Colon Cancer–Specific Cytochrome P450 2W1 Converts Duocarmycin Analogues into Potent Tumor Cytotoxins

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Abstract

Purpose: Cytochrome P450 2W1 (CYP2W1) is a monooxygenase detected in 30% of colon cancers, whereas its expression in nontransformed adult tissues is absent, rendering it a tumor-specific drug target for development of novel colon cancer chemotherapy. Previously, we have identified duocarmycin synthetic derivatives as CYP2W1 substrates. In this study, we investigated whether two of these compounds, ICT2705 and ICT2706, could be activated by CYP2W1 into potent antitumor agents.

Experimental Design: The cytotoxic activity of ICT2705 and ICT2706 in vitro was tested in colon cancer cell lines expressing CYP2W1, and in vivo studies with ICT2706 were conducted on severe combined immunodeficient mice bearing CYP2W1-positive colon cancer xenografts.

Results: Cells expressing CYP2W1 suffer rapid loss of viability following treatment with ICT2705 and ICT2706, whereas the CYP2W1-positive human colon cancer xenografts display arrested growth in the mice treated with ICT2706. The specific cytotoxic metabolite generated by CYP2W1 metabolism of ICT2706 was identified in vitro. The cytotoxic events were accompanied by an accumulation of phosphorylated H2AX histone, indicating DNA damage as a mechanism for cancer cell toxicity. This cytotoxic effect is most likely propagated by a bystander killing mechanism shown in colon cancer cells. Pharmacokinetic analysis of ICT2706 in mice identified higher concentration of the compound in tumor than in plasma, indicating preferential accumulation of drug in the target tissue.

Conclusion: Our findings suggest a novel approach for treatment of colon cancer that uses a locoregional activation of systemically inactive produg by the tumor-specific activator enzyme CYP2W1.

Translational Relevance

Progress in the chemotherapy of advanced colon cancer has been confined to improvements in established classes of cytotoxic agents, notably the platinates, camptothecins, and pyrimidine antimetabolites. It is therefore an attractive option to identify chemotherapeutics with novel mechanisms of action and with lack of conventional resistance that could complement existing therapies without adding to overall toxicity. Here, we present an opportunity for novel chemotherapy that uses the cytochrome P450 2W1 (CYP2W1) with a unique colon cancer-specific expression. This enzyme is shown to efficiently convert inert duocarmycins into potent tumor cytotoxins, without the systemic side effects common to chemotherapeutics.

These results indicate for the first time the possibility for drug design against highly malignant colon cancers using CYP2W1 as a tumor resident target for locoregional activation of a class of ultrapotent chemotherapeutics.

cancers, in particular in the higher grade phenotypes, whereas the expression in healthy tissues remains absent or insignificant (10, 11). Clinical studies indicate that increased levels of CYP2W1 expression positively correlate with the degree of tumor malignancy, as well as with decreased 10-year survival in patients with colon cancer (12, 13). So far, several groups have reported on the metabolism of a few candidate substrates (i.e., benzopyrene, sterigmatocystin, aflatoxin B1, lysophospholipids, and fluorobenzothiazoles) for CYP2W1 expressed in E. coli or mammalian cells (14–19). However, the potential of CYP2W1 as a specific target in cancer chemotherapy remains hitherto unexplored.

