Role of CYP1A1 in Modulation of Antitumor Properties of the Novel Agent 2-(4-Amino-3-methylphenyl)benzothiazole (DF 203, NSC 674495) in Human Breast Cancer Cells

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ABSTRACT

2-(4-Amino-3-methylphenyl)benzothiazole (DF 203, NSC 674495) is a candidate antitumor agent with potent and selective activity against human-derived tumor cell lines in vitro and in vivo. Only sensitive cell lines (e.g., MCF-7) were able to accumulate and metabolize DF 203, forming the main inactive metabolite, 2-(4-amino-3-methylphenyl)-6-hydroxybenzothiazole (6-OH 203). Selective metabolism may therefore underlie its antitumor profile. DF 203 6-hydroxylase activity by MCF-7 cells was not constitutive but induced only after pretreatment of cells with DF 203, 3-methylcholanthrene, or β-naphthoflavone. 6-Hydroxylation was strongly inhibited by either goat antirat cytochrome P450 450A1 (CYP1A1) serum or α-naphthoflavone. Both α-naphthoflavone and 6-OH 203 abrogated DF 203-induced growth inhibition. Microsomes from genetically engineered human B-lymphoblastoid cells expressing CYP1A1, CYP1B1, or CYP2D6 metabolized DF 203 to 6-OH 203. Immunoblot analysis detected significantly enhanced CYP1A1 protein in a panel of sensitive breast cancer cell lines after exposure to DF 203. Neither constitutive expression nor induction of CYP1A1 protein was detected in nonresponsive breast (HBL 100, MDA-MB-435, and MCF-7/ADR) and prostate (PC 3 and DU 145) cancer cell lines. The expression of CYP1B1 was also modulated by DF 203 in the same sensitive cell lines. However, of the two isoforms, only CYP1A1 activity was irreversibly inhibited by DF 203 and significantly inhibited by 6-OH 203. In sensitive cell lines only, [3H]DF 203-derived radioactivity bound covalently to a M r 50,000 protein which was immunoprecipitated by CYP1A1 antisera. The covalent binding of [14C]DF 203 to recombinant CYP1A1 enzyme was NADPH-dependent and reduced by 6-OH 203 and glutathione. CYP1A1 appears essential for the metabolism of DF 203 and may have a pivotal, yet undefined, role in its antitumor activity.

INTRODUCTION

Novel 2-(4-aminophenyl)benzothiazoles (Fig. 1) substituted at the 3’-position with either a methyl group (DF 203) or a halogen atom (Cl, Br, 1) comprise a class of agent with remarkably similar, highly selective profiles of antitumor activity (1–3). Antitumor fingerprints are unique; agents failed to COMPARE (4) with any clinical class of chemotherapeutic agent. No molecular target(s) have been identified.

Selective and superior in vivo antitumor activity of DF 203 against breast (1), ovarian (3) and colon xenograft models has led to its selection as a candidate for clinical consideration. The aryalkylamine structure of these lipophilic compounds suggests susceptibility to metabolism. However, only sensitive cell lines (e.g., MCF-7 and T-47D) retain and biotransform 2-(4-aminophenyl)benzothiazoles. Unresponsive prostate PC 3, DU 145, and breast MDA-MB-435 cancer cell lines showed negligible uptake and metabolism of these compounds. Similarly, radioactivity derived from [14C]DF 203 selectively accumulated and covalently bound to cellular proteins in the aforementioned sensitive cell lines only (6). Selective metabolism and covalent protein binding by sensitive cell lines may therefore be a determinant of antitumor activity.

Rapid and extensive N-acetylation is the major metabolic route of unsubstituted 2-(4-aminophenyl)benzothiazole (CJM 126) in vitro and of DF 203 in vivo in rats (5). However, the major in vitro biotransformation pathway of 3’-substituted analogues is C-oxidation within the benzothiazole nucleus; DF 203 was metabolized by sensitive MCF-7 cells to a major metabolite that cochromatographed with 6-OH 203 (6). Homogenates prepared from untreated MCF-7 or T-47D cells failed to catalyze the 6-hydroxylation of DF 203; the ability to do so was induced only after pretreatment of cells with DF 203.

