Multifaceted cytoprotection by synthetic polyacetylenes inspired by the ginseng-derived natural product, panaxytriol

Ting-Chao Chou[a], Huajin Dong[a], Xiuguo Zhang[a], Xiaoguang Lei[b], John Hartung[b], Yandong Zhang[b], Jun Hee Lee[b], Rebecca M. Wilson[c], and Samuel J. Danishefsky[b,c,1]

[a]Preclinical Pharmacology Core Facility and [c]Bio-Organic Laboratory, Molecular Pharmacology and Chemistry Program, Memorial Sloan–Kettering Cancer Center, New York, NY 10065; and [b]Department of Chemistry, Columbia University, New York, NY 10027

Contributed by Samuel J. Danishefsky, July 13, 2011 (sent for review June 3, 2011)

We describe herein the discovery of a series of panaxytriol (PXT)-derived polyacetylene small molecules with promising cytoprotective activity. In mouse xenograft models, we have demonstrated the capacity of our synthetic analogs to mitigate a range of cancer therapeutic agent-induced toxicities, including body weight loss, lethality, neurotoxicity, and hematotoxicity. Our PXT analogs have also been found to reduce radiation-induced body weight loss and lethality in mouse models. Moreover, several PXT analogs appear to exhibit moderate in vivo antiinflammatory activity as well as in vitro immunoenhancing capabilities. These compounds appear to derive their activity through induction of cancer preventive phase 2 enzymes. The studies described herein suggest that coadministration of a PXT-derived agent with cancer chemotherapeutics or radiation therapy may serve to mitigate a range of therapy-associated toxicities.

chemoprotective | neutraceutical

The progression from structurally novel lead agent to viable drug candidate is a high-risk enterprise of formidable proportions. As the state of contemporary Pharma research can surely attest, the path from discovery to the clinic and beyond is fraught with setbacks. Development candidates exhibiting considerable promise in early laboratory screens fail, for reasons of lack of efficiency or unacceptable toxicity, when introduced to complex living systems. Although current Pharma drug discovery models focus on the identification of lead agents through high-throughput screening of multicompound libraries, the modest success rates that have been realized thus far, in terms of producing approved new drugs, may well warrant reappraisal of this approach (1–3).

By contrast, Nature has historically proven to be a rich source of structurally diverse and biologically active compounds leading to valuable drugs. Thus, even despite the practice of Pharma to deprioritize natural products-based research in recent years, a remarkable proportion of the new drug agents approved by the US Food and Drug Administration continue to be those derived from natural sources, either directly or through modification of a parent natural product (4–6). It is difficult to detail the reasons for the disproportionate success rates achieved by building on natural product-based scaffolds. However, it can be safely surmised that, as a precondition of its existence, the natural product must realize compatibility with a living system. Furthermore, the small-molecule natural product is presumably biosynthesized by its host to perform a specific role, which could well entail binding to the active site of a protein, oligosaccharide, or oligonucleotide. This type of binding characteristic is likely to be required for activity as a drug.

In practice, valuable lead compounds have been gleaned from all reaches of the vast natural product estate. Another productive source of discovery has been realized from traditional medicine. Throughout history, certain plants have been held in particular esteem by their native cultures because of their purported medicinal properties. Scientists have long sought to identify the active components of these medicinal plants in the hopes of using them as lead agents for further drug development. Toward this end, a significant amount of research has been devoted to the investigation of the therapeutic properties of the ginseng plant. For thousands of years, ginseng root has been a staple of the medicinal traditions of its native regions of Asia, and it is still widely used today for the promotion of general well-being and the treatment of a range of specific ailments. There are at least 12 species of botanical ginseng, belonging the Araliaceae family. Among the most prominent is *Panax ginseng* C. A. Meyer, also referred to as Chinese or Korean red ginseng.

Among the many scientific studies on the ginseng root and its individual components, there are indications of its ability to promote general well-being in animal models through prolongation of life span (7), mitigation of stress response (8), and enhancement of serum HDL cholesterol levels (9). The ginseng saponins have been reported to enhance production of IgM antibodies in mice (10), to stimulate serum protein biosynthesis (11), and to promote the expression of GM-CSF from human endothelial cells and monocytes (12). Moreover, ginseng extracts have been shown to exhibit a range of chemoprotective effects that could be of particular value. Notably, *P. ginseng* reduces the rate of adriamycin-induced heart failure in rat models (13) and red ginseng extract has been found to mitigate the side effects of nausea and vomiting associated with cisplatin treatment (14).

Our laboratory has long been devoted to the total synthesis and evaluation of biologically active natural products of potential therapeutic import (15). Toward this end, we took note of the disclosure of a series of biologically active polyacetylene natural products isolated from the *P. ginseng* root. We were originally drawn, in particular, to (3R, 9R, 10S)-panaxytriol (PXT) (16) on the basis of reports of its in vitro cytotoxic activity against a range of human tumor cells, including breast carcinoma (breast M25-SF) (17) and gastric carcinoma (MK-1) (18). In one in vivo study, PXT was reported to suppress the growth of B16 melanoma cells in mouse models (19). On the basis of these reports, we set out to synthesize PXT, anticipating that a successful program directed to this natural product could be adapted to reaching analogs worthy of further investigation.

