C-7 Analogues of Progesterone as Potent Inhibitors of the P-Glycoprotein Efflux Pump

Fabio Leonessa,[†] Ji-Hyun Kim,[†] Alem Ghiorghis,[‡] Robert J. Kulawiec,[‡] Charles Hammer,[‡] Abdelhossein Talebian,[‡,§] and Robert Clarke*[,†]

Departments of Oncology, Physiology and Biophysics, and Lombardi Cancer Center, Georgetown University School of Medicine, 3970 Reservoir Road Northwest, Washington, DC 20007, and Department of Chemistry, Georgetown University, 37th and O Streets Northwest, Washington, DC

Received March 20, 2001

The P-glycoprotein product (Pgp) of the MDR1 gene has been implicated in the multiple drug resistance phenotype expressed by many cancers. Functioning as an efflux pump, P-glycoprotein prevents the accumulation of high intracellular concentrations of substrates. We have taken a rational approach to designing inhibitors of P-glycoprotein function, selecting a natural substrate (progesterone) as our lead compound. We hypothesized that progesterone, substituted at C-7 with an aromatic moiety(s), would exhibit reduced Pgp affinity, significantly increased antiPgp activity, and reduced affinity for progesterone receptors (PGR). We synthesized 7α-[4′-(aminophenyl)thio]pregna-4-ene-3,20-dione (**2**), which comprises a C-7α thiol bridge linking an aminophenyl moiety to progesterone, from pregna-4,6-diene-3,20-dione (**1**). The subsequent addition reaction of **2** with the appropriate isocyanate produced an initial series of compounds (**3**−**6**). Compounds **3**−**5** (respectively, $-CH_2CH_2Cl$; $-CH_2CH_3$; and $-CH(CH_3)C_6H_5$) exhibit a significantly increased ability to inhibit P-glycoprotein. Potency for restoring doxorubicin accumulation in MDR1-transduced human breast cancer cells is increased up to 60-fold as compared with progesterone. Compound **5** has greater potency than verapamil and is equipotent with cyclosporin A, for inhibiting P-glycoprotein function. Furthermore, **5** does not bind to PGR, implying a potential reduction in in vivo toxicity. These data identify C-7-substituted progesterone analogues and **5**, in particular, as rationally designed antiPgp compounds worthy of further evaluation/development.

Introduction

While many cancers are initially responsive to cytotoxic chemotherapy, most acquire a resistant phenotype. This phenotype is often characterized by crossresistance to structurally unrelated drugs to which the tumor has not been exposed. The precise genes that confer this multidrug resistance phenotype are unknown, but there are several strong single-gene candidates. These include several ABC transporters including the P-glycoprotein product of the MDR1 gene (Pgp; gp170),[1] the lung resistance protein,[2] the breast cancer resistance protein,[3] and several members of the multidrug resistance associated protein family.[4,5] The precise contribution of each potential multidrug resistance mechanism is unclear. Indeed, more than one mechanism may operate, either within the same tumor cell subpopulation and/or within different subpopulations of the same tumor.

We have chosen to study Pgp-mediated multiple drug resistance. Pgp confers resistance to drugs by preventing their accumulation within the cell. Pgp's efflux capabilities appear to reflect its ability to bind substrates within the inner leaflet of the plasma membrane.[6] Subsequently, and in a potentially adenosine 5′-triphosphate (ATP)-dependent manner, substrates are expelled from the cell.[7] We have shown by meta analysis that Pgp expression is detected in ≥50% of breast cancers and that this expression is associated with prior chemotherapy, a worse than partial response to chemotherapy, and in vitro resistance to Pgp substrates.[8] These data suggest that where Pgp expression is detected, it likely contributes to multiple drug resistance in some breast cancers. Nonetheless, the likely role of Pgp in conferring drug resistance remains controversial.

The poor activity of current antiPgp agents in patients has been attributed to the presence of resistance factors in addition to Pgp, inappropriate design of clinical trials, toxicity, and/or lack of specificity of antiPgp reagents. The ability of reversing agents to alter the pharmacokinetics of the coadministered cytotoxic drugs, and an inability to achieve adequate levels of some reversing agents, also are problematic.[9] The absence of a series of nontoxic drugs, specifically designed to reverse Pgp, limits the design of clinical trials to reverse this form of multidrug resistance.

One aspect of the controversy regarding the role of Pgp comes from the relatively poor activity of those few Pgp reversing agents evaluated in clinical trials. Most attention has focused on the Pgp reversing agents verapamil, cyclosporin A, and its nonimmunosuppressant analogue PSC833 (valspodar), but the activity of other drugs also has been studied in patients. Few of these compounds were designed as Pgp inhibitors. Thus, severe side effects, often related to either the "normal" function of these agents and/or their ability to influence

* To whom correspondence should be addressed. Tel: (202)687-3755. Fax: (202)687-7505. E-mail: clarker@georgetown.edu.
[†] Departments of Oncology, Physiology and Biophysics, and Lombardi Cancer Center, Georgetown University School of Medicine.
[‡] Department of Chemistry, Georgetown University.
[§] Deceased.

10.1021/jm010126m CCC: $22.00
Published on Web 12/14/2001

additional targets, may be induced at concentrations required to affect Pgp function. Many antiPgp drugs affect the pharmacokinetics of the substrate, significantly increasing cytotoxic drug-induced toxicity.[10] Other antiPgp agents cannot readily be delivered at doses that produce adequate serum levels. For example, the serum levels of verapamil required to produce in vitro reversal of Pgp resistance are rarely achieved in patients, despite administering sufficient doses of verapamil to induce significant cardiotoxicity.[9,11,12] Adequate serum trifluoperazine levels are not reached in patients at doses that induce dose-limiting toxicities.[9,13] Peak plasma levels of the stereoisomer of *cis*-flupenthixol (*trans*-flupenthixol) are 1000-fold less than that necessary to achieve full chemosensitization in vitro.[14,15] Several clinical studies have used patient populations where tumor Pgp expression is unknown, complicating a clear determination of its contribution to multiple drug resistance.

Previously, we have established cellular breast cancer models in which to study Pgp-mediated efflux and evaluate inhibitors of this activity. These models have been generated by inducing a constitutive expression of Pgp, following transduction with retroviral gene expression vectors.[16,17] The major advantage of these models is that unlike cells selected for resistance in vitro, Pgp expression is the only mechanism present to produce the multiple drug resistance phenotype. For example, the widely used $MCF7^{ADR}$ cells, which were selected in vitro for resistance to doxorubicin (DOX) and recently redesignated NCI/ADR-RES,[18] exhibit increased glutathione transferase and topoisomerase II activities.[19,20] Differences in the potency of isomers of fluphenthixol identified in $MCF7^{ADR}$ cells could not be confirmed in MDR1-transfected NIH 3T3 cells.[15]

Using our cellular models, we now describe an initial series of progesterone analogues that exhibit significantly increased antiPgp activity as compared with progesterone and verapamil and comparable to that seen with cyclosporin A. Importantly, the most potent of these analogues has lost its ability to activate progesterone receptors (PGR) and is predicted to exhibit relatively low intrinsic toxicity in vivo.

Chemistry

Conceptualization and Design. We wished to take a rational, structure−function-based approach to design inhibitors of Pgp function. Initially, we hypothesized that a natural substrate for the pump could provide an ideal candidate for rational drug design, since it is likely that Pgp evolved specifically to efflux such molecules. Evidence shows that several molecules with a steroid nucleus are Pgp substrates.[21−23] Pgp is expressed in the uterus[23,24] and the placenta,[25] suggesting a natural role for protecting secretory cells from the toxic effects of high local concentrations of steroids. Progesterone is the most potent of the steroids, including progesterone's metabolites, for reversing the effects of Pgp expression.[23,26,27] Progestins have intrinsically lower toxicity than other reversing agents and are orally active. In addition, progesterone is readily available and cheap, and the chemistry for generating several structural modifications is relatively straightforward.[28,29] Thus, we chose progesterone as our lead compound.

The major beneficial properties we wished to confer included but were not restricted to (i) improved potency for Pgp reversal, (ii) either no change or a reduction in affinity for PGR, and (iii) no agonist (mitogenic) activities. Concurrently, we wished to avoid either a substantial increase in PGR binding or a loss of Pgp reversing potency.