The main oxidizing enzymes in phase I metabolism are CYPs4 that catalyze the initial step in either detoxification or bioactivation of environmental toxins and xenobiotics (7–9). CYP1 isoforms are capable of activating aromatic amines; therefore, extrahepatic and cell line-specific regulation of CYP1A1 and CYP1B1 (10) are major determinants of chemosensitivity or resistance.

Herein, we report identification of CYP isoforms responsible for 6-hydroxylation of DF 203 and specific inhibition by α-NF of 6-hydroxylation activity and growth arrest in MCF-7 cells treated with DF 203. This novel antitumor agent modulates the expression and activity of CYP1A1 and CYP1B1 in sensitive cell lines, and the implications of these observations on its antitumor properties are discussed. In addition, we present evidence to support that CYP1A1 is the covalently bound M r 50,000 protein in sensitive cell lines.

MATERIALS AND METHODS

Reagents. DF 203 and 6-OH 203 were synthesized following published methods (1). Stock solutions (10 mM) were prepared in DMSO or acetonitrile and stored protected from light at 4°C. [14C]DF 203 (26.2 mCi/mmol) was synthesized at the Research Triangle Institute (Research Triangle Park, NC) and stored as 50 mM stock solutions in DMSO at −80°C. Coumarin, 7-ethoxy- coumarin, sulfaphenazole, quinidine, DEDC, TAO, 7-ethoxycoumarin, resoru- fin, NAPD+, NADH, NADPH, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, MTT, 3-MC, and B[a]P were obtained from Sigma Chemical Co. (Poole, United Kingdom; St. Louis, MO); α-NF and β-NF were from Aldrich (Milwaukee, WI), and furafylline was from Ultrafine Chemicals (Manchester, United Kingdom). HPLC-grade acetoneitrile was purchased from Baker (Philipsburg, NJ). All chemicals were of the highest commercial grade. Cell culture media and supplements were supplied by Life Technologies, Inc. (Gaithers- burg, MD; Paisley, United Kingdom) and Costar (Cambridge, MA; Bucking- hamshire, United Kingdom). Abs specific for CYP1A1 (polyclonal antiserum

3 M. C. Bibby and J. A. Double, personal communication.

4 The abbreviations used are: CYP, cytochrome P450; NF, naphthoflavone; DEDC, diethyldithiocarbamate; TAO, triacetylendiaminomycin; MTT, 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide; ECL, enhanced chemiluminescence; Ab, antibody; HPLC, high-performance liquid chromatography; EROD, 7-ethoxycoumarin-O-deeth- ylase; GSH, glutathione; AhR, arylhydrocarbon receptor; ER, estrogen receptor; 3-MC, 3-methylcholanthrene; B[a]P, benzo[a]pyrene; TCA, trichloroacetic acid.
for human CYP1A1/1A2 and CYP1B1 (polycyonal rabbit antihuman CYP1B1 primary Ab), as well as a panel of CYP microsomes, were obtained from Gentest Corp. (Woburn, MA; Cambridge, United Kingdom). ECL kits were purchased from Pierce, and alkaline phosphatase kits were from Upstate Technology (San Diego, CA).

**In Vitro Cell Culture.** Monolayer cells were cultured at 37°C in an atmosphere of 5% CO₂ in RPMI 1640 containing 2 mm L-glutamine and supplemented with 10% FCS, 100 IU/ml penicillin, and 100 µg/ml streptomycin. Cells were maintained in continuous logarithmic growth by routine subculturing twice weekly. Cell lines used include human breast carcinoma cell lines MCF-7, MDA 468, MDA-MB-435, T-47D, SKBR3, ZR 75, and MCF-7/ADR; human prostate carcinoma cell lines PC 3 and DU 145; and a nonmalignant human breast cell line HBL 100.

**MTT Colorimetric Assay.** Cells were seeded onto 96-well microtiter plates at a density of 5 x 10⁴ per well and allowed 24 h to adhere before drugs were added over a concentration range of 0.1 µM to 100 µM (n = 8). Serial dilutions were prepared in media prior to each assay, with final DMSO concentration <0.25%. After 72-h exposure, MTT was added to each well (final concentration, 400 µg/ml) and incubated for 4 h to allow metabolism of MTT by mitochondrial dehydrogenase to an insoluble formazan product. Medium was then aspirated, and formazan was solubilized by the addition of 125 ml of DMSO:glycine buffer (pH 10.5; 4:1). Cell viability was determined as absorbance at 550 nm, read on an Anthos Labtec systems plate reader. An MTT assay performed at the time of drug treatment determined an initial absorbance from which cell growth or drug toxicity could be assessed.