As described in detail below, our investigations served to confirm the modest cytotoxic activity of both PXT and its synthetic analogs. However, as will be seen (*vide infra*), of particular interest to our laboratory was the finding that even at subtherapeutic dosages, the PXT-based compounds are markedly able to alleviate the toxic and neuropathy-inducing side effects

Author contributions: T.-C.C. and S.J.D. designed research; H.D. and X.Z. performed research; X.L., J.H., Y.Z., and J.H.L. contributed new reagents/analytic tools; T.-C.C., H.D., and X.Z. analyzed data; and T.-C.C., J.H., R.M.W., and S.J.D. wrote the paper.

The authors declare no conflict of interest.

[1]To whom correspondence should be addressed. E-mail: s-danishefsky@ski.mskcc.org.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1111332108/-/DCSupplemental.

associated with chemotherapeutic and radiation-based anticancer treatments.

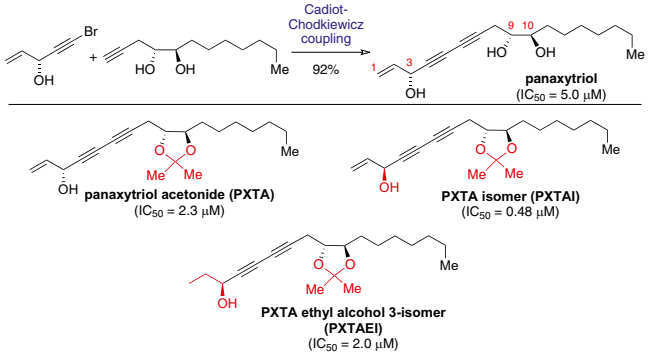
Results and Discussion

Total Synthesis and in Vitro Cytotoxicity Evaluation of PXT and Synthetic Analogs. As outlined in Scheme 1, a rapid and highly efficient total synthesis of optically pure PXT was accomplished (20). Featuring a Cadiot–Chodkiewicz coupling between an alkynyl bromide and an alkyne diol, our total synthesis proceeds in 42% overall yield from commercially available starting materials, providing ready access to multigram quantities of PXT for extensive biological investigations. With synthetic material in hand, we confirmed the in vitro cytotoxic activity reported for the natural product (Table S1, entry 1). We next adapted our synthetic route (21) to allow for the preparation of a range of PXT analogs bearing structural perturbations in the C_1–C_3 and C_9–C_{10} regions (Table S1, entries 2–11).

In an important early finding, we observed that significant enhancement in cytotoxic activity can be achieved through the engagement of the C_9–C_{10} diol as an acetonide. As shown in Scheme 1, in in vitro studies against the human lymphoblastic leukemia cell line CCRF-CEM, panaxytriol acetonide (PXTA, IC_{50} = 2.3 μM) is more cytotoxic than the parent compound, PXT (IC_{50} = 5.0 μM).

A further increase in cytotoxic activity was attained through the synthesis of the C_3 epimer of PXTA. Thus, the PXTA isomer (termed PXTAI) inhibits CCRF-CEM cells with an IC_{50} of 0.48 μM. Finally, PXTAEI, a derivative of PXTAI that contains an ethyl moiety in place of the vinyl group at C_1–C_2, was also found to exhibit high levels of cytotoxicity against CCRF-CEM cell lines (2.0 μM). It is of note that PXT and its derivatives largely retain their activity against Taxol- and vinblastine-resistant CCRF-CEM sublines (Table S1). Moreover, the PXT analogs found to be most reactive against CCRF-CEM were also observed to inhibit, with modest potency, a range of human solid tumor cell lines (Table S2).

Chemoprotective Activity of PXT Analogs. In the light of numerous reports on the capacity of ginseng and its components to promote general well-being through various biological pathways, we sought to examine whether our own synthetic PXT analogs could similarly exhibit beneficial chemoprotective properties. It has been postulated that *P. ginseng* and its components play a role in the prevention of cancer (22), at least in part through the induction of phase 2 enzymes (23). Such enzymes are understood to play a role in chemopreventive pathways by protecting cells against oxidants and other forms of electrophilic attack. The signaling pathway that regulates the phase 2 genes is dependent on repression of the transcription factor Nrf2 by Keap1. The repressor, Keap1, comes "equipped" with reactive cysteine residue sensors; on interaction with appropriate inducers, these sensors render Keap1 unable to repress Nrf2. Thus released, the Nrf2 transcription factor then binds to the antioxidant response elements of the phase 2 genes, thereby activating the transcription pathway (24). Phase 2 enzyme induction assays are often based on the measurement of Nrf2-dependent enzymes, such as nicotinamide quinone oxireductase 1 (NQ01) (25, 26). Along these lines, we recently demonstrated, through an NQ01-based assay, that PXT does indeed induce phase 2 enzymes (27). More recently, we have established the phase 2 induction capacities of a number of our synthetic PXT analogs. Because most Nrf2 inducers are capable electrophiles, it is of interest that the relatively nonelectrophilic PXTs appear to operate through the Nrf2 pathway. It may perhaps be speculated that the carbinol functionality undergoes in vivo oxidation, rendering a competent Michael acceptor. Moreover, an ene-diyne may itself have electrophilic capabilities.



Scheme 1. Synthesis and in vitro cytotoxicities of PXT and synthetic analogs. The IC_{50} (μM) against human lymphoblastic leukemia sublines (CCRF-CEM) is shown.

Of particular interest to our laboratory was the prospect that the PXT compounds might be used to alleviate the toxic side effects that typically attend exposure to cytotoxic cancer chemotherapeutic agents. Thus, although cytotoxic agents remain the most efficacious class of compounds available for the treatment of cancer, the utility of such agents can be severely compromised by the onset of significant accompanying toxic side effects, including nausea-induced weight loss, peripheral neuropathy, and hematotoxicity. In recent decades, a number of agents have been developed that alleviate impairments in the aftermath of cancer chemotherapy. For instance, antiemetics are used to reduce vomiting and nausea, Epogen and Neupogen are prescribed for the treatment of chemotherapy-induced anemia, and steroids and antihistamines are used for the suppression of allergic reactions (a common side effect of Cremophor treatment).