Unfortunately, the precise structure−function characteristics of Pgp reversing agents are unknown. This is not surprising, given the remarkable structural diversity of Pgp substrates.[30] Nonetheless, several characteristics are apparent, providing generic guidelines for the design of Pgp reversing agents. Lipophilicity appears central, with increased lipophilicity strongly associated with increased antiPgp activity.[31−37] Planar aromatic rings are commonly found in substrates, and these may contribute to lipophilicity.[37] Amphipathicity also is common, as is the presence of a basic amine, where primary amines appear most effective.[31,33,35,36,38] Size, for example, as determined by calculated molar refractivity, appears an important factor in several classes of compounds.[32,35,39] C21-aminosteroids have a structural similarity to progesterone, and in these compounds, the steroid moiety, lipophilicity, and amphipathicity are considered important attributes.[40] For compounds composed of two structures joined by a molecular spacer, the length of the spacer seems important.[15,31,34,41,42] This suggests that some part of the molecule may be oriented into a "pocket" in Pgp.[31] This pocket may have specific requirements for lipophilicity, size, and charge.

A C-7 addition to the steroid 17β-estradiol, as occurs in the antiestrogens ICI 182,780 and ICI 164,384, produces compounds with low toxicity and potentially appropriate pharmacokinetics.[43,44] Limited evidence suggests that ICI 164,384 can reverse Pgp-mediated resistance,[45] despite the apparent inability of 17β-estradiol to do so.[23] Thus, a bulky C-7 substitution on a steroid nucleus might increase antiPgp activity. C-7-substituted progesterone analogues were synthesized 20 years ago, but several exhibit antiprogestational activity.[46] Data from these studies suggest that bulky additions at C-7, when these include an aromatic ring, reduce PGR affinity by approximately 10−1000-fold.[46] Consequently, it may be possible to reduce the endogenous toxicity of progesterone by reducing/eliminating its ability to bind PGR.

On the basis of the various structure−function observations noted above, we hypothesized that progesterone, substituted at C-7 with an aromatic moiety(s), would exhibit both reduced Pgp affinity and significantly increased antiPgp activity. Thus, we designed an initial compound from which we could derive an appropriate series of progesterone analogues for evaluation. This compound, 7α-[4′-(aminophenyl)thio]pregna-4-ene-3,20-dione (**2**), has a C-7 thiol bridge linking an aminophenyl moiety to progesterone. Subsequent additions to the amine with the appropriate isocyanate generated the corresponding Pgp analogues. For our initial series of compounds, we selected isocyanates that would provide analogues with predicted differences in the size, lipophilicity, and charge of their C-7 additions.

Synthesis. Compound **1** (Scheme 1) was prepared from progesterone by a modified Turner and Ringold's

Scheme 1. Synthesis of C-7 Progesterone Analogues

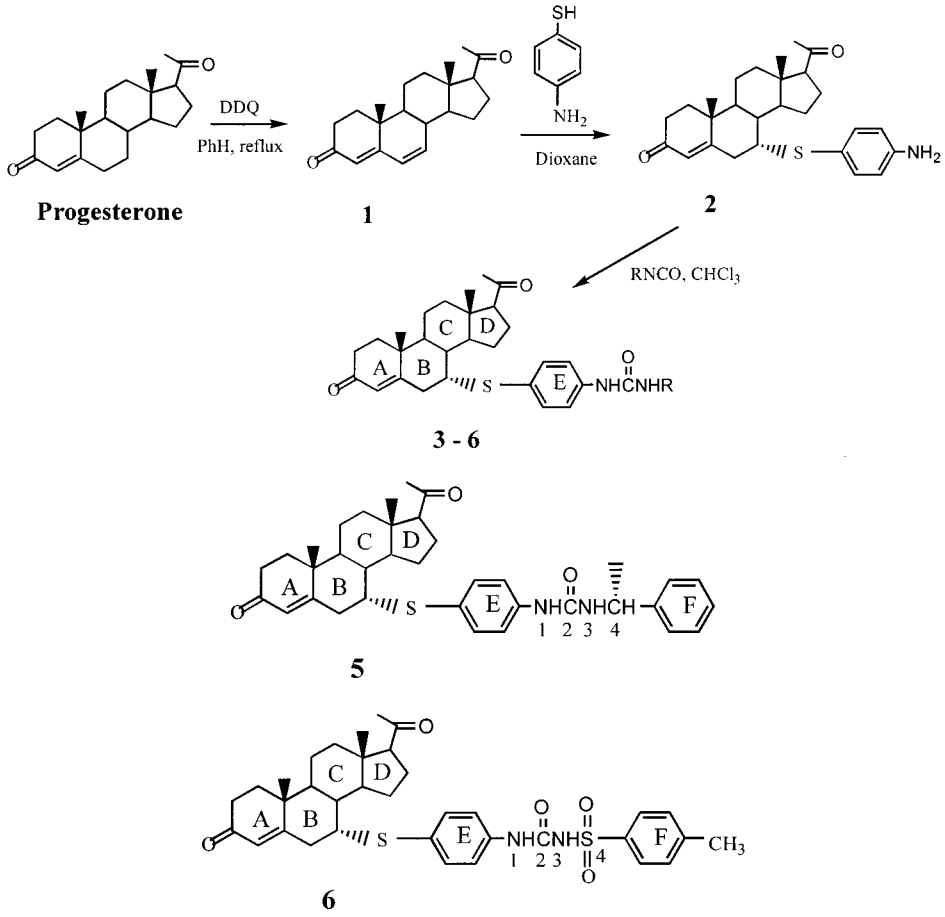

Table 1. Structure and Physical Properties of C-7 Progesterone Analogues[a]

compd	R	mp (deg)	R_f [b]
2	N/A	228−230	0.23
3	$-CH_2CH_2Cl$	137−141	0.47
4	$-CH_2CH_3$	130−135	0.36
5	$-CH(CH_3)C_6H_5$	146−149	0.46
6	$-SO_2C_6H_4CH_3$	128−132	0.29

[a] See Scheme 1 for the structures of **2**−**6**. [b] R_f in hexane−ethyl acetate (2:3).

method,[47−49] using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) as an oxidizing agent and *p*-toluenesulfonic acid (*p*-TsOH) in refluxing benzene via Dean−Stark distillation. Purification of the crude **1** on silica gel gives 6-dehydroprogesterone (**1**) as a yellow solid (35%, R_f = 0.44, 2:3 hexanes−ethyl acetate, mp = 143−145 °C). Reaction of compound **1** with 4-aminothiophenol and NaOH pellets, in degassed dioxane as solvent for 6 days at 74 °C, provided 7α-[(4′-aminophenyl)thio]pregna-4-ene-3,20-dione (**2**) as an ivory solid. Crude crystals of **2** were precipitated from a mixture of hexanes−ethyl acetate and purified by flash column chromatography to yield 790 mg of white solid (61%, R_f = 0.23, 2:3 hexanes−ethyl acetate, mp = 228−230 °C).

The additional C-7 progesterone analogues (**3**−**6**) were obtained by reacting compound **2** with the appropriate isocyanate (Table 1). The general reaction was performed under a N_2 atmosphere for 12 h, until no **2** was detected by thin-layer chromatography (TLC), and the solvent was removed under reduced pressure. All crude analogues were purified by flash column chromatography to yield the corresponding ureas as a white solid (40−83%). The physical properties of these analogues are provided in Table 1 as mp and R_f on silica gels (2:3 hexanes−ethyl acetate).

Results and Discussion

Substrate Accumulation Studies. Because Pgp is an efflux pump, we measured the ability of our compounds to influence the intracellular accumulation of the cytotoxic drugs vinblastine (VBL) and DOX. Both drugs are widely used clinically and are efficiently effluxed by Pgp.[50] Activity was evaluated in MDR1-transduced human breast cancer cells (MDA435/LCC6^{MDR1}), using the parental cells (MDA435/LCC6) as the Pgp-negative control. Potency of the compounds was compared with that of progesterone and the established Pgp inhibitors verapamil and cyclosporin A. VBL content in Pgp-positive cells, exposed to media containing 5 nM [^{3}H] VBL, was approximately 6-fold lower than in parental Pgp-negative cells. Cellular content of DOX, in cells exposed to 4 μM DOX, was about 8-fold lower in the presence than in the absence of Pgp.