**Metabolism of DF 203 by Cell Homogenates.** Homogenates were prepared from cells pretreated for 24 h with DF 203, 3-MC (1 µM), β-NF (10 µM), or vehicle control (0.1% DMSO). Metabolites of DF 203 were detected by HPLC. A typical reaction mixture consisted of 100 µM DF 203, 100 mM Tris-Cl (pH 7.4), 5 mM MgCl₂, 0.5 mM NADH, NADPH-generating system (1 mM NAD⁺, 5 mM glucose 6-phosphate, and 1 unit/ml glucose 6-phosphate dehydrogenase) and MCF-7 homogenate (1 mg/ml) in a final volume of 0.2 ml. DF 203 was dissolved in acetonitrile and added to the incubation mixture at a final acetonitrile concentration of 1%. The reaction mixture was preincubated at 37°C for 5 min, and the reaction was started by the addition of NADH and NADPH-generating system. After 30 min incubation at 37°C, the reaction mixture was centrifuged at 14,000 rpm for 10 min, and supernatant (100 µl) was analyzed by HPLC.

The HPLC system consisted of a Hewlett Packard 1050 Series Module (solvent delivery pump, autosampler, and multiple wavelength detector; Hewlett Packard, Palo Alto, CA) and a Hewlett Packard 1046A fluorescence detector. Compounds were separated at room temperature on a C18 reversed-phase column (YMC-ODS-AQ, 150 x 4.6 mm inside diameter, S-5 µm; YMC Inc., Wilmington, NC). The mobile phase composition was changed linearly during 40 min from 10:90:1 to 80:20:1 (acetonitile:water:acetic acid). Mobile phase was continuously degassed with nitrogen, and the flow rate through the column was 1 ml/min. UV detection was at 330 nm, and fluorescence detection was at λex 344 nm and λem 434 nm.

**Metabolism of DF 203 by Specific Human P450 Isoforms.** Microsomes from human B-lymphoblastoid cells expressing human CYP isoforms 1A1, 1A2, 1B1, 2A6, 2B6, 2C8, 2C9-Ar, 2C9-Cys, 2C19, 2D6, 2E1, and 3A4 (11) were tested for their ability to metabolize DF 203. Microsomes from cells carrying the expression vector without P450 cDNA were used as a negative control. Reactions were conducted as described above but with incubation periods of 2 h.

**Inhibition of DF 203-induced Metabolism.** A typical incubation mixture contained 100 mM Tris-Cl buffer (pH 7.4), 5 mM MgCl₂, NADPH-generating system, and 1 mg/ml MCF-7 cell homogenate (pretreated for 24 h with 1 µM DF 203). α-NF, furafylline, coumarin, 7-ethoxycoumarin, sulfaphenazole, quinidine, DEDC, or TAO were each added to the incubation mixture (100 µM in DMSO vehicle; final DMSO concentration, 0.5%) as relatively specific inhibitors of P450 isoforms. Each inhibitor was preincubated for 15 min with the reaction mixture before the reaction was initiated by addition of DF 203.

For immune-inhibition studies, homogenates of DF 203-treated MCF-7 cells were preincubated with varying concentrations of human CYP1A1-selective polyclonal goat antiserum CYP1A1 serum or normal goat serum at room temperature for 30 min before incubations were carried out as described.

**Assay of EROD Activity.** A sensitive and rapid HPLC method was used to measure EROD activity (12). The incubation mixture consisted of 100 mM Tris-HCl (pH 7.4), 5 mM MgCl₂, NADPH-generating system, and either MCF-7 cell homogenate (1 mg/ml) or microsomes expressing recombinant CYP isoforms (0.1 mg/ml) and 80 µM 7-ethoxyresorufin in a final volume of 0.2 ml. The mixture was preincubated for 5 min at 37°C, and the reaction was initiated by addition of the NADPH-generating system. After incubation (15 min for recombinant CYP1A1 and 30 min for recombinant CYP1B1 and MCF-7 homogenate), 0.6 ml of ice-cold acetonitrile was added to stop the reaction. The reaction mixture was centrifuged at 14,000 rpm for 10 min, and supernatant (100 µl) was analyzed by HPLC using a mobile phase of 25 mM phosphate buffer (pH 7.0):acetonitrile (75:25% v/v) at a constant flow rate of 1 ml/min. Resorufin fluorescence was detected at λex 530 nm and λem 580 nm.