By contrast, we envisioned a setting wherein subtherapeutic doses of our PXT compounds would be coadministered with the cytotoxic agent, with the goal of attenuating the toxic side effects of the anticancer drug, thereby enhancing its therapeutic index. In principle, the PXT analog would be administered at subtherapeutic doses, such that the relationship of the PXT compound to the cytotoxic drug would be defined as "augmentation" as opposed to "synergism" (28, 29). In fact, as described below, in in vivo studies, several PXT analogs were found to mitigate a range of toxic side effects associated with high-profile cytotoxic agents.

Attenuation of body weight loss and improved recovery of body weight following cotreatment of Taxol and iso-fludelone with PXT analogs. Nausea-induced body weight loss is a common and, at times, treatment-limiting side effect of Taxol-based chemotherapy. We sought to evaluate whether coadministration with our synthetic PXT analog, PXTAEI, might serve to attenuate the severity of body weight loss in mouse xenograft settings. Thus, nude mice bearing a human mammary carcinoma (MX-1) xenograft were treated with 25 mg/kg Taxol (i.v. injection), either alone or with subtherapeutic levels (30 mg/kg) of PXTAEI (i.v.) according to the schedule shown (Fig. 1). Although both sets of mice achieved complete remission by day (D) 33, significant reductions in body weight loss ($P < 0.05$) were observed in the PXTAEI-cotreated subjects (Fig. 1). As shown, the greatest effect was observed in the recovery phase and during the second round of Taxol treatments. Of further potential clinical import were the results of an analogous study, shown in Fig. S1, involving Taxol-treated nude mice bearing an MX-1 xenograft, which revealed that Taxol-induced body weight losses could also be attenuated through oral treatment with 50 mg/kg PXTAEI (administered every 3 d, as shown). In both of these studies, it was confirmed that at the dosage levels examined, PXTAEI on its own does not have an impact on the rate of tumor growth.

In the context of our research program directed toward the discovery of improved anticancer agents, we have developed a series of synthetic microtubule-targeting epothilone analogs that we con-

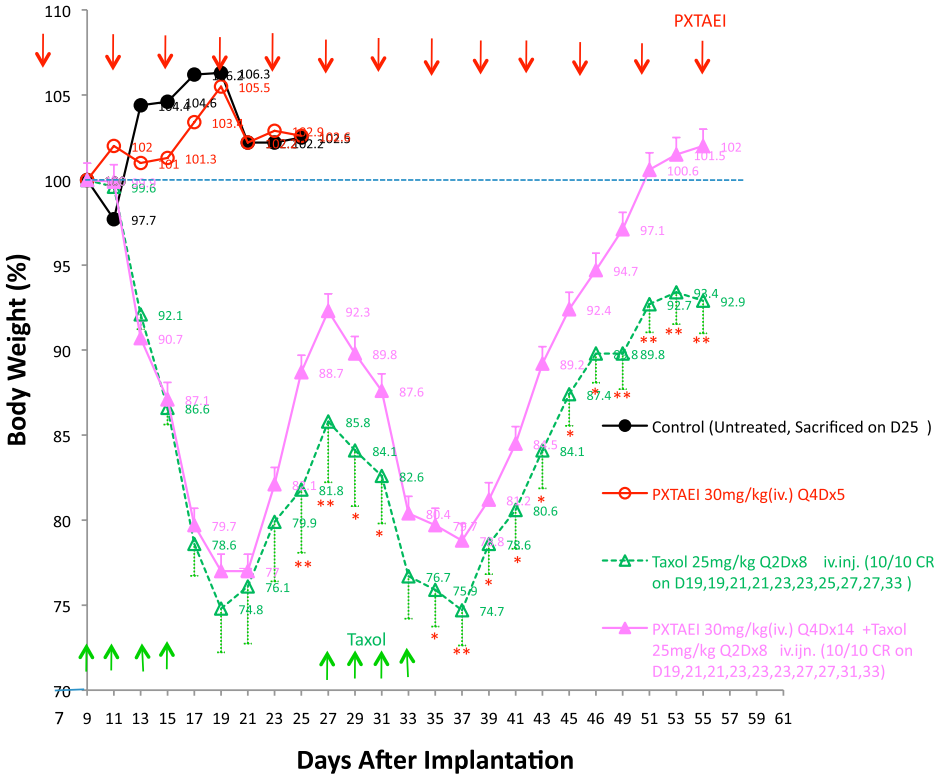


Fig. 1. Alleviation of Taxol-induced body weight losses by PXTAEI in nude mice bearing a human mammary carcinoma MX-1 xenograft. The doses of Taxol and PXTAEI and their schedules of i.v. injection are shown (n = 10). Taxol was used as a Cremophor formulation (60 mg of Taxol in 2.5 mL of ethanol and 2.5 mL of Cremophor). The saline-diluted Taxol solution was used within 2 h of its preparation; 0.2 mL of the solution was introduced through i.v. injection via the tail vein. The PXTAEI stock solution was prepared in 10 mg/mL DMSO. The solution was diluted with saline containing 0.5% Tween 80 for i.v. injection into mice. This formulation caused no detectible toxicity in mice. The Taxol-induced body weight losses (green △) were reduced by coadministration of PXTAEI (pink ▲) with a P value <0.05 (**) or <0.1 (*) as shown. The numbers indicate the average body weight of 10 mice (mean ± SE). Both treated groups achieved complete remission of xenograft tumors by D33. The PXTAEI and control groups were killed on D25 because of excessive tumor burden. All P values were determined by the Student t test for paired comparisons (Excel; Microsoft Corp.).