Results of VBL and DOX accumulation studies, summarized in terms of EC_{50}, are presented in Table 2. Because these data were estimated from dose response curves, representative curves are shown in Figure 1. Treatment with progesterone analogues **3**−**6** reverses the difference in VBL and DOX content between Pgp-positive and Pgp-negative cells. Analogues **3**−**5** exhibit significantly increased antiPgp potency as compared

Table 2. Potency of C-7 Progesterone Analogues, Progesterone, Verapamil, and Cyclosporin A in Reversing the Difference in VBL and DOX Accumulation between Pgp-Negative and Pgp-Positive Cells

	reversal of [^{3}H] VBL accumulation		reversal of DOX accumulation	
compd	EC_{50} μM[a] (relative potency)[b]	Pgp-specific EC_{50}[c] μM (relative potency)	EC_{50} μM (relative potency)	Pgp-specific EC_{50} μM (relative potency)
progesterone	18.7 ± 3.7[d] (1)	21.0 ± 4.2 (1)	22.3 ± 2.0 (1)	42.2 ± 7.2 (1)
3	0.8 ± 0.2 (22.5)	0.9 ± 0.2 (23.5)	0.6 ± 0.1 (40.5)	0.7 ± 0.2 (60.2)
4	1.3 ± 0.1 (14.2)	1.5 ± 0.2 (14.0)	0.7 ± 0.07 (31.3)	1.0 ± 0.06 (42.7)
5	0.8 ± 0.2 (24.5)	0.7 ± 0.2 (28.2)	0.6 ± 0.07 (37.2)	0.9 ± 0.09 (44.8)
6	34.8 ± 8.6 (0.5)	33.4 ± 5.2 (0.6)	14.7 ± 3.2 (1.5)	37.0 ± 6.5 (1.1)
verapamil	1.2 ± 0.2 (16.1)	3.1 ± 0.9 (6.8)	2.4 ± 0.3 (9.2)	4.1 ± 0.5 (10.2)
cyclosporin A	0.6 ± 0.06 (32.5)	0.6 ± 0.06 (32.5)	0.5 ± 0.1 (41.9)	0.7 ± 0.2 (60.6)

[a] EC_{50} = drug concentration that reduces the difference in drug accumulation between MDA435/LCC6 and MDA435/LCC6^{MDR1} cells by 50%; obtained by interpolation on dose response curves. Representative curves are shown in Figures 1 ([^{3}H] VBL) and 2 (DOX). [b] Values in parentheses represent the potency of each compound relative to the lead compound progesterone. [c] Pgp-specific EC_{50} = data corrected for any effect of test compound on drug accumulation in MDA435/LCC6 (Pgp-negative) cells. [d] Values represent the mean ± SE obtained from at least three independent experiments.

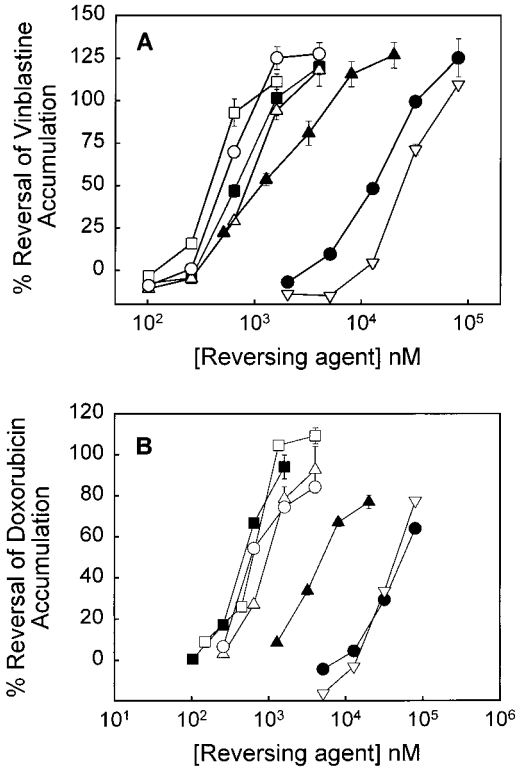

Figure 1. Ability of C-7 progesterone analogues to affect [^{3}H] VBL accumulation (A) and DOX accumulation (B) in MDA435/LCC6 and MDA435/LCC6^{MDR1} cells. Data (mean ± SE) are from one of three or more representative experiments used to obtain the ED_{50} values presented in Table 2. Progesterone = ●, cyclosporin A = ○, verapamil = ▲, **3** = ■, **4** = △, **5** = □, and **6** = ▽.

with progesterone, being 14−60-fold more potent. In marked contrast, **6** is only equipotent with progesterone. Three compounds (**3**−**5**) are significantly more potent than verapamil, when Pgp-specific EC_{50}s are compared for both VBL and DOX accumulation. Compounds **3** and **5** were equipotent with cyclosporin A ($p > 0.05$ for all comparisons). Recently, we have established a chromatographic approach for assessing relative Pgp binding affinities.[51,52] Studies to measure the affinity of these analogues are in progress.

While **3** and **5** tend to be slightly more potent than **4**, the difference is not statistically significant. This suggests that addition of either a Cl (**3**) or a second aromatic ring (**5**) does not further increase activity. In marked contrast, the presence of the sulfonyl group in **6** eliminates the gain in activity conferred by the C-7 moiety. Thus, the increased activity in **5** is not simply due to the presence of an aromatic F ring (Scheme 1). Further structural modifications will allow us to test further the structure−activity relationships of C-7 progesterone analogues for Pgp reversal.

A major problem with many existing antiPgp compounds is their intrinsic toxicity. We wished to obtain an in vitro assessment of the toxicity of our compounds relative to progesterone, verapamil, and cyclosporin A. We used our breast cancer cell models because they do not express PGR and would provide a simple model for assessing PGR-independent cytotoxicity. Furthermore, any reduction in toxicity seen in the MDA435/LCC6^{MDR1} cells, as compared with the MDA435/LCC6 cells (relative resistance of Pgp-positive cells in Table 3), would suggest that the compounds were Pgp substrates, not simply Pgp inhibitors. Results are summarized in terms of IC_{50} in Table 3; representative dose response curves are shown in Figure 2.

To estimate relative activity, each drug's intrinsic cytotoxicity was expressed relative to its antiPgp activity (IC_{50}/EC_{50}; Table 3). We did not detect cytotoxicity for compounds **4** and **5**, due to their low solubility, rendering our ratios underestimates based on the highest (noncytotoxic) concentration tested. Nonetheless, **4** produces ~40% inhibition of proliferation at 20 μM. In marked contrast, 20 μM **5** does not inhibit proliferation significantly in either untreated cells or MDA435/LCC6 and MDA435/LCC6^{MDR1} cells.

When adjusted for cytotoxicity, cyclosporin A and progesterone exhibit approximately equivalent relative activities. The low estimates for cyclosporin A reflect its substantial toxicity. Compound **6** is the least active compound, and **5** is the most active despite the overestimation of its cellular toxicity. While VBL and DOX may have different recognition sites in Pgp,[53] **5** shows broadly comparable activity against both drugs, as does cyclosporin A.

