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Nonsteroidal Human Progesterone Receptor Modulators from the Marine Alga *Cymopolia barbata*

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SUMMARY

The co-transfection assay is a novel functional assay using cells transiently transfected with plasmids encoding intracellular receptors and corresponding reporter genes. Using this assay, natural product extracts were tested to identify compounds that modulate intracellular receptor activity, measured as changes in reporter gene activity. A crude extract of the marine alga *Cymopolia barbata* was found to inhibit progesterone-stimulated reporter gene expression in cells transfected with the human progesterone receptor (hPR) and an appropriate reporter construct. Purification of the active constituents of the extract, guided by the co-transfection assay, yielded two diastereomers of cyclocymopol monomethyl ether, possessing opposing pharmacological activities with the hPR. The antagonist (3*R*)-cyclocymopol monomethyl ether (LG100127) blocked 1 nM progesterone-stimulated reporter gene expression with an IC_{50} value of 549 ± 55 nM in the co-transfection assay. The agonist (3*S*)-cyclocymopol monomethyl ether (LG100128) had efficacy similar to that of progesterone and an EC_{50} value of 35 ± 2 nM. Stimulation by progesterone of the hPR in the human breast cancer cell line T-47D results in enhanced expression of alkaline phosphatase; LG100127 blocked alkaline phosphatase expression stimulated either by progesterone or by LG100128, and LG100128 mimicked progesterone in this assay. Both diastereomers displaced [^{3}H]progesterone from baculovirus-expressed hPR. LG100127 and LG100128 each interacted with the human androgen receptor but did not interact with the human glucocorticoid receptor, estrogen receptor, vitamin D receptor, or retinoid receptors. In summary, these *in vitro* studies describe the first nonsteroidal pharmacophores for the hPR and demonstrate the use of the co-transfection assay in their discovery.

Progesterone plays a central role in preparing the uterus for implantation of fertilized ova and induces proliferation of a variety of tissues, including the mammary gland. The biological actions of progesterone are mediated through the PR, a member of the intracellular receptor superfamily of ligand-dependent transcription factors (1). When progesterone or related agonists bind to the PR, receptor is released from associated heat-shock proteins, inducing the formation of a ligand-receptor complex that binds to the progesterone response element in the promoters of progesterone-responsive genes. This cascade results in the modulation (usually up-modulation) of transcriptional activity.

Acute administration of a PR antagonist, such as mifepristone (RU-486) (along with a prostaglandin to cause uterine contractions), effectively induces termination of pregnancy (<9 weeks after fertilization) (2). Chronic administration of antiprogestins, by blocking the proliferative actions of progesterone, has shown promise in treating PR-positive breast cancers, uterine fibroids, endometriosis, and meningiomas (3–9). Besides possessing antiprogestin activity, RU-486 and related compounds have direct antiglucocorticoid and antiandrogen effects as well as indirect antiestrogen effects (10–13). With chronic administration, the antiglucocorticoid effects are dose limiting. The development of selective antiprogestins (especially those devoid of antiglucocorticoid activity), therefore, may be more effective for the chronic treatment of progesterone-dependent proliferative disorders. To this end, we used the co-transfection assay (1), a functional cell-based gene expression assay, as a screen to identify and characterize novel PR agonists and antagonists with less

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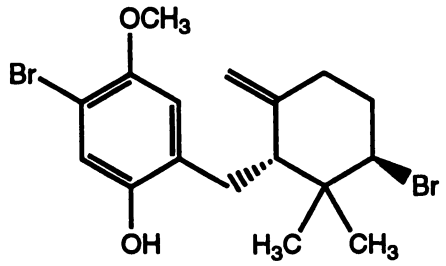
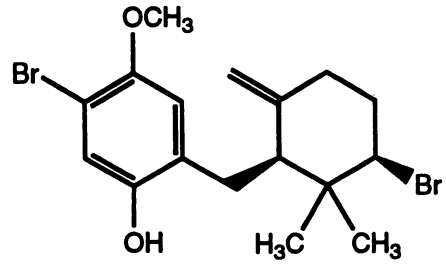
ABBREVIATIONS: PR, progesterone receptor(s); MTV, mouse mammary tumor virus; LUC, luciferase; β-Gal, β-galactosidase; hVDR, human vitamin D receptor(s); hER, human estrogen receptor(s); hGR, human glucocorticoid receptor(s); hRAR, human retinoic acid receptor(s); hRXR, human retinoid X receptor(s); hAR, human androgen receptor(s); hGR, human glucocorticoid receptor(s); hPR, human progesterone receptor(s); MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide; LTR, long terminal repeat; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]propanesulfonate.