sider to possess truly remarkable therapeutic potential at the clinical level (30–32). One such candidate, termed iso-fludelone (iso-flu), is currently being readied for phase I clinical trials. As outlined in Fig. 2, sets of nude mice bearing a human colon carcinoma (HCT-116) xenograft were treated with iso-flu (25 mg/kg administered via i.v. infusion every 12 d from D9 to D45), either on its own or with the synthetic PXT analog, PXTAI (10 mg/kg administered as an i.v. injection according to the schedule shown). The body weights of the mice were monitored. Once more, cotreatment with even low levels of PXTAI resulted in alleviation of iso-flu–induced body weight losses over the four rounds of treatment.

Mitigation of Taxol-induced neurotoxicity by PXTAEI. A limitation of Taxol-based cancer chemotherapy has been the high incidence of peripheral neuropathy reported as a significant side effect of treatment. Along these lines, we took note of a number of promising studies suggesting a neuroprotective role for various ginseng components. Specifically, ginsenoside Rd has been shown to exhibit neuroprotective effects against oxygen-glucose deprivation in cultured hippocampal neurons (33). It has been postulated that these effects may be attributed to the antioxidative activity of the compound, which serves to reduce intracellular reactive oxygen species, increase glutathione content, and enhance the antioxidant activities of a range of enzymes (catalase, superoxide dismutase, and glutathione peroxidase). Given the reported neuroprotective activity of these ginseng extracts, we sought to examine whether our PXT-based compounds might possess valuable neuroprotective activity, which would allow for the alleviation of Taxol-induced neurotoxicity.

Twenty nude mice bearing MX-1 xenografts were divided into two groups. The control group (n = 10) received repeated high-dose i.v. injections of Taxol (25 mg/kg) on D9, D11, D13, and D15 following tumor implantation. The treated group (n = 10) received the same Taxol treatment but were coadministered PXTAEI [30 mg/kg administered as an i.v. injection every 4 d throughout the course of the study, including pretreatment on D(−4)]). In the first round (cycle 1), peripheral neuropathy was scored from D9 to D27 (Table S3). The following rating system was used for the evaluation of neuropathy: −, no detectable neurotoxicity; +, slight impairment of balance; ++, paralysis of two hind legs; and +++, paralysis of all four legs, which would lead to death without intervention with food and water. On D27, the control group, receiving treatment with Taxol alone, carried an aggregate neuropathy score of 53, whereas the group receiving cotreatment with PXTAEI received a score of only 27. Thus, in cycle 1, a significant reduction of peripheral neuropathy was achieved through cotreatment with PXTAEI. Moreover, the observed neuroprotective effects of PXTAEI were maintained throughout the course of a second round of Taxol treatment. Thus, on D29, when the mice had mainly recovered from the neurotoxic effects induced by the first round of treatment, a second cycle analogous to the first was commenced. As shown in Table S4, this second treatment cycle led to elevated levels of neuropathy in both sets of mice, presumably attributable to carryover toxicity accumulated from the first round. Nonetheless, coadministration with PXTAEI did measurably serve to attenuate the severity of the peripheral neuropathy. The aggregate neurotoxicity score of the control group in cycle 2 was calculated to be 181, whereas the

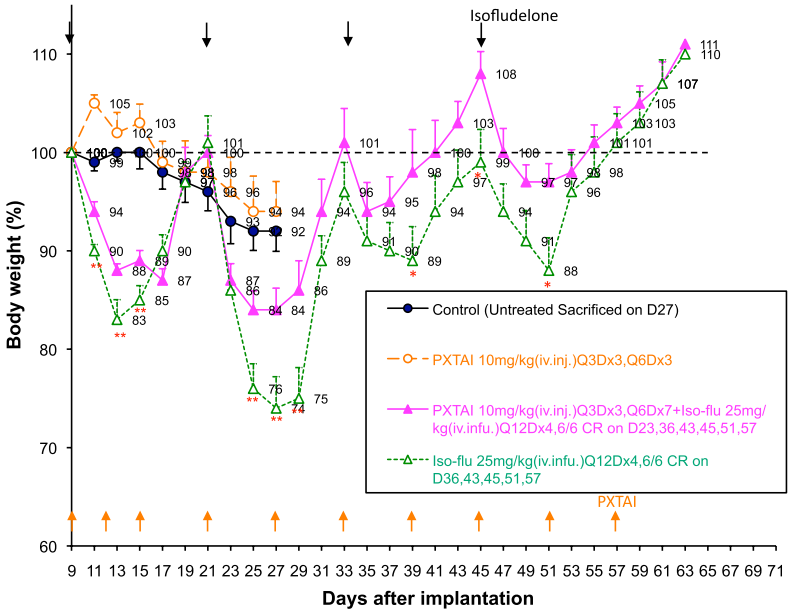


Fig. 2. Alleviation of iso-flu–induced body weight losses by PXTAI in nude mice bearing human colon carcinoma HCT-116 xenografts. The doses of iso-flu (i.v. infusion) and PXTAI (i.v. injection) and their schedules are shown ($n = 6$). The iso-flu–induced body weight losses were reduced, and recovery was improved by coadministration of PXTAI with $P < 0.05$ (**) and $P < 0.1$ (*) as shown. The numbers indicate the average body weight of six mice (mean ± SE). The iso-flu–treated groups (green △) achieved complete remission of xenograft tumors between D36 and D57, and the cotreated group (pink ▲) achieved complete remission between D23 and D57. The PXTAI control groups (orange ○) were killed on D27 because of excessive tumor burden.