Recently, we have reported a synthesis of a novel chloromethylindolodine ICT2700 as the first example of a duocarmycin analogue whose cytotoxic activity is dependent on metabolic activation by CYP1A1 (20). The mechanism behind the cytotoxicity involves CYP-mediated oxidation of ICT2700 followed by spirocyclization and production of an N3-adenine covalent adduct. Because the substrate recognition site of CYP2W1 shares the highest sequence identity with CYP1A1 (21), we hypothesized that certain chloromethylindolodines might also represent CYP2W1 substrates. Our initial screen has identified several derivatives of ICT2700 as first candidate CYP2W1 substrates (Sheldrake and colleagues, submitted, under review). In the present study, we have analyzed the interaction of 3 chloromethylindolodines with CYP2W1 in a colon cancer cell milieu. We show that the chloromethylindodine ICT2726 is metabolized by CYP2W1 into innocuous metabolites, whereas ICI2705 and ICI2706 are specifically converted into cytotoxic products capable of inducing rapid tumor cell death. We show the enzyme-dependent cytotoxic effect of these prodrugs using CYP2W1-transfected cell lines and a human colon cancer xenograft murine model. This communication represents a first report on CYP2W1 targeting by novel chloromethylindolodines both in vitro and in vivo and establishes a basis for a possible novel antitumor strategy in the treatment of colon cancer.

Materials and Methods

Chemicals

The chloromethylindolodine analogues of duocarmycin used in this study were (1-(chloromethyl)-1,2-dihydroprrolo[3,2-e]indol-3(6H)-yl)(5-fluoro-1H-indol-2-yl)methanone (ICI2705), (5-chloro-1H-indol-2-yl)(1-(chloromethyl)-1,2-dihydropyrrolo[3,2-e]indol-3(6H)-yl)methanone (ICI2706), and (8-(chloromethyl)-7,8-dihydro-6H-furo[3,2-e]indol-6-yl)(5-fluoro-1H-indol-2-yl)methanone (ICI2726; Supplementary Fig. S1), synthesized in-house and shown to be authentic and more than 97% pure using nuclear magnetic resonance, mass spectrometry, and high-performance liquid chromatography (Sheldrake and colleagues, submitted, under review).

cDNA constructs

The cDNA of CYP2W1 was cloned into the HindIII and EcoRV sites of both Flp-In System expression vector
pcDNA5/FRT (Invitrogen) generating the pcDNA5/FRT/2W1 construct, and pcDNA3.1 vector generating pcDNA5/FRT/2W1 construct.

Cell lines and transfections
The Flp-In system (Invitrogen) was used for the generation of the human colon cancer SW480-2W1 cell line stably expressing CYP2W1. SW480 Flp-In host cells were kindly provided by Céline Gongora (IRCM INSERM, France; ref. 22). Cotransfection of pcDNA5/FRT/2W1 vector with Flp Recombinase expression plasmid (pOG44) yielded SW480-2W1 cell line, whereas the cotransfection of an empty pcDNA5/FRT vector with pOG44 generated SW480-mock cells. After transfections, zeocin (Invitrogen) was omitted from the medium and stable clones were selected for hygromycin B (Invitrogen) resistance (50 μg/mL). Colo320 human colon cancer cell line (American Type Culture Collection) was maintained in RPMI-1640 medium supplemented with 10% FBS, penicillin 100 U/mL, and streptomycin 100 μg/mL (Invitrogen). Colo320-2W1 cells transiently expressing CYP2W1 were generated with pcDNA3.1-2W1 plasmid using Lipofectamine 2000 reagent (Invitrogen). Corresponding Colo320-mock cells were generated by transient transfection with empty pcDNA3.1 vector.

Cell viability assay
Cells were seeded on 48-well plates and incubated with chloromethylindolines for 60 hours in triplicate. Compounds were dissolved in dimethyl sulfoxide DMSO (Sigma) whose final concentration in the cell medium did not exceed 0.25%. Viability of the cells following incubation was determined using EZ4U assay, according to manufacturer’s guidelines (Biomedica).

Bystander effect
SW480-2W1 cells were incubated with ICT2706, DMSO (vehicle), and NP40 (negative viability control) for 30 hours. Medium from all incubations was collected, centrifuged for 10 minutes at 13,000 rpm, and then transferred to SW480-mock cells whose intact medium was previously aspirated. SW480-mock cells were incubated with the conditioned media for 50 hours before their viability was determined using the EZ4U assay.