LG100127



LG100128

Fig. 1. Structures of the cyclocymopols LG100127 [(3*R*)-cyclocymopol monomethyl ether] and LG100128 [(3*S*)-cyclocymopol monomethyl ether].

glucocorticoid antagonist activity. We have discovered two nonsteroidal diastereomeric secondary metabolites from a marine alga that are capable of modulating the actions of progesterone *in vitro*.

Materials and Methods

Co-transfection assays. CV-1 cells (African green monkey kidney fibroblasts) were initially obtained from the American Type Culture Collection (Rockville, MD) and were grown in Dulbecco's modified Eagle medium (BioWhittaker, Walkersville, MD) containing 10% (v/v) charcoal resin-stripped fetal bovine serum (Hyclone, Logan, UT), 2 mM L-glutamine, and 55 μg/ml gentamicin. Cells were maintained in an environment of 4% carbon dioxide and routinely passaged from T-225 flasks to 96-well microtiter plates (1.5×10^5 cells/well, 70% confluent) 1 day before transfection.

Cells were transiently transfected, by the standard calcium phosphate co-precipitation procedure (14, 15), with 50 ng/well of each of the following plasmids: hPR B-isoform (pRShPR-B1), MTV-LUC reporter, pRS-β-Gal, and filler DNA (Rous sarcoma virus chloramphenicol acetyltransferase). pRShPR-B1 is a pBR322 plasmid containing hPR-B (from D. McDonnell and B. O'Malley, Baylor College of Medicine) with the τ-1 region of hGR (14) inserted as a *Bgl*II fragment into the amino-terminal region of hPR at the unique *Hinc*II site, using *Bgl*II linkers. This resulted in 175 amino acids of hGR being inserted after amino acid 456 of hPR-B. In addition, two amino acids specified by the linker were included on both sides of the τ-1 domain. Constitutive expression of hPR-B1 is under control of the Rous sarcoma virus LTR. The reporter, MTV-LUC, contains the cDNA for expression of firefly LUC under control of the MTV LTR, a conditional promoter containing a progesterone response element. pRS-β-Gal, encoding constitutive expression of *Escherichia coli* β-Gal, was included as an internal control for evaluation of transfection efficiency and compound cytotoxicity. Co-transfection studies with the hGR, hRAR-α, hRXR-α, hAR, hER, and hVDR were carried out as described previously (15–18).