PXTAEI cotreated group scored 145. Over the course of both cycles, the PXTAEI-treated group achieved an overall reduction in peripheral neuropathy of ∼26%.

Mitigation of 5-fluorouracil–induced toxicity and lethality through cotreatment with PXT analogs. Among the common side effects of treatment with the anticancer drug 5-fluorouracil (5-FU) are low RBC and WBC counts, which can lead to increased risk for infection as well as anemia. We set out to determine whether the hematotoxicity associated with 5-FU might be mitigated through exposure to the PXT analog PXTAEI. Normal CD-1 mice were treated with either 5-FU alone (50 mg/kg administered i.v. QD for 4 d) or 5-FU with PXTAEI (30 mg/kg administered i.v. on D(−2), D1, D4, and D7), and blood samples were taken on D8. As expected, following treatment with 5-FU alone, the levels of WBCs, lymphocytes, RBCs, platelets, and hemoglobin were all found to be markedly diminished (Fig. 3). Interestingly, cotreatment with PXTAEI led to alleviation of hematotoxicity, as demonstrated by enhanced blood cell counts, in comparison with the 5-FU–treated group.

In contrast to Taxol and the epothilone-based cytotoxic agents, cotreatment with 5-FU and PXT analogs did not result in significant attenuation of body weight losses. However, coadministration with PXTAI did lead to a marked reduction in 5-FU–induced lethality. Thus, nude mice bearing MX-1 xenograft tumors were treated with 5-FU (50 mg/kg administered as an i.v. injection QD for 5 d) either alone or coadministered with PXTAI (10 mg/kg administered via i.v. injection every 4 d). In both sets of mice, significant body weight losses were observed (Fig. S2). However, of the eight 5-FU–treated mice, five died during the course of treatment (2 on D18 and 1 each on D19–D21), although only two of the eight mice cotreated with PXTAI died (1 each on D19 and D20). These findings raise the intriguing possibility that PXTAI is perhaps operating through an as yet undefined chemoprotective pathway.

Reduction of radiation-induced toxicity by PXTAEI. Several ginseng-derived natural products, including the polysaccharide ginsan (34) and several ginsenosides (35), have been reported to exhibit radioprotective effects in irradiated mice. Having established the chemoprotective abilities of our PXT compounds in cotreatment with cytotoxic agents, we speculated that these agents might demonstrate the capacity to alleviate similar side effects associated with radiation therapy, namely, body weight loss, lethality, and hematotoxicity.

Thus, in a control group, CD-1 mice irradiated with 7 Gy suffered significant body weight losses, peaking at 12.3% on D15 following irradiation. A separate group of mice treated with 7 Gy were coadministered PXTAEI (30 mg/kg administered as an i.v. injection every 4 d, as shown). As outlined in Fig. 4, significant alleviation of body weight loss was observed on D3–D8 ($P < 0.05$). Over the course of this study, comparison of the body

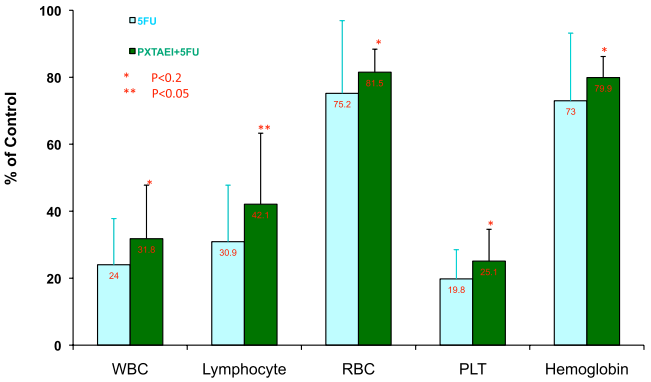


Fig. 3. Reduction of 5-FU–induced hematotoxicity by PXTAEI in normal CD-1 mice. Heavy and light bars indicate with or without PXTAEI treatment, respectively. The PXTAEI dose was 30 mg/kg administered i.v. on D(−2), D1, D4, and D7, and the 5-FU dose was 50 mg/kg administered i.v. QD for 4 d. All measurements were taken on D8 ($n = 17$). Blood was collected in 100 μL of potassium EDTA in a round-bottomed inner tube (Microvette 100). Blood cell counts in samples (2.0 μL) were determined by a flow Multispecies Hematology System (Hemavet HV950FS). The lymphocyte reduction was significantly alleviated by PXTAEI ($P < 0.05$), whereas other parameters showed a trend toward significance ($P < 0.2$). PLT, platelet.