**Inactivation of EROD Activity.** Microsomes from human B-lymphoblastoid cells expressing human CYP1A1 or CYP1B1 were incubated with NADPH-generating system in the presence or absence of 100 µM DF 203 or concentrations of 6-Oh DF 203 at 37°C for 30 min before EROD activity was measured. To investigate EROD inactivation by DF 203, microsomes were incubated with NADPH-generating system in the presence or absence of 100 µM DF 203 (37°C for 30 min) and 0.8 ml of hypotonic buffer added. The reaction mixtures were ultracentrifuged at 100,000 × g for 30 min, and supernatant was removed. The microsomal precipitate was resuspended in 1 ml of hypotonic buffer and ultracentrifuged again. The resulting microsome precipitate was then assayed for EROD activity.

**Western Blot Protocol.** Whole-cell lysates were prepared for examination of CYP1A1 and CYP1B1 expression. After appropriate treatment, cells were detached by trypsinization, washed in PBS, and counted. Cells were pelleted by centrifugation (5 min at 12,000 rpm) and lysed by the addition of lysis buffer containing 20 mM Tris-HCl (pH 7.4), 2 mM EDTA (pH 7.4), 2 mM EGTA (pH 7.4), 6 mM β-mercaptoethanol, 10 µg/ml leupeptin, 2 µg/ml aprotinin, and 1% v/v NP40. Cell lysates were sonicated (3 x 10-s bursts, setting 25 of MSE Soniprep 150 sonicator, United Kingdom), protein content was estimated by the Bradford assay (13), and sample buffer was added. Samples were boiled at 95°C for 5 min, and solubilized proteins (50 µg) were separated on 10% gels by SDS-PAGE before electroblotting to polyvinylidene difluoride membranes (Bio-Rad, United Kingdom). Membranes were blocked for 1 h in PBS-0.1% Tween 20, 10% dried milk and then washed 3 x 5 min with PBS 0.1% before incubation (2 h) with goat antirabbit polyclonal primary Ab (1:500 in PBS 0.02% T, 1% milk). After washing, membranes were incubated for 1 h with alkaline phosphatase-conjugated rabbit antigoat secondary Ab (1:5000 in PBS 0.02% T, 1% milk). CYP1A1 was detected after 10 min incubation with substrates (bromochloroindolyl phosphate and nitro blue tetrazolium) in alkaline phosphatase buffer.

For CYP1B1 immunoblots, nonspecific binding was blocked with 5% dried skimmed milk in TBS (0.05 M, pH 7.9)-0.1% T for 1 h at room temperature. After washing with TBS-0.1% T, membranes were probed with CYP1B1 specific primary Ab (1:500 in TBS-0.01% T, 0.5% dried skimmed milk) for 1 h at room temperature. Membranes were rinsed with TBS-0.1% T and incubated for 1 h with horseradish peroxidase-conjugated goat antirabbit secondary Ab (1:500 in TBS-0.01% T, 0.5% dried milk). After rinsing the membranes (3 x 5 min with TBS-0.1% T and 2 x 5 min with TBS), CYP1B1 was detected by ECL.
Separation of Protein Bound to [14C]DF 203-derived Radioactivity. Cells were grown in 150-mm tissue culture Petri dishes to 50–70% confluency before use. Laemmli buffer (25 mM Tris-HCl (pH 7.4), 2 mM MgCl₂, 1 mM NADPH, and 10 mM β-NF) was solubilized by addition of lysis buffer containing 50 mM HEPES buffer (pH 7.4), 50 mM NaCl, 1% Triton X-100, 10 mM β-glycerophosphate, 2 mM phenylmethylsulfonyl fluoride, 1 mM Na₃VO₄, 10 μg/ml aprotinin, and 10 μg/ml leupeptin. After centrifugation at 14,000 rpm for 15 min at 4°C, the supernatant was collected for estimation of protein content, and Laemmli’s buffer was added. Samples were boiled for 5 min, and 200 μg of protein were loaded onto 4–20% gradient SDS-polyacrylamide slab gel. SDS-PAGE was carried out according to the method of Laemmli (14). After electrophoresis, the gel was fixed for 1 h with 40% TCA, 30% methanol, and 10% acetic acid and then kept for 1 h in 1% SDS and treated with 0.1, 1, and 10 mM fluoride, 100 μM Na₃VO₄, 50 mM 3-MC, and 10 μM [14C]DF 203. After 24 h incubation, medium was removed, and cells were rinsed with cold PBS (pH 7.4). Protein content was measured. Data are plotted as means of three experiments. Bars, SD.