Having established that the C-7 addition significantly increased antiPgp activity, we wished to evaluate the PGR activity of our best compound. Overall, **3** and **5** have antiPgp activity comparable to cyclosporin A. Because **3** exhibits significant cellular toxicity, we chose to evaluate the relative affinity of **5** for binding to PGR. We compared the ability of **5**, progesterone, and unlabeled ORG2058, a synthetic progestin, to compete with [^{3}H] ORG2058 for binding to PGR. The data in Figure

Table 3. Growth Inhibitory Activity of C-7 Progesterone Analogue, Progesterone, Verapamil, and Cyclosporin A on MDA435/LCC6 (Pgp-Positive) and MDA435/LCC6^{MDR1} (Pgp-Negative) Human Breast Cancer Cells

	IC_{50} μM			IC_{50}/EC_{50}[c]	
compd	MDA435/LCC6 (relative cytotoxicity)[a]	MDA435/LCC6^{MDR1} (relative cytotoxicity)	relative resistance of Pgp-positive cells[b]	VBL activity	DOX activity
progesterone	27.4 ± 7.9[d](1.0)	36.4 ± 8.3 (1.0)	1.4 ± 0.09	1.3	0.6
3	3.2 ± 0.09 (8.5)	7.3 ± 2.5 (5.0)	2.2 ± 0.7	3.6	3.2
4	>20.0 (ND)[e]	>20.0 (ND)	ND	>13.3	>20.0
5	>20.0 (ND)	>20.0 (ND)	ND	>28.6	>22.2
6	22.1 ± 1.6 (1.2)	38.2 ± 0.2 (1.0)	1.7 ± 0.1	0.7	0.6
verapamil	65.8 ± 0.04 (0.4)	63.4 ± 1.2 (0.6)	1.0 ± 0.02	21.2	16.0
cyclosporin A	1.0 ± 0.5 (26.9)	1.1 ± 0.2 (33.6)	1.3 ± 0.4	1.7	1.4

[a] Relative cytotoxicity = ability of compounds to inhibit cell growth relative to progesterone. [b] Relative resistance of Pgp-positive = ratio of toxicity in resistant and control cells. Relative resistance > 1 suggests that the compound is at least partly effluxed by Pgp. [c] This ratio relates the effect of each drug on either VBL or DOX accumulation to its intrinsic cellular toxicity; higher ratios suggest a greater degree of safety. [d] Values represent the mean ± SE obtained from at least three independent experiments. [e] ND = no data; an IC50 was not reached at the highest concentration tested (limited by solubility). Some values (>) are underestimates based on nontoxic concentrations.

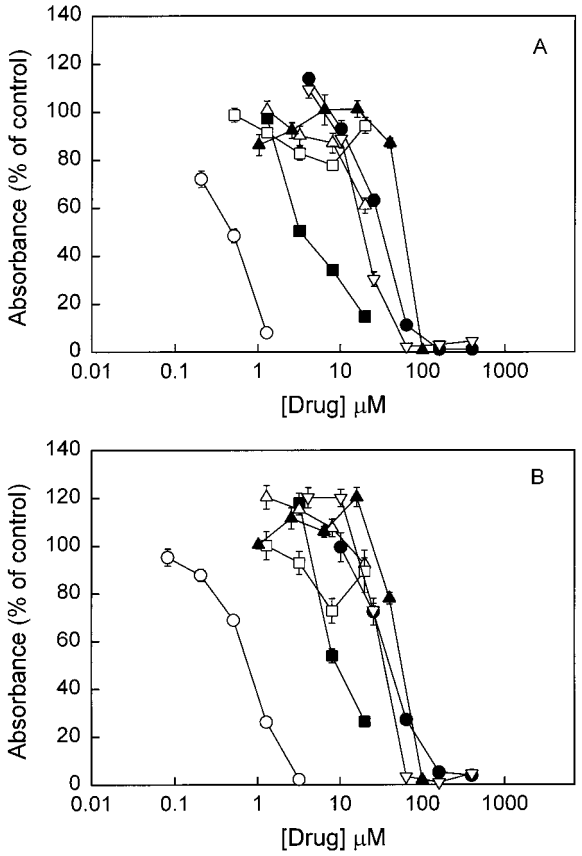

Figure 2. Cytotoxicity of progesterone analogues in MDA435/LCC6 (A) and MDA435/LCC6^{MDR1} cells (B). Data (mean ± SE) are from one of three or more representative experiments used to obtain the IC_{50} values presented in Table 3. Progesterone = ●, cyclosporin A = ○, verapamil = ▲, **3** = ■, **4** = △, **5** = □, and **6** = ▽.

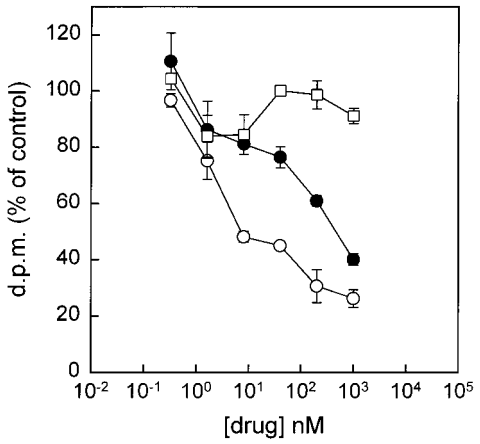

Figure 3. Competitive binding of progesterone, ORG2058, and **5** to PGR. [^{3}H] ORG2058 was used as the radiolabeled ligand. Progesterone = ●, ORG2058 = ○, and **5** = □.

3 show that **5**, at concentrations up to its EC_{50}, does not significantly compete with ORG2058. This represents a reduction of >100-fold in its PGR affinity as compared with ORG2058. Thus, **5** has antiPgp activity comparable to cyclosporin A, exhibits potentially low intrinsic cellular toxicity, and does not bind to its predicted cellular target (PGR) at its EC_{50} for inhibition of Pgp activity.

Conclusions

While we cannot draw definitive structure−activity conclusions, some potentially useful preliminary observations can guide future studies. The molecules are clearly amphipathic, with lipophilicity greatest around the "E" and/or "F" rings and the polarity greatest around C-17−C-21. These observations suggest that effective substrates may concurrently interact with both hydrophilic and hydrophobic regions. However, it is not clear whether these are both in Pgp as previously suggested[31] or whether they represent pockets at the plasma membrane/Pgp interface. The possibility that drugs are removed from within the plasma membrane[6] may favor the model that invokes a plasma membrane component to the binding interaction.

Compounds **3**−**5** are significantly more potent than progesterone at specifically increasing Pgp substrate accumulation. These observations are consistent with our initial hypothesis that aromatic C-7 substitutions of progesterone will increase activity and with the known contribution of aromatic moieties in other modulating agents.[37,42] Compound **6** also contains a C-7 aromatic addition but is essentially equipotent with progesterone. Perhaps the simplest explanation is that this compound is the least lipophilic of the analogues, since lipophilicity appears to be a major factor in the activity of other Pgp substrates.[31−35]

The significant increase in potency observed with compounds **3**−**5** supports our initial structure−function-based hypothesis, based on previous published observations. The activity of our existing compounds already compares well with that of cyclosporin A. C-7 progest-

erone analogues have the potential to provide more potent, selective, and safe inhibitors of Pgp function than others that have currently completed clinical trials. We believe that the observations reported here, combined with the lack of receptor binding activity, identify **5** as the next logical lead compound for further development and provide valuable clues for the further optimization of this structure. We are currently synthesizing a larger series of compounds to further optimize the MDR1 reversing potency and effectively define the structure−activity relationships of these compounds.

Our ability to increase the potency of progesterone up to 60-fold (**3**; Pgp-specific EC_{50} for DOX accumulation) supports the use of relatively limited structure−function data in the design of effective antiPgp compounds. Furthermore, by including structure−activity information on the binding characteristics of the lead's natural intracellular target (PGR) in our conceptualization, we reduced affinity of **5** for a target that could produce toxicity in normal cells. We are now poised to evaluate our compounds in vivo, to pursue further modifications that may increase antiPgp activity, and to explore the structure−activity relationship for C-7 progesterone analogues in detail. Overall, the data in this study identify C-7-substituted progesterone analogues and **5**, in particular, as rationally designed antiPgp compounds worthy of further evaluation/development.

Experimental Section

Chemistry. General Procedures. All reactions were carried out under an atmosphere of nitrogen using standard Schlenk techniques.[54] Benzene and chloroform were distilled from CaH_2, stored over 3D molecular sieves, and deaerated by purging with nitrogen immediately before use. TLC was performed using Merck glass plates precoated with F_{254} silica gel 60; compounds were visualized by UV and/or with *p*-anisaldehyde stain solution. Flash chromatography was performed using EM Science silica gel 60, following the procedure of Still,[55] with the solvent mixtures indicated. Melting points were measured on a Thomas-Hoover capillary melting point apparatus and are uncorrected (Table 1). The broad melting points for compounds **3**−**6** suggest the presence of minor impurities. All reagents were purchased from commercial suppliers and used as received unless indicated otherwise. Dioxane was purchased from Aldrich in Sure-Seal bottles.