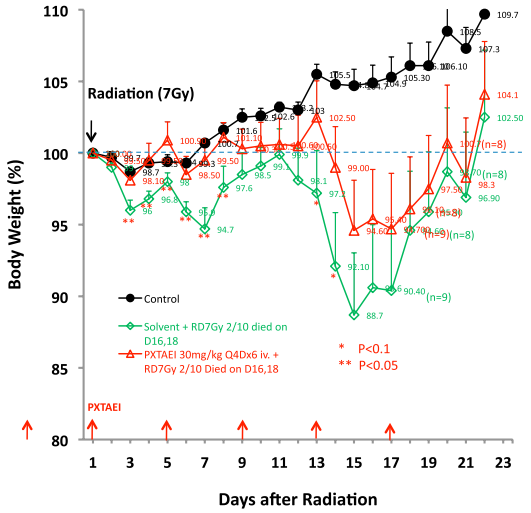


Fig. 4. Reduction of radiation-induced body weight loss by PXTAEI in CD-1 mice. Female CD-1 mice, 10 per group, were treated with 0 Gy of X-radiation (●); 7 Gy of X-radiation using an X-RAD320 irradiator with an IACUC-approved protocol and radiation user's license (green ◇); and 7 GY of X-radiation plus 30 mg/kg of PXTAEI administered i.v. on D(−4), D1, D5, D9, D13, and D17 (orange △). Two mice in the radiation-treated group and the cotreated group died on D16 and D18. The cotreated group had significantly ($P < 0.05$) less body weight losses than the radiation-treated group.

weight losses of PXTAEI-treated and untreated mice suggests a trend toward significance.

In a separate study, the X-irradiation dose was increased to 8 Gy, leading to the eventual death of all the CD-1 mice examined, regardless of whether they were cotreated with PXTAEI or not (Fig. 5). However, the mean postirradiation survival times increased from 12.4 ± 1.5 d for the untreated mice to 14.0 ± 1.3 d for mice cotreated with PXTAEI [30 mg/kg administered i.v. on D(−2), D1, D5, and D9]. Thus, cotreatment with PXTAEI led to a statistically meaningful ($P < 0.03$) increase in life span following high levels of irradiation.

Finally, we examined the ability of PXTAEI to alleviate X-irradiation–induced hematotoxicity. Thus, three sets of CD-1 mice were irradiated with 8 Gy. The control group received no further treatment, a second group was treated with the free radical scavenger and well-established radioprotective agent Amifostine (100 mg/kg), and the third group received treatment with PXTAEI (30 mg/kg). Both Amifostine and PXTAEI were administered by i.v. injection on D(−2), D0, D4, and D8. On D10, blood samples were collected from each group and blood cell counts were determined through hematocytometric analysis (Fig. 6). As expected, the untreated control group exhibited significantly diminished WBC, lymphocyte, RBC, hemoglobin, and platelet levels. Notably, Amifostine and PXTAEI were both able to attenuate the hematotoxic effects of irradiation, with generally comparable levels of efficacy.

Conclusion

In summary, we have described herein the discovery and evaluation of a promising class of biologically active compounds based on the ginseng-derived natural product PXT. Of particular interest to our laboratory is the ability of this unique series of polyacetylene agents to alleviate a range of toxic side effects associated with standard cancer treatments. In particular, in mouse models, we observed mitigation of body weight loss, lethality, peripheral neuropathy, and hematotoxicity, all of which are side effects that may be associated with treatment with standard chemotherapeutic agents, including Taxol, vincristine, epothilones, cyclophosphamide, and 5-FU, as well as radiation

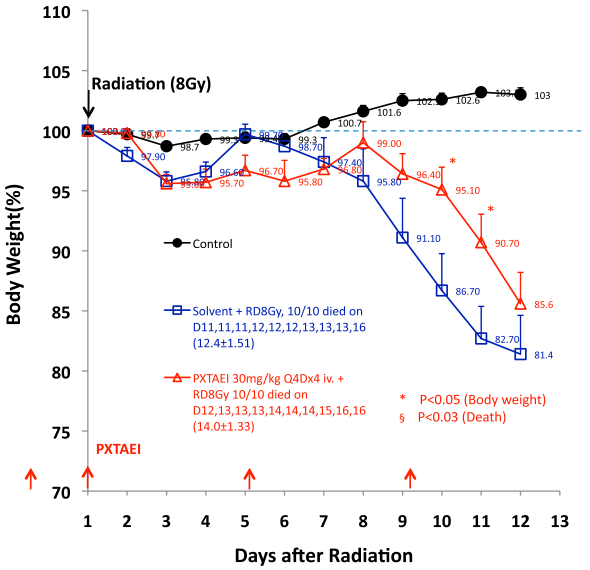


Fig. 5. Extension of life span for lethal dose radiation-treated CD-1 mice by PXTAEI. Female CD-1 mice, 10 per group, were treated with (●) 0 Gy of X-radiation; 8 Gy of X-radiation using an X-RAD320 irradiator with an IACUC-approved protocol and radiation user's license (blue □); or 8 Gy of X-radiation plus 30 mg/kg of PXTAEI administered i.v. on D(−2), D1, D5, and D9 (orange △). All 10 mice died in both the radiation-only group and the PXTAEI-cotreated group. However, the cotreated group had longer survival times (14.0 ± 1.33 d) than the radiation-only group (12.4 ± 1.51 d) ($P < 0.03$). In addition, the body weight loss of the cotreated group was significantly less than that of the radiation-only group ($P < 0.05$).