Western blot analysis
Colo320 and SW480 cell lines transfected with CYP2W1 or mock transfected, were lysed at 4°C for 15 minutes in 1% Triton X-100 in PBS with addition of a protease inhibitors cocktail (Roche), or in radioimmunoprecipitation assay buffer with subsequent centrifugation at 13,000 × g. Snap-frozen xenograft tumor samples were homogenized using the Bullet Blender Homogenizer (Next Advance Inc.) in a buffer containing 100 mmol/L Tris–HCl and 1 mmol/L EDTA, pH 7.5, with addition of protease inhibitors cocktail. Cell lysates and tumor homogenates were centrifuged at 13,000 × g for 10 minutes. The aliquots of supernatants were subjected to SDS-PAGE and subsequent immunoblotting using CYP2W1, γH2AX, or ERp29 antibodies (Supplementary Methods).

CYP2W1 functional activity assay
SW480-2W1 and corresponding mock-transfected cells were incubated with ICT2726 (4 μmol/L) for 24 hours, and ICT2706 (2 μmol/L) for 6 hours. Following incubations, the cell media were collected while the cells were harvested and centrifuged at 3,000 rpm for 5 minutes. Cell pellets were resuspended with an equal volume of ice-cold acetone, vortexed, and centrifuged at 13,300 rpm for 20 minutes. The supernatant was collected, mixed with collected medium, and centrifuged again at 13,300 rpm for 20 minutes. Combined cell extract and cell medium was analyzed by liquid chromatography/mass spectrometry (LC/MS; Supplementary Methods).

Human colon cancer xenograft model
All in vivo studies were approved by the local animal ethical committee in Stockholm, Sweden, and animal care was carried out in accordance with Institutional guidelines. For treatment with ICT2706 or vehicle, 20 severe combined immunodeficient (SCID) female mice (MTC, Karolinska Institutet, Sweden) were divided into test and control groups. The test group was inoculated subcutaneously in both flanks with SW480-2W1 cells (7 × 10⁶) and the control group with SW480-mock cells (7 × 10⁶). In both test and the control group, 20 tumors developed and were allowed to grow to average volume of 60 mm³ before the onset of treatment. ICT2706 (100 mg/kg) was dissolved in DMSO and further diluted in corn oil. In the single injection volume of 200 μL, DMSO solvent accounted for 10%. Vehicle formulation comprised of corn oil and 10% DMSO. Half of the animals from both test and control groups received intraperitoneal (i.p.) injections of 100 mg/kg ICT2706 once daily for 8 days, whereas the remaining animals received intraperitoneal injections of vehicle following the same dosing schedule. The tumor volumes were measured by caliper (width² × length × 0.52 = mm³), with the final measurement conducted on the day 9, 18 hours after the last treatment dose. Extracted tumors were weighed and divided into 2 equal parts with one being snap-frozen and the other preserved in paraformaldehyde.

ICT2706 pharmacokinetics
12 SCID female mice were inoculated subcutaneously with SW480-2W1 cells (7 × 10⁶) in both flanks, and the xenografts were allowed to grow to an average volume of 70 mm³ before the animals received a single intraperitoneal dose of 100 mg/kg ICT2706. Following treatment, animals were sacrificed at consecutive time points (t = 0 minute, 5 minutes, 30 minutes, 1 hour, 4 hours, and 24 hours), 2 animals per time point, and plasma, tumor, and liver tissue were removed, snap-frozen in liquid nitrogen, and analyzed for the presence of ICT2706 (Supplementary Methods).

Immunohistochemistry
Formalin-fixed xenograft tissue sections were rehydrated, incubated in 3% hydrogen peroxide, and blocked using...
2.5% horse serum for 20 minutes (Vector Laboratories, Inc.), followed by incubation with CYP2W1 antibody (11) in a dilution of 1:1,250 at 4°C overnight. After rinsing, the sections were incubated with ImmPRESS reagent for 30 minutes (Vector Laboratories, Inc.), followed by 3,3-diaminobenzidine enzymatic development (Invitrogen). Sections were mounted and visualized by light microscopy.