Nuclear magnetic resonance (NMR) spectra were measured on Nicolet NT 270 and Varian Mercury 300 MHz instruments at the Georgetown NMR Facility. Chemical shifts are reported in units of parts per million relative to Me_4Si. All spectra are recorded in $CDCl_3$. Significant ^{1}H NMR data are tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), coupling constants in Hertz, and number of protons. ^{13}C NMR spectra were recorded at frequencies of 67.9 and 75.6 MHz. Infrared (IR) spectra were measured on a MIDAC Corp. or a Mattson Galaxy 2020 Series FTIR, as neat films; absorption bands are reported in cm^{-1}. Low-resolution mass spectra were measured on a Fisons Instruments MD 800 quadrupole mass spectrometer, with 70 EV electron ionization and a GC 8000 Series gas chromatograph inlet, and using a J & W Scientific DB-5MS column (15 m length, 0.25 mm internal diameter, 0.25 μm film thickness). Mass spectra data are given as mass-to-charge ratio, with the relative peak height following in parentheses. All new compounds were characterized by ^{1}H NMR, IR, and ^{13}C NMR spectroscopies. Fast atom bombardment mass spectra (FABMS) were recorded at the University of Maryland College Park of Mass Spectrometry Facility. Literature references are given for all known compounds, except for those that are commercially available; all known compounds were identified by ^{1}H NMR spectroscopy. Elemental analysis was performed by Atlantic Microlab (Norcross, GA).

Pregna-4,6-diene-3,20-dione (1). Compound **1** was prepared by the method of Tuner and Ringold.[47,49] Thus, *p*-TsOH monohydrate (11.0 g, 63.9 mmol) was dehydrated in freshly distilled benzene (320 mL) by azeotropic refluxing using a Dean−Stark trap. After 1 h, the solution was cooled for 0.5 h, and progesterone (5.0 g, 15.9 mmol) and DDQ (4.6 g, 20.3 mmol) were added. The olive mixture was refluxed for 3 h and then filtered through a pad of Celite. The filtrate was washed with saturated NaCl (5 × 20 mL) followed by 1% NaOH solution until it gave clear solution and dried over anhydrous $MgSO_4$. Solvent was removed under reduced pressure, and the filtrate was purified by chromatography; 1.69 g of product (35%, R_f = 0.44, 2:3 hexanes−ethyl acetate); yellow solid (mp = 143−145 °C). ^{1}H NMR: δ 6.12 (s, 1H), 5.69 (s, 1H), 2.84−1.12 (complex, 12H), 2.17 (s, 3H), 2.14 (s, 3H), 1.12 (s, 3H), 1.10 (s, 1H), 1.00 (s, 1H), 0.72 (s, 3H). IR: 3855, 3745, 3678, 2953, 1700, 1663, 1457, 1361, 1223, 875, 754.

7α-[4′-(Aminophenyl)thio]pregna-4-ene-3,20-dione (2). We obtained **2** using the method of Brueggemeier et al.[56] Briefly, **1** (1.65 g, 5.28 mmol), 4-aminothiophenol (1.32 g, 10.56 mmol), and NaOH (pellet, 116 mg, 2.9 mmol) were placed in a Schlenk tube, which was purged with a constant flow of N_2(g). Deoxygenated anhydrous dioxane (25 mL) was added and heated at 74 °C for 6 days. The mixture was concentrated under reduced pressure and purified by chromatography; 790 mg white solid (61%, R_f = 0.23, 2:3 hexanes−ethyl acetate); mp = 228−230 °C). ^{1}H NMR: δ 7.26−7.21 (q, J = 8.5 Hz, 2H), 6.64−6.61 (q, J = 8.5 Hz, 2H), 5.73 (s, 1H), 3.77 (s, 2H), 3.24 (s, 1H), 2.14 (s, 3H), 2.63−1.10 (complex, 11H), 1.19 (s, 3H), 0.69 (s, 3H). IR: 3420, 3360, 3250, 2930, 1700. ^{13}C NMR: δ 209.3, 199.0, 167.6, 147.1, 136.6, 127.3, 121.2, 115.7, 63.4, 17.7, 13.1.

General Procedure for the Preparation of Progesterone Analogues. A suspension of **2** in degassed chloroform was treated with the appropriate isocyanates under N_2. The mixture was stirred for 12 h and then chromatographed directly on silica gel to afford the corresponding ureas as oil. The resulting oil was stirred in anhydrous ether until white powder came out.

7α-[4′-(*N*-Chloroethylaminoacylaminophenyl)thio]-pregna-4-ene-3,20-dione (3). Reaction of **2** (0.10 g, 0.23 mmol) with 2-chloroethylisocyanate (38 μL, 0.46 mmol) in $CHCl_3$ (3.0 mL) for 12 h gave 50 mg of product (40%, mp = 137−141 °C, R_f = 0.47, 2:3 hexanes−ethyl acetate). ^{1}H NMR: δ 7.34−7.25 (m, 4H), 5.69 (s, 1H), 5.18 (s, 1H), 3.68−3.62 (m, 4H), 3.38 (s, 1H), 2.64−0.84 (complex, 18H), 2.14 (s, 3H), 1.19 (s, 3H), 0.69 (s, 3H). IR: 3312, 2964, 1700, 1630, 1587, 1517, 1488, 1449, 1394, 1238, 1013, 831, 734. ^{13}C NMR: δ 231.5, 210.3, 196.2, 193.9, 181.9, 156.5, 149.3, 146.4, 141.4, 132.9, 125.1, 119.5, 118.5, 103.2, 94.2, 75.9, 75.8, 71.9, 69.3, 49.0, 35.8, 24.2, 14.4. MS: m/e = 543 (24, $M^+ + 1$), 507 (10), 313 (27), 230 (23), 185 (50), 149 (69), 125 (57), 119 (23), 107 (38), 105 (48), 91 (50), 81 (50), 57 (73), 55 (100). HRMS: calcd for $C_{30}H_{39}N_2O_3SCl$ $[M + H]^+$, 543.24481; found, 543.24248. Anal. Calcd for ($C_{30}H_{40}O_3N_2SCl$): C, 66.22; H, 7.41; N, 8.82; S, 6.52. Found: C, 66.38; H, 7.27; N, 8.78; S, 6.28.

7α-[4′-(*N*-Ethylaminoacylaminophenyl)thio]pregna-4-ene-3,20-dione (4). Reaction of **2** (0.10 g, 0.23 mmol) with ethylisocyanate (37 μL, 0.46 mmol) in $CHCl_3$ (3.0 mL) for 12 h gave 78 mg of product (67%, mp = 130−135 °C, R_f = 0.36, 2:3 hexanes−ethyl acetate). ^{1}H NMR: δ 7.36−7.25 (m, 4H), 6.38 (s, 1H), 5.69 (s, 1H), 4.18−4.03 (m, 2H), 3.38−3.26 (m, 2H), 2.67−0.68 (complex, 17H), 2.14 (s, 3H), 2.05 (s, 2H), 1.20 (s, 3H), 0.69 (s, 3H). IR: 3855, 3745, 3678, 3373, 2953, 2359, 1700, 1663, 1539, 1457, 1223. ^{13}C NMR: δ 228.5, 222.5, 193.9, 171.5, 141.5, 135.0, 128.3, 127.0, 123.4, 118.5, 108.7, 96.2, 84.5, 69.3, 67.7, 66.0, 62.6, 52.2, 48.4, 46.3, 43.9, 39.8, 34.1, 22.9, 21.2, 13.4. MS: m/e = 509 (62, $M^+ + 1$), 438 (8), 313 (32), 196 (47), 125 (100), 117 (57), 97 (52), 95 (85), 79 (68), 71 (59). HRMS: calcd for $C_{30}H_{40}N_2O_3S$ $[M + H]^+$, 509.28378; found, 509.28372. Anal. Calcd for ($C_{30}H_{41}O_3N_2S$): C, 70.69; H, 8.11; N, 5.49; S, 6.29. Found: C, 70.46; H, 8.06; N, 5.52; S, 6.20.