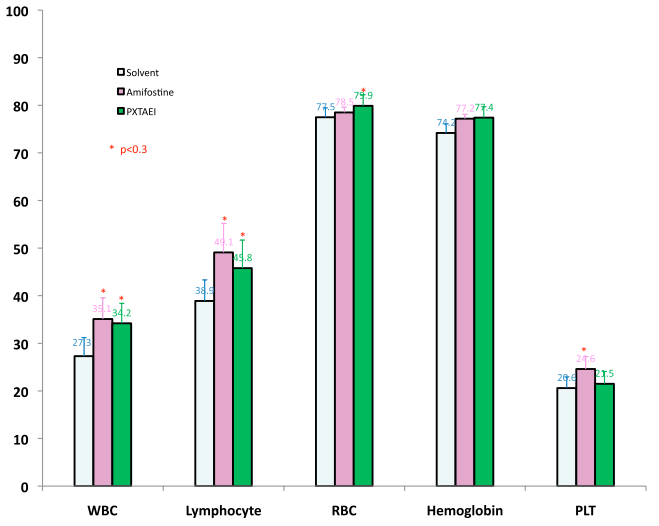


Fig. 6. Effects of PXTAEI on X-irradiation–induced hematological suppression in CD-1 mice. CD-1 mice underwent X-irradiation with 8 Gy, and blood samples were collected on D10. PXTAEI at a dose of 30 mg/kg was administered i.v. on D(−2), D0, D4, and D8 (green bars). The well-established radiation protection agent, Amifostine (Sigma–Aldrich) was administered i.v. at a dose of 100 mg/kg on D(−2), D0, D4, and D8 to serve as the positive control (pink bars). The solvent-treated group (open bars) served as the negative control. Each group comprised 20 CD-1 mice. Blood cell counts in samples (2.0 μL) were determined by a flow Multispecies Hematology System (Hemavet HV950FS). Although all parameters tested showed consistent alleviation of X-irradiation–induced hematological suppression on D10, the P value calculated for $n = 20$ did not reach the level of significance.

therapy. The indications for these treatments cover a broad spectrum of cancer types. Through coadministration of a subtherapeutic dose of PXT-based drug agent, it is conceivably possible to increase the therapeutic indices of these commonly used cytotoxic agents. Based on these findings, we are optimistic that a PXT-derived lead agent may provide a clinically viable means by which to enhance chemotherapeutic and radiation-based treatments. We do not claim to have yet developed the optimal candidate structure. Indeed, investigations along these lines are ongoing in our laboratories. However, we believe that this "cytoprotection"-based approach may well represent a promising paradigm in the development of cancer treatment strategies. Accordingly, we have expanded our structure-activity relationship (SAR) study, and the results will be described in due course. Studies addressed to the possible mode of action of these compounds are in progress. Already, however, the results shown here serve to help validate the idea that small molecule natural products provide a menu of possibilities for drug discovery (15). Above, we have demonstrated the possibility of combining chemistry and biology to advance from anecdotally based neutraceuticals to the more challenging domain of pharmaceuticals.

Materials and Methods

The PXTs and iso-flu were synthesized in-house according to previously reported methods (17, 24). In vitro cell growth inhibition was measured by cell counting kit 8 (CCK-8) assay (Dojindo Molecular Technologies Inc.) following a 72-h incubation using a Powerwave XS microplate spectrophotometer (BioTek Instruments, Inc.). IC_{50} values were determined in duplicate or triplicate from the dose–effect relationship at six or seven concentrations of each drug using CompuSyn software (ComboSyn, Inc.) based on the median-effect principle and plot and serial deletion analysis.

In in vivo studies with xenograft-bearing nude mice, Taxol was used as a Cremophor formulation (60 mg of Taxol in 2.5 mL of ethanol and 2.5 mL of Cremophor). The saline-diluted Taxol solution was used within 2 h of its preparation; 0.2 mL of solution was introduced through i.v. injection via the tail vein. The PXT stock solutions were prepared in 10 mg/mL DMSO. The solutions were diluted with saline containing 0.5% Tween 80 for i.v. injection into mice. Iso-flu was introduced in the tail vein through a 6-h i.v. infusion. For the 5-FU– and radiation-induced hematotoxicity studies, blood was collected in 100 μL of potassium EDTA in a round-bottomed inner tube (Microvette 100; Sarstedt). Blood cell counts in the sample (2.0 μL) were determined by a flow Multispecies Hematology System (Hemavet HV950FS; Drew Scientific, Inc.). The radiation studies were performed with an X-RAD320 irradiator (Precision X-Ray) with an Institutional Animal Care and Use Committee (IACUC)-approved protocol and radiation user's license. All animal studies were conducted in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Animals, and the protocol was approved by the Memorial Sloan–Kettering Cancer Center's IACUC.

ACKNOWLEDGMENTS. We thank Grace Kang for assistance in the preparation of this manuscript and Quen-Hui Tan and Luan-Ing Chen for technical assistance. This work is supported, in part, by National Institutes of Health Grant HL25848 (to S.J.D.) and by the Sloan-Kettering Institute General Fund (to T.-C.C.) from the Memorial Sloan–Kettering Cancer Center.