Statistical analyses
The GraphPad Prism 5 software package was used for all statistical analyses. Results were compared using a Student’s t-test, one-way or a two-way ANOVA (Dunnett or Bonferroni). All data were expressed as means ± SEM. Differences were considered statistically significant at a P < 0.05.

Results
CYP2W1 sensitizes colon cancer cells to cytotoxic prodrugs in vitro
To identify substrates metabolized by CYP2W1 to potent chemotherapeutics, we have synthesized and screened a library of chloromethylindolines (Sheldrake and colleagues, submitted, under review). In the present study, we investigate ICT2705, ICT2706, and ICT2726 as substrates for CYP2W1 to examine their potential as colon cancer-selective cytotoxins. For this purpose, we established 2 colon cancer cell lines, SW480-2W1 with a stable expression of CYP2W1, and Colo320-2W1 with transient CYP2W1 expression. Presence of CYP2W1 protein in both cell lines was confirmed by immunodetection (Fig. 1A and B,

Figure 1. CYP2W1 mediates cytotoxicity of ICT2705 and ICT2706. Human colon cancer cell lines SW480 (A) and Colo320 (B), expressing wild-type CYP2W1 and corresponding mock-transfected cells, were seeded on 48-well plates and treated with ICT2705 (2705), ICT2706 (2706), and ICT2726 (2726). Cell viability was analyzed using EZ4U assay after 60-hour incubation. NP40-treated cells were used as a negative control for viability. Values represent the means of at least 3 independent experiments ± SEM, each conducted in triplicate. Statistically significant differences in viability between enzyme-transfected and mock-transfected cells are depicted by asterisks (*, P < 0.05; ***, P < 0.001). Inserts, immunodetection of CYP2W1 protein following transfection. C, chloromethylindoline-induced DNA damage. SW480-2W1 and SW480-mock cells were treated with 4 μmol/L ICT2705, ICT2706, ICT2726, and doxorubicin (DOX; positive control) for the indicated time periods. Cell lysates containing 80 μg of total protein were subjected to 15% SDS-PAGE with subsequent immunodetection of γH2AX.

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Incubation of ICT2705 and ICT2706 in either of the cell line resulted in a significant dose-dependent loss of viability, whereas the treatment with ICT2726 had no detrimental effect. The dependence on CYP2W1 expression for the observed cytotoxicity was confirmed by the absence of effect on mock-transfected cells (Fig. 1A and B).

**Treatment with ICT2705 and ICT2706 induces DNA damage in CYP2W1-expressing SW480 cells**

To show whether DNA damage is associated with the cellular effects of the 3 chloromethylindolines, we analyzed the appearance of phosphorylated H2A.X histone (γH2A.X), a positive indicator of DNA damage (23). SW480-2W1 and SW480-mock cells were treated with ICT2705, ICT2706, ICT2726, and doxorubicin (positive control), for 0, 8, 24, and 48 hours. Using immunodetection of γH2A.X, we show a time-dependent increase in DNA damage following treatment with ICT2705 and ICT2706 in SW480-2W1 cells, but not in SW480-mock cells. Significant accumulation of γH2A.X was not detectable after treatment with ICT2726 consistent with its lack of cytotoxicity (Fig. 1C).

**Metabolism of ICT2706 and ICT2726 by CYP2W1**

LC/MS analysis of the extracts of SW480-2W1 cells incubated with ICT2706 reveals a specific metabolic product (MW 363.2, m/z 364.2), consistent with a hydroxylated derivative of ICT2706 (Fig. 2A). As the cytotoxicity of the chloromethylindolines is known to be mediated by a regiospecific hydroxyl group (20), the cytotoxicity observed for ICT2706-treated SW480-2W1 cells is consistent with formation of a cytotoxic metabolite generated in the mono-oxygenase reaction typical for CYP enzymes.