7α-[4′-(*N*-α-(+)-Methylbenzylaminoacylaminophenyl)-thio]pregna-4-ene-3,20-dione (5). Reaction of **2** (0.10 g, 0.23 mmol) with (*R*)-(+)-α-methylbenzylisocyanate (66 μL, 0.46 mmol) in $CHCl_3$ (3.0 mL) for 12 h gave 56 mg of product (46%, mp = 146−149 °C, R_f = 0.46, 2:3 hexanes−ethyl acetate). ^{1}H NMR: δ 7.32−7.25 (m, 5H), 5.79−5.77 (m, 1H), 5.70−5.68 (s, 1H), 4.97−4.92 (m, 1H), 4.13−4.06 (m, 1H), 3.28 (s, 1H), 2.64−1.49 (complex, 7H), 2.14 (s, 3H), 1.45 (d, J = 9.3 Hz, 3H), 1.19 (s, 3H), 0.68 (s, 3H). IR: 3353, 3273, 2949, 2854, 2362, 2340, 1700, 1653, 1595, 1539, 1457, 1460, 1376, 1343, 1159, 1089, 916. ^{13}C NMR: δ 209.4, 199.0, 167.6, 147.0, 136.6, 127.2, 121.2, 115.8, 63.4, 17.7, 13.1. MS: *m*/*e* = 585 (11, $M^+ + 1$), 135 (12), 125 (20), 105 (100), 103 (22), 91 (29), 77 (22), 55 (26). HRMS: calcd for $C_{36}H_{44}N_2O_3S$ $[M + H]^+$, 585.31506; found, 585.31501. Anal. Calcd for ($C_{36}H_{45}O_3N_2S$): C, 73.81; H, 7.74; N, 4.78; S, 5.47. Found: C, 73.76; H, 7.79; N, 4.81; S, 5.39.

7α-[4′-(*N*-*p*-Toluenesulfonylaminoacylaminophenyl)-thio]pregna-4-ene-3,20-dione (6). Reaction of **2** (0.10 g, 0.23 mmol) with *p*-toluenesulfonylisocyanate (59 μL, 0.46 mmol) in $CHCl_3$ (3.0 mL) for 12 h gave 120 mg of product (83%, mp = 128−132 °C, R_f = 0.29, 2:3 hexanes−ethyl acetate). ^{1}H NMR: δ 8.38 (s, 1H), 7.88 (d, J = 8.4 Hz, 2H), 7.80 (d, J = 8.3 Hz, 2H), 7.37−7.25 (m, 4H), 5.70 (s, 1H), 3.36 (s, 1H), 2.67−1.13 (complex, 20H), 2.41 (s, 3H), 2.15 (s, 3H), 1.55 (m, 2H), 1.20 (s, 3H), 0.69 (s, 3H). IR: 3855, 3745, 2359, 1700, 1539, 1457, 1160, 1086, 668. ^{13}C NMR: δ 198.6, 148.6, 141.4, 136.6, 134.6, 129.9, 129.7, 129.6, 127.7, 127.2, 126.4, 120.5, 118.5, 92.4, 76.1, 69.3, 63.3, 52.1, 51.1, 47.0, 46.3, 39.8, 39.4, 38.5, 38.1, 35.4, 34.0, 31.6, 23.7, 22.9, 21.8, 21.1, 17.9, 13.4. MS: *m*/*e* = 635 (29, $M^+ + 1$), 313 (39), 155 (33), 135 (36), 125 (65), 119 (64), 91 (100), 85 (92), 77 (47), 59 (50), 47 (45). HRMS: calcd for $C_{35}H_{42}N_2O_5S_2$ $[M + H]^+$, 635.26135; found, 635.26130. Anal. Calcd for ($C_{35}H_{43}O_5N_2S_2$): C, 66.11; H, 6.82; N, 4.41; S, 10.09. Found: C, 66.05; H, 6.79; N, 4.45; S, 10.02.

Pharmacology. Cell Lines. For the studies of antiPgp activity, we used cells transduced with a retroviral vector directing the constitutive expression of the Pgp gene (MDA435/LCC6^{MDR1}) and their parental, Pgp-negative, MDA435/LCC6 human breast cancer cells.[17] Both MDA435/LCC6 and MDA435/LCC6^{MDR1} cells are estrogen receptor and PGR negative and grow as monolayer cultures in vitro and as rapidly proliferating solid tumors and malignant ascites in vivo in nude mice.[17] We used MCF-7 human breast cancer cells[57] to measure binding to PGR. These cells were routinely grown in vitro in Improved Minimal Essential Media (Biofluids) containing 5% fetal bovine serum in a 5% CO_2:95% air atmosphere.

Substrate Accumulation Assays. Pgp reversing activity of all test agents was evaluated by measuring the ability of the agents to affect accumulation of DOX and VBL in MDA435/LCC6^{MDR1} (resistant) and MDA435/LCC6 (control) cells. Cells were plated at 2.5×10^5 cells/well into 24 well culture dishes, in routine growth media, and incubated at 37 °C. Forty-eight hours after they were plated, cells were treated by replacing growth media with media containing the test compounds at five different concentrations and either DOX (4 μM) or [^{3}H] VBL (5 nM). All treatments were carried out in triplicate. After 3 h of incubation, treatments were stopped by washing each well with 0.5 mL of ice-cold NaCl (0.15 M). Cells from reference wells in each plate were counted to enable accumulation to be corrected for cell number.

For the DOX accumulation assays, DOX was extracted from cell monolayers by adding 1.5 mL of 20% trichloroacetic acid to each well and incubating overnight at 4 °C in the dark. DOX concentrations in the extracts were evaluated fluorometrically. Thus, extracts were transferred into 13mm × 100 mm borosilicate glass tubes, placed in the 10 × 10 rack of a Hitachi A3000 Autosampler and connected to a Hitachi F-4500 fluorescence spectrophotometer. Fluorescence was read at 500 nm excitation and 580 nm emission wavelengths. Concentrations of DOX were obtained by interpolation on a DOX standard curve and normalized on the extraction volume and number of cells per well. For the VBL accumulation assays, at the end of treatment, wells were rinsed with phosphate-buffered saline (0.5 mL/well) and left to dry at room temperature. Cell monolayers were removed by trypsinization and diluted with 10 mL of scintillation fluid (Ultima Gold XR, Packard Bioscience, Meriden, CT). Drug accumulation was radiometrically assessed by scintillation spectrometry.

Results of substrate accumulation assays were plotted as cellular concentration of substrate against the concentration of the respective test compound. Pgp reversing potency was expressed as the EC_{50}, defined as the concentration of a test drug that reduced the difference in substrate accumulation between Pgp-negative and Pgp-positive cells by 50%. Progesterone and the standard Pgp reversing agents verapamil and cyclosporin A were used as positive controls and as a reference to establish relative potency.

Cytotoxicity. Twenty-four hours after they were plated in 96 well plates, MDA435/LCC6 and MDA435/LCC6^{MDR1} cells were exposed to growth media containing different concentrations of the test agents for 5 days. Cell cultures were then fixed/stained by incubation in a 0.5% (w/v) crystal violet solution in 25% methanol (v/v). After plates had dried, the dye was extracted in 0.1 M sodium citrate in 25% methanol (v/v) and absorbance was read at 540 nm using a microplate spectrophotometer. Absorbance directly correlates with cell number in this assay.[50] Cell survival curves were obtained by plotting absorbance values (as percent of untreated controls) against drug concentration. The toxicity of each drug was expressed as an IC_{50}, defined as the concentration inhibiting cell density by 50% at the end of the treatment period. To estimate the extent of resistance conferred by Pgp, the ratio of each drug's IC_{50} in MDA435/LCC6^{MDR1} and MDA435/LCC6 cells (relative resistance of Pgp-positive cells) was used for those drugs that produced a detectable IC_{50} value.

Radioligand Binding Studies. These were performed as previously described, using a whole cell competitive binding assay.[57,58] Briefly, MCF-7 cells were grown in 24 well dishes and incubated at 37 °C with 100 nM hydrocortisone for 30 min, before determining PGR binding, to eliminate residual binding to glucocorticoid receptors. Subsequently, cells were incubated for 60 min at 37 °C with 5 nM [^{3}H] ORG2058 (specific activity 50.6 Ci/mmol) in the absence or presence of increasing concentrations of unlabeled competitor (0.5 nM−1 μM; progesterone, ORG2058, **5**). Radioactivity was extracted into ethanol and measured in a liquid scintillation spectrometer.