RESULTS

Induction of DF 203 6-Hydroxylase Activity. Concentration- and time-dependent 6-hydroxylation of DF 203 was induced in MCF-7 cells treated with DF 203 (Fig. 2A). Maximum activity occurred after 24 h exposure to 1 μM DF 203, levels of 6-hydroxylase activity decreased with DF 203 concentrations >10 μM or prolonged drug exposure. In MCF-7 cells, induction was observed as early as 2 h after DF 203 treatment (Fig. 2B). In addition, DF 203 6-hydroxylase activity was efficiently induced after treatment of MCF-7 cells (24 h) with 3-MC (1 μM) or β-NF (10 μM; Fig. 2C). However, 6-hydroxylation was not detected in either vehicle-treated MCF-7 cells or unresponsive MDA-MB-435 homogenates, regardless of treatment conditions.

Identification of 6-Hydroxylase. Specific CYP inhibitors were used to aid determination of CYP isoform(s) induced by DF 203 in MCF-7 cells treated with 1 μM DF 203 for 24 h. The respective specific inhibitors of CYP1A1/CYP1B1, CYP1A2, CYP2A6, CYP2B, CYP2C9, CYP2D6, CYP2E1, and CYP3A used were: α-NF (15, 16), furafylline (17), coumarin (18), 7-ethoxycoumarin (19), sulphanilic acid (20), quinidine (21), DEDC (22), and TAO (23). α-NF strongly inhibited DF 203 6-hydroxylation, whereas furafylline was a
weak inhibitor (Fig. 3), implicating an oxidative role for CYP1A1 and/or CYP1B1. Human CYP1A1-selective polyclonal goat antirat CYP1A1 serum inhibited the 6-hydroxylase activity induced in homogenates of MCF-7 cells treated with 1 μM DF 203 for 24 h (Fig. 4). At the highest antisera concentration (0.3 ml/mg protein), 6-hydroxylation of DF 203 was inhibited by 90%. These data suggest CYP1A1 as the major isoform that catalyzes 6-hydroxylation of DF 203.

The characteristic biphasic dose-response of DF 203 was abrogated when MCF-7 cells were cotreated with DF 203 and 10 μM α-NF (Fig. 5A). This protective effect against growth inhibition was similarly observed with 6-OH DF 203 (Fig. 5B). Furafylline had no effect on DF 203-induced growth inhibition.

**Metabolism of DF 203 by Human CYP Isoforms.** Metabolism of DF 203 was studied using microsomes from human B-lymphoblastoid cells expressing specific human P450 isoforms (CYP1A1, CYP1A2, CYP1B1, CYP1A6, CYP2C8, CYP2C9-Arg, CYP2C9-Cys, CYP2C19, CYP2D6, CYP2E1, and CYP3A4). Microsomes from cells carrying the expression vector alone served as negative control. 6-Hydroxylation of DF 203 was extensively catalyzed by CYP1A1 (Fig. 6A) and to a lesser extent by CYP1B1, CYP2C9-ARG, and CYP2D6. Other unidentified oxidation products of DF 203 were also produced. The \( K_m \) s for 6-hydroxylation by CYP1A1, CYP1B1, and CYP2D6 were estimated by Lineweaver-Burk plots (Fig. 6B): 4.2 μM (CYP1A1), 4.5 μM (CYP1B1), and 1 mM (CYP2D6). Thus, CYP1A1 and CYP1B1 possess greater affinity for DF 203, more readily catalyzing 6-hydroxylation under experimental conditions.

