group and bars are SE. **C**, Female *scid* mice implanted 1 day previously with a 17-β-estradiol 90 day slow release pellet were inoculated s.c. with 10^7 MCF-7 human breast cancer cells. When tumors were 175 mm^3 on

day 8 (arrow) dosing was begun with (○) vehicle alone; (◆) PX-916 22.5 mg/kg iv daily for 5 doses; (▲) PX-916 27.5 mg/kg iv every other day for 5 doses; and (■) PX-916 22.5 mg/kg po daily for 5 doses. Values are the mean of 8 mice per group and bars are SE.

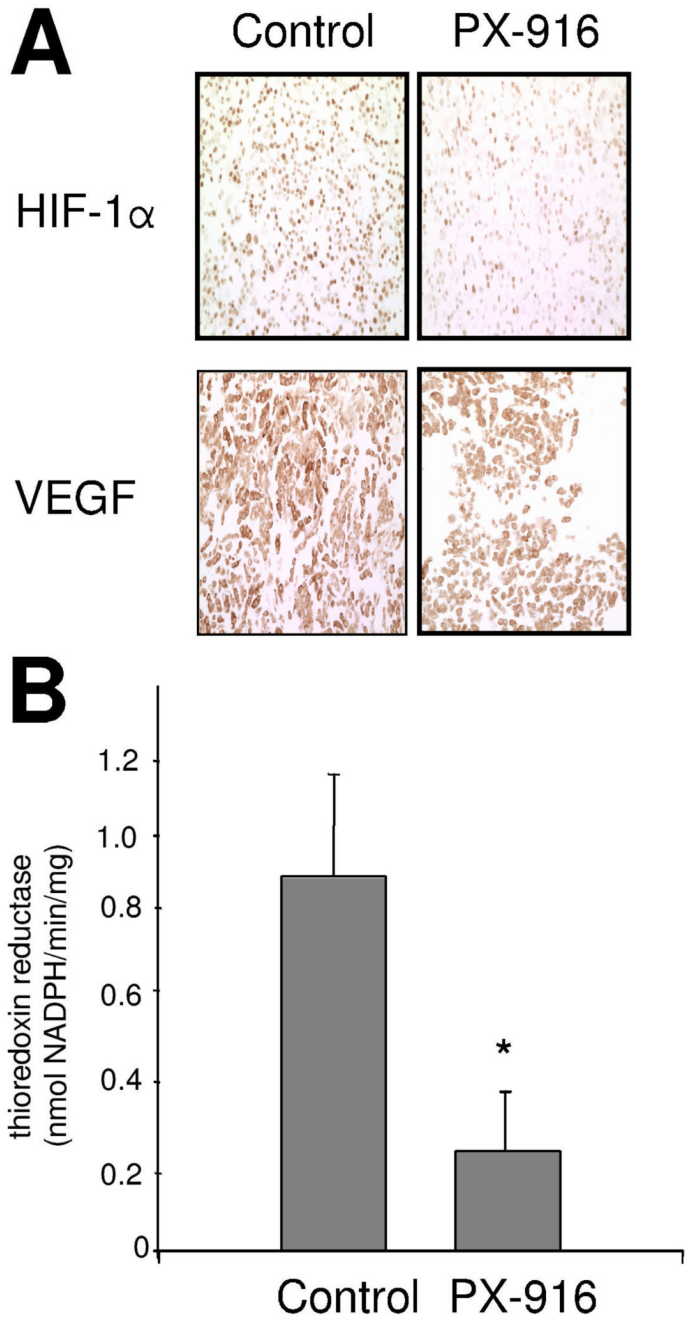

Figure 4.

Inhibition of tumor HIF1*α*, VEGF and TrxR by repeated administration of PX-916.

Female *scid* mice implanted 1 day previously with a 17-β-estradiol 90 day slow release pellet were inoculated sc with 10^7 MCF-7 human breast cancer cells. When the tumors were 300 mm^3, vehicle or PX-916 of 25 mg/kg was administered iv daily for 5 doses. Twenty four hr later the tumors were removed and **A**, stained by immunohistochemistry for HIF1α and VEGF; or **B**, assayed for TrxR activity. Values are the mean of 4 mice and bars are S.E. * p = < 0.05.

Table 1

Inhibition of TrxR-1 and cell growth by palmarumycin CP1 analogs

Compound	Inhibition of human TrxR-1 IC_{50} (μM)	Inhibition of MCF-7 breast cancer cell growth IC_{50} (μM)
palmarumycin CP1	0.35	1.0
PX-911	3.2	9.2
PX-916	0.28	3.1
PX-960	0.27	4.1

Table 2

Toxicity of PX-916 in scid mice PX-916 was administered to female scid mice at 25 mg/kg iv daily for 5 days, and mice were killed 24 hr after the last dose. There were 4 mice per group and values are the mean ± SE. * = $p < 0.05$

Schedule	Dose mg/kg	Δbody wt g	ALT U/l	AST U/l	WB K/µl	NE K/µl	LY K/µl	MO K/µl	RBC M/µl	Hb g/dl	PLT K/µl
Control		-1.2 ± 1.5	30.3 ± 10	154.1 ± 62.4	3.0 ± 0.6	2.5 ± 0.5	0.4 ± 0.1	0.11 ± 0.05	9.4 ± 0.5	15.5 ± 0.7	773 ± 67
QD × 5 iv	25	-0.6 ± 0.3	39.8 ± 12.8	166.9 ± 29.8	1.3 ± 0.6*	0.9 ± 0.3*	0.3 ± 0.1	005 ± 0.01	8.9 ± 0.4	14.9 ± 0.4	436 ± 142*