1. Strohl WR (2000) The role of natural products in a modern drug discovery program. *Drug Discov Today* 5:39–41.
2. Cordell GA (2002) Natural products in drug discovery—Creating a new vision. *Phytochem Rev* 1:261–273.
3. Rouhi AM (2003) Rediscovering natural products. *Chem Eng News* 81:77–91.
4. Newman DJ, Cragg GM, Snader KM (2000) The influence of natural products upon drug discovery. *Nat Prod Rep* 17:215–234.
5. Newman DJ, Cragg GM, Snader KM (2003) Natural products as sources of new drugs over the period 1981-2002. *J Nat Prod* 66:1022–1037.
6. Butler MS (2004) The role of natural product chemistry in drug discovery. *J Nat Prod* 67:2141–2153.
7. Bittles AH, Fulder SJ, Grant EC, Nicholls MR (1979) The effect of ginseng on lifespan and stress responses in mice. *Gerontology* 25:125–131.
8. Rai D, Bhatia G, Sen T, Palit G (2003) Anti-stress effects of Ginkgo biloba and Panax ginseng: a comparative study. *J Pharmacol Sci* 93:458–464.
9. Yamamoto M, Uemura T, Nakama S, Uemiya M, Kumagai A (1983) Serum HDL-cholesterol-increasing and fatty liver-improving actions of Panax ginseng in high cholesterol diet-fed rats with clinical effect on hyperlipidemia in man. *Am J Chin Med* 11: 96–101.
10. Mita A, Shida R, Kasai N, Shoji J (1979) Enhancement and suppression in production of IgM-antibody in mice treated with purified saponins. *Biomedicine* 31:223–227.
11. Oura H, Hiai S, Odaka Y, Yokozawa T (1975) Studies on the biochemical action of ginseng saponin. I. Purification from ginseng extract of the active component stimulating serum protein biosynthesis. *J Biochem* 77:1057–1065.
12. Chen D, Wang SL, Wang YP (2003) [Experimental study on expression of GM-CSF from human endothelial cells and monocytes induced by total saponins of panax ginseng]. *Zhongguo Zhong Xi Yi Jie He Za Zhi* 23:845–847.
13. You JS, Huang HF, Chang YL (2005) Panax ginseng reduces adriamycin-induced heart failure in rats. *Phytother Res* 19:1018–1022.
14. Kim JH, et al. (2005) Effects of Korean red ginseng extract on cisplatin-induced nausea and vomiting. *Arch Pharm Res* 28:680–684.
15. Wilson RM, Danishefsky SJ (2006) Small molecule natural products in the discovery of therapeutic agents: the synthesis connection. *J Org Chem* 71:8329–8351.
16. Kitagawa I, Yoshikawa M, Yoshihara M, Hayashi T, Taniyama T (1983) Chemical studies on crude drug procession. 1. On the constituents of Ginseng radix rubra. *Yakugaku Zasshi* 103:612–622.
17. Matsunaga H, Saita T, Nagumo F, Mori M, Katano M (1995) A possible mechanism for the cytotoxicity of a polyacetylenic alcohol, panaxytriol: inhibition of mitochondrial respiration. *Cancer Chemother Pharmacol* 35:291–296.
18. Saita T, et al. (1995) Screening of polyacetylenic alcohols in crude drugs using the ELISA for panaxytriol. *Biol Pharm Bull* 18:933–937.
19. Katano M, et al. (1990) [Cell growth inhibitory substance isolated from Panax ginseng root: panaxytriol]. *Gan To Kagaku Ryoho* 17:1045–1049.
20. Yun HD, Danishefsky SJ (2003) Straightforward synthesis of panaxytriol: an active component of Red Ginseng. *J Org Chem* 68:4519–4522.
21. Yun H, et al. (2005) Total synthesis as a resource in drug discovery: the first in vivo evaluation of panaxytriol and its derivatives. *J Org Chem* 70:10375–10380.
22. Helms S (2004) Cancer prevention and therapeutics: Panax ginseng. *Altern Med Rev* 9: 259–274.
23. Halim M, Yee DJ, Sames D (2008) Imaging induction of cytoprotective enzymes in intact human cells: coumberone, a metabolic reporter for human AKR1C enzymes reveals activation by panaxytriol, an active component of red ginseng. *J Am Chem Soc* 130:14123–14128.
24. Kensler TW, Wakabayashi N, Biswal S (2007) Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. *Annu Rev Pharmacol Toxicol* 47:89–116.
25. Talalay P, et al. (2007) Sulforaphane mobilizes cellular defenses that protect skin against damage by UV radiation. *Proc Natl Acad Sci USA* 104:17500–17505.
26. Liu H, Dinkova-Kostova AT, Talalay P (2008) Coordinate regulation of enzyme markers for inflammation and for protection against oxidants and electrophiles. *Proc Natl Acad Sci USA* 105:15926–15931.
27. Ng F, et al. (2008) (3R, 9R, 10R)-Panaxytriol: A molecular-based nutraceutical with possible application to cancer prevention and treatment. *Tetrahedron Lett* 49: 7178–7179.
28. Chou TC, Talalay P (1984) Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv Enzyme Regul* 22: 27–55.
29. Chou TC (2006) Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. *Pharmacol Rev* 58: 621–681.
30. Rivkin A, Chou TC, Danishefsky SJ (2005) On the remarkable antitumor properties of fludelone: how we got there. *Angew Chem Int Ed Engl* 44:2838–2850.
31. Chou TC, Dong H, Zhang X, Tong WP, Danishefsky SJ (2005) Therapeutic cure against human tumor xenografts in nude mice by a microtubule stabilization agent, fludelone, via parenteral or oral route. *Cancer Res* 65:9445–9454.
32. Chou TC, et al. (2008) Therapeutic effect against human xenograft tumors in nude mice by the third generation microtubule stabilizing epothilones. *Proc Natl Acad Sci USA* 105:13157–13162.
33. Ye R, et al. (2009) Neuroprotective effects of ginsenoside Rd against oxygen-glucose deprivation in cultured hippocampal neurons. *Neurosci Res* 64:306–310.
34. Song JY, et al. (2003) Radioprotective effects of ginsan, an immunomodulator. *Radiat Res* 159:768–774.
35. Lee JH, et al. (2006) Effects of ginsenosides and their metabolites on voltage-dependent Ca(2+) channel subtypes. *Mol Cells* 21:52–62.