**Modulation of EROD Activity by DF 203.** CYP1A1 specifically catalyzes the deethylation of 7-ethoxyresorufin (24). No constitutive EROD activity was detected in homogenates of untreated MCF-7 cells. Treatment of MCF-7 cells with various concentrations of DF 203 for 24 h induced EROD activity in cell homogenates (Fig. 7). The concentration-dependent profile of EROD activity is similar to that of DF 203 6-hydroxylation, with maximum activity observed after 1 μM drug exposure.

Simultaneous incubation of CYP1A1 or CYP1B1 microsomes with 7-ethoxyresorufin and 100 μM DF 203 inhibited EROD activity (Fig. 8). After removal of DF 203 and recovery of microsomes by ultracentrifugation, microsomes expressing human CYP1A1 showed...
little recovery of EROD activity. In contrast, EROD activity was significantly recovered in microsomes expressing human CYP1B1. Thus, DF 203 irreversibly inhibited CYP1A1 but not CYP1B1. In addition, 6-OH 203 inhibited EROD activity of CYP1A1 but not CYP1B1 microsomes (Fig. 9).

Covalent Binding to a Mr 50,000 Protein. Sensitive cell lines only accumulated, and covalently bound radioactivity was derived from [14C]DF 203 (6). After treatment of sensitive MCF-7 and T-47D cells with [14C]DF 203, autoradiography of separated (SDS-PAGE) cellular proteins showed concentration of radioactivity on a Mr 50,000 band (Fig. 10). This was absent from insensitive MDA-MB-435 cells. This covalently bound, Mr 50,000 protein was immunoprecipitated by CYP1A1 (but not CYP1B1) antiserum, providing evidence that it is the CYP1A1 enzyme (Mr 52,000). An in vitro covalent binding assay using [14C]DF 203 and recombinant CYP1A1 microsomes showed NADPH-dependent binding of radioactivity to CYP1A1 protein (Fig. 11). Coincubation with the inactive metabolite 6-OH 203 (100 µM) or GSH (1 mM) reduced covalent binding by 74 and 33%, respectively.

Modulation of CYP1A1 and CYP1B1 Expression. The constitutive expression and induction of CYP1A1 and CYP1B1 were de-
termined in our panel of cell lines. Goat antirat CYP1A1 serum detected a Mr 52,000 protein band in sensitive cell lines only (MCF-7, MDA 468, T-47D, and SKBR3), after pretreatment of cells with DF 203 (3 mM, 72 h; Fig. 12), β-NF (10 μM), or 3-MC (1 μM; results not shown). Expression of CYP1A1 in sensitive cell lines was strictly dependent upon induction by drug treatment. CYP1A1 protein could be detected in MCF-7 cell lysates after 4 h exposure to low (100 nM) concentrations of DF 203 (results not shown).

CYP1B1, however, was constitutively expressed in sensitive breast cell lines tested (MCF-7, MDA 468, T-47D, and SKBR3). In MCF-7 and MDA 468 cells, the expression of CYP1B1 was modulated in a manner distinct from that of CYP1A1; at concentrations <300 nM, CYP1B1 was down-regulated with induction occurring only at DF 203 concentrations >3 μM (Fig. 12). Induction in both of these cell lines occurred within 24 h of drug exposure. DMSO solvent (0.5% v/v) did not alter expression of either enzyme (results not shown).

Neither CYP1A1 nor CYP1B1 were expressed constitutively or induced in cell lines unresponsive to 2-(4-aminophenyl)benzothiazoles (breast MDA-MB-435, HBL 100, and MCF-7/ADR; prostate PC 3 and DU 145).

**DISCUSSION**

Novel 2-(4-aminophenyl)benzothiazoles represent a distinct mechanistic class of antitumor agent. They are sequestered and metabolized by sensitive cells only, suggesting a pivotal role of metabolism in their, as yet, undefined mode of action. Thus, knowledge of metabolic capacities of sensitive cell lines and the expression of major xenobiotic metabolizing enzymes are valuable in mechanism elucidation. In a panel of human tumor cell lines with differential responses to 2-(4-aminophenyl)benzothiazoles, we have observed differential expression of N-acetyltransferases (5).