Data Analysis. DOX accumulation and cytotoxicity dose response data were processed and graphed using SigmaPlot 4.0 (SPSS Science, Chicago, IL). EC_{50} (DOX accumulation assays) and IC_{50} values (cytotoxicity assays) were calculated by interpolation on the respective dose response curves. The EC_{50} and IC_{50} values reported in Tables 2 and 3 represent the mean and standard error (SE) obtained from at least three independent experiments. Descriptive statistics were obtained using SigmaStat 2.0 (SPSS Science).

Acknowledgment. This work was supported in part by the Department of Defense, United States Army Medical Research and Materiel Command, Award RP950649, and by a grant from the Susan G. Komen Breast Cancer Foundation, Award PDF 2000 186.

References

(1) Ueda, K.; Clark, D. P.; Chen, C. J.; Roninson, I. B.; Gottesman, M. M.; Pastan, I. The human multidrug resistance (MDR1) gene. cDNA cloning and transcription initiation. *J. Biol. Chem.* **1987**, *262*, 505−508.

(2) Pohl, G.; Filipits, M.; Suchomel, R. W.; Stranzl, T.; Depisch, D.; Pirker, R. Expression of the lung resistance protein (LRP) in primary breast cancer. *Anticancer Res.* **1999**, *19*, 5051−5055.

(3) Doyle, L. A.; Yang, W.; Abruzzo, L. V.; Krogmann, T.; Gao, Y.; Rishi, A. K.; Ross, D. D. A multidrug resistance transporter from human MCF-7 breast cancer cells. *Proc. Natl. Acad. Sci U.S.A.* **1998**, *95*, 15665−15670.

(4) Nooter, K.; de la Riviere, B. G.; Look, N. P.; van Wingerden, K. E.; Henzen-Logmans, S. C.; Scheper, R. J.; Flens, M. J.; Klijn, J. G. M.; Stoter, G.; Foekens, J. A. The prognostic significance of expression of the multidrug resistance-associated protein (MRP) in primary breast cancer. *Br. J. Cancer* **1997**, *76*, 486−493.

(5) Borst, P.; Evers, R.; Kool, M.; Wijnholds, J. A family of drug transporters: the multidrug resistance-associated proteins. *J. Natl. Cancer Inst.* **2000**, *92*, 1295−1302.
(6) Raviv, Y.; Pollard, H. B.; Bruggemann, E. P.; Pastan, I.; Gottesman, M. M. Photosensitized labeling of a functional multidrug transporter in living drug-resistant tumor cells. *J. Biol. Chem.* **1990**, *265*, 3975−3980.
(7) Nuti, S. L.; Mehdi, A.; Rao, U. S. Activation of the human P-glycoprotein ATPase by trypsin. *Biochemistry* **2000**, *39*, 3424−3432.
(8) Trock, B. J.; Leonessa, F.; Clarke, R. Multidrug resistance in breast cancer: a meta analysis of MDR1/gp170 expression and its possible functional significance. *J. Natl. Cancer Inst.* **1997**, *89*, 917−931.
(9) Murren, J. R.; Hait, W. N. Why have not we cured multidrug resistant tumors? *Oncol. Res.* **1992**, *1*, 1−6.
(10) Horton, J. K.; Thimmaiah, K. N.; Houghton, J. A.; Horowitz, M. E.; Houghton, P. J. Modulation by verapamil of vincristine pharmacokinetics and toxicity in mice bearing human tumor xenografts. *Biochem. Pharmacol.* **1989**, *38*, 1727−1736.
(11) Miller, T. P.; Grogan, T. M.; Dalton, W. S.; Speir, C. M.; Schepner, R. J.; Salmon, S. E. P-glycoprotein expression in malignant lymphoma and reversal of clinical drug resistance with chemotherapy plus high-dose verapamil. *J. Clin. Oncol.* **1991**, *9*, 17−24.
(12) Dalton, W. S.; Grogan, T. M.; Meltzer, P. S.; Scheper, R. J.; Durie, B. G. M.; Taylor, C. W.; Miller, T. P.; Salmon, S. E. Drug-resistance in multiple myeloma and non-Hodgkin's lymphoma: detection of P-glycoprotein and potential circumvention by addition of verapamil to chemotherapy. *J. Clin. Oncol.* **1989**, *7*, 415−424.
(13) Miller, R. L.; Bukowski, R. M.; Budd, G. T.; Purvis, J.; Weick, J. K.; Shepard, K.; Midha, K. K.; Ganapathi, R. Clinical modulation of doxorubicin resistance by the calmodulin-inhibitor, trifluoperazine: a phase I/II trial. *J. Clin. Oncol.* **1988**, *6*, 880−888.
(14) Jorgenson, A.; Fredricson, O. K. Clopenthixol and flupenthixol depot preparations in outpatient schizophrenics. III. Serum levels. *Acta Psychiatr. Scand.* **1980**, *279*, 41−54.
(15) Ford, J. M.; Bruggemann, E. P.; Pastan, I.; Gottesman, M. M.; Hait, W. N. Cellular and biochemical characterization of thioxanthenes for reversal of multidrug resistance in human and murine cell lines. *Cancer Res.* **1990**, *50*, 1748−1756.
(16) Clarke, R.; Currier, S.; Kaplan, O.; Lovelace, E.; Boulay, V.; Gottesman, M. M.; Dickson, R. B. Effect of P-glycoprotein expression on sensitivity to hormones in MCF-7 human breast cancer cells. *J. Natl. Cancer Inst.* **1992**, *84*, 1506−1512.
(17) Leonessa, F.; Green, D.; Licht, T.; Wright, A.; Wingate-Legette, K.; Lippman, J.; Gottesman, M. M.; Clarke, R. MDA435/LCC6 and MDA435/LCC6^{MDR1}: ascites models of human breast cancer. *Br. J. Cancer* **1996**, *73*, 154−161.
(18) Scudiero, D. A.; Monks, A.; Sausville, E. A. Cell line designation change: multidrug-resistant cell line in the NCI anticancer screen. *J. Natl. Cancer Inst.* **1998**, *90*, 862−863.
(19) Batist, G.; Tuple, A.; Sinha, B. K.; Katki, A. G.; Myers, C. E.; Cowan, K. H. Overexpression of a novel anionic glutathione transferase in multidrug-resistant human breast cancer cells. *J. Biol. Chem.* **1986**, *261*, 15544−15549.
(20) Sinha, B. K.; Mimnaugh, E. G.; Rajagopalan, S.; Myers, C. E. Adriamycin activation and oxygen free radical formation in human breast tumor cells: protective role of glutathione peroxidase in adriamycin resistance. *Cancer Res.* **1989**, *49*, 3844−3848.
(21) van Kalken, C. K.; Broxterman, H. J.; Pinedo, H. M.; Feller, N.; Dekker, H.; Lankelman, J.; Giaccone, G. Cortisol is transported by the multidrug resistance gene product P-glycoprotein. *Br. J. Cancer* **1993**, *67*, 284−289.
(22) Wolf, D. C.; Horwitz, S. B. P-glycoprotein transports corticosterone and is photoaffinity- labeled by the steroid. *Int. J. Cancer* **1992**, *52*, 141−146.
(23) Yang, C.-P. H.; DePinho, S. G.; Greenberger, L. M.; Arceci, R. J.; Horwitz, S. B. Progesterone interacts with P-glycoprotein in multidrug-resistant cells and in the endometrium of gravid uterus. *J. Biol. Chem.* **1989**, *264*, 782−788.
(24) Arceci, R. J.; Croop, J. M.; Horwitz, S. B.; Housman, D. The gene encoding multidrug resistance is induced and expressed at high levels during pregnancy in the secretory epithelium of the uterus. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4350−4354.
(25) MacFarland, A.; Abramovich, D. R.; Ewen, S. W.; Pearson, C. K. Stage-specific distribution of P-glycoprotein in first-trimester and full-term human placenta. *Histochem. J.* **1994**, *26*, 417−423.
(26) Naito, M.; Yusa, K.; Tsuruo, T. Steroid hormones inhibit binding of Vinca alkaloid to multidrug resistance related P-glycoprotein. *Biochem. Biophys. Res. Commun.* **1989**, *158*, 1066−1071.
(27) Ichikawa-Haraguchi, M.; Sumizawa, T.; Yoshimura, A.; Furukawa, T.; Hiramoto, S.; Sugita, M.; Akiyama, S.-I. Progesterone and its metabolites: the potent inhibitors of the transporting activity of P-glycoprotein in the adrenal gland. *Biochim. Biophys. Acta* **1993**, *1158*, 201−208.
(28) Neef, G.; Beier, S.; Elger, W.; Henderson, D.; Wiechert, R. New steroids with antiprogestational and antiglucocorticoid activities. *Steroids* **1984**, *44*, 349−372.
(29) Teutsch, G.; Philibert, D. History and perspectives of antiprogestins from the chemist's point of view. *Hum. Reprod.* **1994**, *9* (Suppl. 1), 12−31.
(30) Ford, J. M.; Hait, W. N. Pharmacology of drugs that alter multidrug resistance in cancer. *Pharmacol. Rev.* **1990**, *42*, 155−199.
(31) Hait, W. N.; Aftab, D. T. Rational design and pre-clinical pharmacology of drugs for reversing multidrug resistance. *Biochem. Pharmacol.* **1992**, *43*, 103−107.
(32) Zamora, J. M.; Pearce, H. L.; Beck, W. T. Physical-chemical properties shared by compounds that modulate multidrug resistance in human leukemic cells. *Mol. Pharmacol.* **1988**, *33*, 454−462.
(33) Granzen, B.; Graves, D. E.; Baguley, B. C.; Danks, M. K.; Beck, W. T. Structure−activity studies of amsacrine analogues in drug resistant human leukemia cell lines expressing either altered DNA topoisomerase II or P-glycoprotein. *Oncol. Res.* **1992**, *4*, 489−496.
(34) Ford, J. M.; Prozialeck, W. C.; Hait, W. N. Structural features determining activity of phenothiazines and related drugs for inhibition of cell growth and reversal of multidrug resistance. *Mol. Pharmacol.* **1988**, *35*, 105−115.
(35) Tang-Wai, D. F.; Brossi, A.; Arnold, L. D.; Gros, P. The nitrogen of the acetamido group of colchicine modulates P-glycoprotein-mediated multidrug resistance. *Biochemistry* **1993**, *32*, 6470−6476.
(36) Klopman, G.; Srivastava, S.; Kolossvary, I.; Epand, R. F.; Ahmed, N.; Epand, R. M. Structure−activity study and design of multidrug reversal compounds by a computer automated structure evaluation methodology. *Cancer Res.* **1992**, *52*, 4121−4129.
(37) Dellinger, M.; Pressman, B. C.; Calderon-Higginson, C.; Savaraj, N.; Tapiero, H.; Kolonias, D.; Lampidis, T. J. Structural requirements of simple cations for recognition by multidrug-resistant cells. *Cancer Res.* **1992**, *52*, 6385−6389.
(38) Nogae, I.; Kohno, K.; Kikuchi, J.; Kuwano, M.; Akiyama, S.-I.; Kiue, A.; Suzuki, K.-I.; Yoshida, Y.; Cornwell, M.; Pastan, I.; Gottesman, M. M. Analysis of structural features of dihydropyridine analogues needed to reverse multidrug resistance and to inhibit photoaffinity labeling of P-glycoprotein. *Biochem. Pharmacol.* **1989**, *38*, 519−527.
(39) Horton, J. K.; Thimmaiah, K. N.; Harwood, F. C.; Kuttesch, J. F.; Houghton, P. J. Pharmacological characterization of *N*-substituted phenoxazines directed toward reversing *Vinca* alkaloid resistance in multidrug-resistance cancer cells. *Mol. Pharmacol.* **1993**, *44*, 552−559.
(40) Abraham, I.; Wolf, C. L.; Sampson, K. E. Nonglucocorticoid steroid analogues (21-aminosteroids) sensitize multidrug resistant cells to vinblastine. *Cancer Chemother. Pharmacol.* **1993**, *32*, 116−122.
(41) Dhainaut, A.; Régnier, G.; Atassi, G.; Pierré, A.; Léonce, S.; Kraus-Berthier, L.; Prost, J.-F. New triazine derivatives as potent modulators of multidrug resistance. *J. Med. Chem.* **1992**, *35*, 2481−2496.
(42) Pearce, H. L.; Safa, A. R.; Bach, N. J.; Winter, M. A.; Cirtain, M. C.; Beck, W. T. Essential features of the P-gycoprotein pharmacophore as defined by a series of reserpine analogues that modulate multidrug resistance. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 5128−5132.
(43) Wakeling, A. E.; Dukes, M.; Bowler, J. A potent specific pure antiestrogen with clinical potential. *Cancer Res.* **1991**, *51*, 3867−3873.
(44) Wakeling, A. E.; Bowler, J. Novel antioestrogens without partial agonist activity. *J. Steroid Biochem.* **1988**, *31*, 645−653.
(45) Hu, X. F.; Nadalin, G.; De Luise, M.; Martin, T. J.; Wakeling, A.; Huggins, R.; Zalcberg, J. R. Circumvention of doxorubicin resistance in multi-drug resistant human leukaemia and lung cancer cells by the pure antioestrogen ICI 164384. *Eur. J. Cancer* **1991**, *27*, 773−777.
(46) Beyer, B.; Terenius, L.; Counsell, R. E. Synthesis of potential antiprogestins. *Steroids* **1980**, *35*, 481−488.
(47) Turner, A. B.; Ringold, H. J. Applications of high-potential quinones; the mechanism of dehydrogenation of steroidal ketones by 2,3-dichloro-5,6-dicyanobenzoquinone. *J. Chem. Soc.* **1967**, (C), 1720−1730.
(48) Darby, M. V.; Lovett, J. A.; Brueggemeier, R. W.; Groziak, M. P.; Counsell, R. E. 7α-substituted derivatives of androstenedione as inhibitors of estrogen biosynthesis. *J. Med. Chem.* **1985**, *28*, 803−807.