As a part of the present investigation, we also characterized a metabolite of ICT2726 in SW480-2W1 cells. The metabolite formed (m/z 351) was consistent with the conversion of the chloromethyl moiety to the nontoxic hydroxymethyl derivative (Fig. 2B). Such a metabolite is rationalized not to undergo spirocyclization to the cyclopropane moiety, a process previously shown to be necessary for triggering the formation of the chloromethylindoline cytotoxic metabolite (20). No metabolites of ICT2706 and ICT2726 were detected in the mock-transfected cells (Fig. 2A and B) confirming the requirement for CYP2W1 in the cytotoxicity of these agents.
ICT2706 inhibits SW480-2W1 tumor growth in vivo

Next, we examined the effect of the chloromethylindolines on growth of human SW480-2W1 colon cancer xenografts established as solid tumors in SCID mice. ICT2706 was selected as a lead compound because in SW480 cells it possessed the highest potency (Fig. 1A). Consecutive daily doses of ICT2706 (100 mg/kg i.p.) produced a persistent inhibition of tumor growth throughout the 8-day dosing period (Fig. 3A). SW480-mock xenografts, subjected to the same treatment regimen, showed no growth retardation in comparison with vehicle-treated tumors (Fig. 3B). The ICT2706 multiple dosing treatment used in this study was well tolerated in all animals as judged by appearance, behavior, and body weight changes of less than 10% (results not shown). After termination of the in vivo experiment on day 9, tumors were excised and weighed. CYP2W1-expressing tumors treated with ICT2706 showed marked depletion of CYP2W1-positive cells, as compared with the vehicle-treated SW480-2W1 tumors (Fig. 4A).

Growth inhibition in SW480-2W1 xenografts is associated with ICT2706-induced DNA damage

Using immunodetection of γH2A.X, we examined the levels of treatment-induced DNA damage in tumor samples resected from mice. The data show that ICT2706 treatment of SW480-2W1 tumors results in a strong γH2A.X signal. In comparison, vehicle-treated SW480-2W1 tumors produced little or no effect on γH2A.X formation, and no difference was found between the levels of γH2A.X in SW480-mock tumors subjected to either ICT2706 or vehicle treatment (Fig. 4B). Overall, a 50% increase in γH2A.X was measured in ICT2706-treated versus vehicle-treated SW480-2W1 tumors using quantitative densitometric analysis (data not shown).

Tissue distribution of ICT2706

To show tumor uptake of ICT2706 and determine the fate of this agent in vivo, we compared the distribution of ICT2706 in SW480-2W1 tumors, plasma, and liver. The distribution of ICT2706 in these tissues was monitored for up to 24 hours following its administration as a single intraperitoneal dose of 100 mg/kg. A summary of the...
pharmacokinetic parameters for ICT2706 is given in Supplementary Table S1, and the data are shown graphically in Fig. 5. The absorption from the peritoneum was delayed with a $T_{\text{max}}$ of 4 hours in plasma and tissue, however, ICT2706 was well distributed throughout the tissues. Tumor concentrations peaked at 7.7 mg/mL, which is 4-fold higher than the concentrations detected in plasma (1.78 mg/mL), though peak concentrations were considerably higher in liver (324 µg/g). All AUCs are approximated because of the limited number of sampled points. The results show also that the ICT2706 concentration–time curves followed a similar clearance profile. A direct comparison of AUCs calculated from this data showed that the AUC for ICT2706 was higher in tumor compared with plasma, whereas the highest AUC associated with the liver showed hepatic clearance to be an important route of elimination.