Sensitive breast cancer cell lines metabolized DF 203 to its inactive 6-hydroxy derivative (6). Data herein report DF 203 to be efficiently oxidized by microsomal CYP1A1 and CYP1B1 expressed in human...
B-lymphoblastoid cells and to a lesser extent by CYP1A2 and CYP2D6. Moreover, induction of CYP1A1 and modulation of CYP1B1 expression occurred specifically in breast cell lines sensitive to growth inhibition by 2-(4-aminophenyl)benzothiazoles, irrespective of ER status. Further experimental evidence implicates CYP1A1 as the major isozyme catalyzing 6-hydroxylation of DF 203, following its induction in sensitive breast cell lines. Neither CYP1A1 expression, EROD, nor DF 203 6-hydroxylase activities were detected constitutively; in contrast, CYP1B1 protein was constitutively expressed in sensitive breast cell lines. Profiles of 6-hydroxylation and EROD activity in MCF-7 cells were biphasic and inverse to growth inhibition and induction of 6-hydroxylation activity by DF 203 was inhibited by CYP1A1-selective goat antirat CYP1A1 serum and α-NF. Coincubation of MCF-7 cells with DF 203 and α-NF abolished DF 203-induced growth inhibition. Thus, a mechanistic link between CYP1A1 catalytic activity and DF 203-induced growth inhibition may be inferred.

CYP-catalyzed metabolism of DF 203 appears to produce both mitogenic and inhibitory metabolites, such as in the case of estradiol (25). Indeed, 6-OH 203 not only protected cells from growth inhibition by DF 203 but evoked a mitogenic response in MCF-7 cells cultured under conditions suboptimal for growth (phenol red-free medium supplemented with 5% charcoal-stripped FCS).5 In addition, CYP1B1 is regulated by AhR (28). After ligand binding, AhR heterodimerizes with its protein partner, arylhydrocarbon receptor nuclear translocator (29, 30). This heterodimer complex binds xenobiotic responsive elements located within the 5′-flanking region of responsive genes to stimulate the synthesis of their protein products (31, 32). Most breast cancer cell lines express AhR and the arylhydrocarbon receptor nuclear translocator, 2,3,7,8-Tetrachlorodibenzo-p-dioxin is a strong inducer of CYP1B1 in both ER+ and ER− breast cancer cell lines (33, 34), whereas CYP1A1 induction by 2,3,7,8-tetrachlorodibenzo-p-dioxin appears dependent on ER status (34–36). This suggests differences in the regulatory controls of CYP1A1 and CYP1B1. Indeed, the profile of CYP1B1 modulation by DF 203 differed from that of CYP1A1; concentrations of DF 203 <300 nM down-regulated CYP1B1 expression in both MCF-7 and MDA 468 cell lines. Induction of CYP1B1 occurred at concentrations of DF 203, which elicited the second proliferative phase of the in vitro dose-response curve (2), suggesting a possible correlation between these two observations. That activity of CYP1B1 was neither irreversibly inhibited by DF 203 (Fig. 8) nor inhibited by 6-OH 203 (Fig. 9) corroborates this deduction.

Human mammary tumors express CYP1A1 and CYP1B1, both of which contribute significantly to the bioactivation of carcinogenic polycyclic aromatic hydrocarbons (37–40). Known inducers of CYP1A1, such as 3-MC, induced DF 203 6-hydroxylase activity in MCF-7 lysates as efficiently as DF 203 (Fig. 2C). However, 3-MC and B[a]P displayed very poor profiles of growth inhibition in the National Cancer Institute in vitro drug screen and, more importantly, did not COMPARE with DF 203.

Significantly, regulation of CYP1A1 and CYP1B1 by 2-(4-aminophenyl)benzothiazoles is cell line specific and may determine drug sensitivity. Induction and inactivation of CYP1A1 by DF 203 has potentially important clinical implications. Because CYP1A1 metabolizes many drugs and xenobiotics and can be induced in human liver, manipulation of CYP1A1 levels can potentially influence drug interactions and toxicities. Polymorphisms exist within the CYP gene family, e.g., individuals (~5% of the population) possessing a CYP2D6 defect metabolize drugs more slowly, causing accumulation of toxic drugs (41). Thus, pharmacogenomic assessment will provide essential information to reduce adverse drug effects and optimize therapeutic efficacy in patients with favorable genetic profiles.

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