(49) Haynex, R. K.; Jackson, W. R.; Stragalinon, S. Conversion of enone into diones via allylpalladium complexes. *Aust. J. Chem.* **1980**, *33*, 1537−1544.
(50) Leonessa, F.; Jacobson, M.; Boyle, B.; Lippman, J.; McGarvey, M.; Clarke, R. The effect of tamoxifen on the multidrug resistant phenotype in human breast cancer cells: isobologram, drug accumulation and gp-170 binding studies. *Cancer Res.* **1994**, *54*, 441−447.
(51) Zhang, Y.; Leonessa, F.; Clarke, R.; Wainer, I. W. Development of an immobilized P-glycoprotein stationary phase for on- line liquid chromatographic determination of drug-binding affinities. *J. Chromatogr., B* **2000**, *739*, 33−37.
(52) Lu, L.; Leonessa, F.; Clarke, R.; Wainer, I. W. Competitive and allosteric interactions in ligand binding to P-glycoprotein as observed on an immobilized P-glycoprotein liquid chromatographic stationary phase. *Mol. Pharmacol.* **2001**, *59*, 62−68.
(53) Gros, P.; Dhir, R.; Croop, J.; Talbot, F. A single amino acid substitution strongly modulates the activity and substrate specificity of the mouse mdr1 and mdr3 drug efflux pumps. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 7289−7293.
(54) Shriver, D. F.; Drezdon, M. A. *The Manipulation of Air-Sensitive Compounds*; Wiley: New York, 1986.
(55) Still, W. C.; Kahn, M.; Mitra, A. Rapid chromatographic technique for preparative separations with moderate resolution. *J. Org. Chem.* **1978**, *43*, 3, 2923−2925.
(56) Brueggemeier, R. W.; Floyd, E. E.; Counsell, R. E. Synthesis and biochemical evaluation of inhibitors of estrogen biosynthesis. *J. Med. Chem.* **1978**, *21*, 1007−1011.
(57) Clarke, R.; Brünner, N.; Katzenellenbogen, B. S.; Thompson, E. W.; Norman, M. J.; Koppi, C.; Paik, S.; Lippman, M. E.; Dickson, R. B. Progression from hormone dependent to hormone independent growth in MCF-7 human breast cancer cells. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 3649−3653.
(58) Clarke, R.; Morwood, J.; van den Berg, H. W.; Nelson, J.; Murphy, R. F. The effect of cytotoxic drugs on estrogen receptor expression and response to tamoxifen in MCF-7 cells. *Cancer Res.* **1986**, *46*, 6116−6119.

JM010126M