Figure 4. Expression of CYP2W1 and treatment-induced DNA damage in xenograft samples. Upon termination of xenograft experiment, SW480-2W1 tumors treated with ICT2706 or vehicle were analyzed by immunohistochemistry (A) using CYP2W1 antibodies. Immunohistochemical images are shown in magnification $\times 10$ and $\times 60$. B, resected SW480-2W1 and SW480-mock tumors treated with ICT2706 or vehicle were analyzed for the presence of H2AX, reflecting the levels of DNA damage in the tumor tissue. Representative samples are shown (SW480-2W1 and SW480-mock tumors treated with ICT2706, 2706 1-3; and vehicle, vhcl 1-3). SW480 cells incubated with doxorubicin (2 µmol/L) for 24 hours were used as a positive control (Ctrl+). Loading control was accomplished by the immunodetection of ERp29.

Figure 5. Pharmacokinetic analysis of ICT2706 in tumor, plasma, and liver following intraperitoneal administration to SW480-2W1 tumor-bearing mice. SW480-2W1 xenograft tumor bearing mice received a single dose of ICT2706 (100 mg/kg, i.p.) and were sacrificed at consecutive time points up to 24 hours. The ICT2706 content in plasma, tumor, and liver tissue was analyzed by LC/MS as described in the Supplementary Methods.
SW480-2W1 cells were incubated with 1 and 4 μmol/L ICT2706, DMSO (solvent; final concentration 0.25%), and NP40 (negative viability control; final concentration 1%), for 30 hours. Following the incubation, SW480-mock cell culture medium was replaced by the supernatants of centrifuged media from the differentially treated SW480-2W1 cells. The viability of the SW480-mock cells incubated with the conditioned media was determined using EZ4U assay after 50 hours of incubation. Values represent the means of 3 independent experiments ± SEM. Statistically significant differences in viability between differentially treated SW480-mock cells are depicted by asterisks (* P < 0.05; ***, P < 0.001).

**Bystander effect of ICT2706 in vitro**

The formation of a cytotoxic metabolite of ICT2706 in a tumor cell is predicted to kill the cell in which it is produced. However, even though the majority of CYP2W1-positive cells have been eliminated from SW480-2W1 xenografts during ICT2706 treatment (Fig. 4A), significant DNA damage is still observed in the residual tumor tissue (Fig. 4B). We therefore investigated whether bystander cytotoxicity can occur, i.e., whether the active metabolite of ICT2706 is likely to transfer from one tumor cell to others in close proximity. SW480-mock cells incubated with the medium from ICT2706-treated SW480-2W1 cells show significant loss of viability, whereas no cytotoxicity occurs in the SW480-mock cells incubated with medium from the cells treated with vehicle (Fig. 6).

**Discussion**

The conventional treatment of a variety of tumor types with CYP-activated agents (e.g., CPA, IFA, and nitrosoureas) is associated with substantial host toxicity due to the metabolism of the prodrug by several CYPs and the presence of these enzymes in the liver and other tissues (24). Ongoing efforts to improve the therapeutic index for drugs like CPA are based on viral CYP2B6 gene delivery to tumor tissue, pioneered by Waxman and colleagues (25–27). This GDEPT approach does significantly sensitize the cancer cells to administered prodrug; however, the toxicity problem remains. In contrast to such issues, confinement of CYP2W1 to cancer cells circumvents the need for gene delivery as well as the unwanted effects resulting from hepatic prodrug activation.

In the present study, we evaluate the potential of CYP2W1 targeting in a preclinical setting. Novel chloromethylindoline prodrug design has yielded ICT2705 and ICT2706 (Supplementary Fig. S1) as substrates for the CYP2W1 enzyme that can be metabolically activated into potent cytotoxic agents. The hydroxylation of these compounds most likely triggers their alkylation of DNA, as chloromethylindolines have previously been shown to mediate this event (20). We show that upon treatment with ICT2705 and ICT2706, cancer cells expressing CYP2W1 enzyme suffer substantial DNA damage with concomitant loss of viability.

In mice, xenograft growth is inhibited from the onset of ICT2706 dosing by continuous eradication of CYP2W1-expressing cells (Fig. 3A). The requirement of CYP2W1 enzyme was absolute in this study, as an identical treatment of mock-transfected xenografts produced no antitumor effect (Fig. 3B). After the cessation of ICT2706 dosing, CYP2W1-positive cells were undetectable in extracted SW480-2W1 tumors (Fig. 4A). This tumor tissue most probably represents a remnant of the cell population that has initially circumvented the stable expression of CYP2W1 and subsequently proliferated in the absence of antibiotic pressure. Nonetheless, significant levels of ICT2706-induced DNA damage can be detected in this residual tumor tissue through immunodetection of γH2A.X (Fig. 4B). The comprehensive antitumor effect and the accumulation of DNA damage observed despite the destruction of CYP2W1-expressing cells, speak in favor of potential bystander cytotoxicity. For instance, the cytosine deaminase/5-fluorocytosine suicide-prodrug system triggers colon cancer xenograft regression in vivo with only 4% of successfully transfected cells (28). Our in vitro analyses suggest that a similar bystander mechanism might augment CYP2W1 targeting since SW480-mock cells suffer significant loss of viability after incubation with SW480-2W1–conditioned medium (Fig. 6). As in the case of CPA, whose CYP-generated 4-hydroxy metabolite alkylates the DNA of surrounding cells (27), the antitumor effect of ICT2706 is likely to be facilitated by the dissemination of the toxic metabolite, affecting even tumor tissue scarcely populated with CYP2W1-expressing cells.

In vivo, ICT2706 is distributed to the xenograft tissue at a concentration that is sufficient to produce a sustained antitumor effect. Analysis of ICT2706 from dosed mice identified higher concentration of the compound in tumor than in plasma, indicating preferential accumulation of drug in the target tissue. Furthermore, ICT2706 is cleared within 24 hours, inferring that on each day of treatment the tumor was exposed to an equal concentration of the prodrug. Predominantly higher concentration of ICT2706 in liver is consistent with hepatic elimination of highly lipophilic compounds (Fig. 5).
CYP2W1 enzyme is shown to be expressed at high levels, albeit in 30% of malignant colorectal cancers (11). Therefore, there is a need to identify the patients who would benefit from CYP2W1-targeted therapy. In this regard, ICT2726 is a novel chloromethylindolone that has potential as a biomarker for functional CYP2W1. We show that CYP2W1 oxidizes ICT2726 to an identified nontoxic metabolite in colon cancer cells (Fig. 2B). Unlike the chloromethylindolones that are converted to DNA-binding cytotoxins, ICT2726 is attractive for assessing the activity of CYP2W1 without the complication of induction of toxicity in the test system and with ease of extraction for quantitative detection.

The active site homology between CYP2W1 and CYP1A1 enzymes (21) implies a potential overlap in their substrate specificities. Expression of CYP1A1 is however confined to tumor tissues and generally dependent on xenobiotic induction, allowing for targeting by anticancer prodrugs such as Phortress (8). In the occurrence of in vivo targeting of CYP2W1 by ICT2706, no signs of unwanted host toxicity were apparent indicating that the activation of this agent by systemic CYPs is not contributing to the cytoxicity observed.

The importance of CYP2W1 as a promising drug target is supported by in vitro studies of the metabolism of 2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole (GW-610, NSC721648) to a reactive intermediate shown to have a cytotoxic effect in selected breast and colon cancer cell lines (15, 16). However, the relevance of this to its CYP2W1 mediated antitumor activity remains to be verified by in vivo studies.

In summary, our study describes the first example of CYP2W1 targeting, using newly generated chloromethylindolone prodrugs, both in vitro and in vivo. We identify ICT2706 as a potential therapy, which could be trialed in an adjuvant setting of colon cancer to further prevent tumor growth or metastasis or in a neoadjuvant setting to assist tumor shrinkage or ablation before surgery. Endogenous cancer-confined CYP2W1 expression in combination with ICT2706 prodrg may present a novel candidate system for selective colon tumor treatment, minimizing the risk of systemic toxicity.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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References