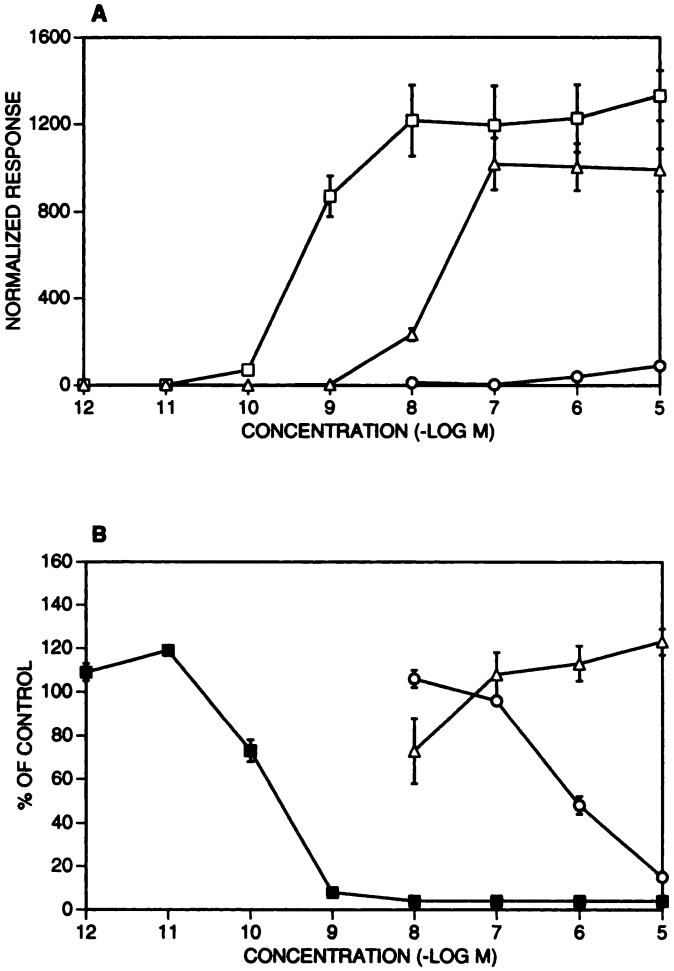


Fig. 2. Modulation of LUC expression in CV-1 cells by progesterone agonists and antagonists. A, African green monkey kidney CV-1 cells were added to 96-well plates (1.5×10^5 cells/well). One day later, the cells were transiently transfected with plasmids coding for hPR-B (hPR-B1) and pRS-β-Gal under constitutive control and a reporter (MTV-LUC) containing a response element for PR. After 6 hr, the medium was replaced with medium containing progesterone (□), LG100127 (○), or LG100128 (△). At the completion of the 40-hr incubation, cells were lysed. LUC activity was determined by chemiluminescence and β-Gal activity was determined spectrophotometrically. The normalized response represents LUC level/β-Gal activity. The results represent the mean ± standard error of six experiments, each having three replicates. B, CV-1 cells were transiently transfected with the same plasmids as described for A. After transfection, the medium was replaced with medium containing 1 nM progesterone and RU-486 (■), LG100127 (○), or LG100128 (△). *Ordinate* (percentage of control), LUC activity (normalized response) as a function of the control (1 nM progesterone). The results represent the mean ± standard error of six experiments, each having three replicates.

At the completion of each assay, wells were washed with phosphate-buffered saline and the cells were lysed with a Triton X-100-based buffer (15). An aliquot of lysate (20 μl) was then transferred to Dynatech (Chantilly, VA) 96-well plates containing 1.6 mM ATP (final concentration). LUC activity (chemiluminescence upon addition of luciferin substrate) was determined using a Dynatech ML1000 luminometer, according to the equation LUC units = relative LUC units $\times 10^4$. β-Gal activity was determined from the remaining lysate in the original 96-well plates. The substrate, *o*-nitrophenol-β-galactoside, was added to the plates, followed by incubation at 37°. The incubation was terminated by addition of sodium carbonate when the average absorbance, as determined by visual observation of the yellow product (*o*-nitrophenol), was within a standard

TABLE 1

Potencies of LG100127, LG100128, progesterone, and RU-486 for the modulation of intracellular receptor-mediated gene expression, determined using the co-transfection assay

Each value represents the mean ± standard error of separate experiments.

	Potency					
	LG100127		LG100128		Progesterone, Agonist EC_{50}	RU-486, Antagonist IC_{50}
	Agonist EC_{50}	Antagonist IC_{50}	Agonist EC_{50}	Antagonist IC_{50}		
	nM					
hPR-B1	>10,000	549 ± 55	35 ± 2	>10,000	0.67 ± 0.11	0.15 ± 0.01
T-47D cells	>10,000	401 ± 93	353 ± 96	>10,000	0.94 ± 0.08	0.41 ± 0.02
hAR	>10,000	449 ± 11	>10,000	238 ± 106	1,497 ± 129	2.5 ± 0.43
hGR	>10,000	>10,000	>10,000	>10,000	ND[a]	0.56 ± 0.22
hER	>10,000	>10,000	>10,000	>10,000	ND	735 ± 293
hRAR-α	>10,000	>10,000	>10,000	>10,000	ND	ND
hRXR-α	>10,000	>10,000	>10,000	>10,000	ND	ND
hVDR	>10,000	ND	>10,000	ND	ND	ND

[a] ND, not determined.

range. Absorbance at a wavelength of 415 nm was then quantified spectrophotometrically (Biomek). β-Gal rates were calculated according to the following equation: β-Gal rate = β-Gal absorbance × 10^5/β-Gal incubation time. For each set of replicate wells, normalized response was calculated according to the following equation: normalized response = LUC units/β-Gal rate.

In all experiments, agonist activity was determined by examining the amount of LUC expression (normalized response). The effective concentration that produced 50% of the maximal response (EC_{50}) was quantified. The efficacy was a function of the LUC expression relative to the maximal LUC expression produced by the reference agonist, e.g., progesterone for hPR. Antagonist activity was determined by testing the amount of LUC expression in the presence of a fixed concentration (equal to its agonist EC_{50}) of reference agonist. The concentration of test compound that inhibited by 50% the gene expression induced by the reference compound was quantified (IC_{50}). In addition, the efficacy of antagonists was determined as a function of maximal inhibition (LUC expression = basal activity). The reference compounds and concentrations used for each of the receptors were as follows: PR, progesterone (1×10^{-9} M); GR, dexamethasone (1×10^{-9} M); AR, dihydrotestosterone (5×10^{-9} M); ER, estradiol (1×10^{-8} M).

T-47D cell alkaline phosphatase assays. T-47D breast carcinoma-derived cells (obtained from the American Type Culture Collection) were grown in RPMI 1640 medium (BioWhittaker) with 10% (v/v) fetal bovine serum (Hyclone), 2.5 mM L-glutamine, 60 μg/ml gentamicin, and 0.2 μg/ml insulin (19). Three days before experiments, cells were transferred from 10-cm dishes to 96-well plates (1×10^4 cells/well). At the beginning of each experiment, the medium was removed and replaced with fresh medium (with 2%, v/v, charcoal resin-stripped fetal bovine serum) containing either test compound or test compound plus progesterone (1×10^{-9} M), and cells were returned to the incubator (5% carbon dioxide). After 18–20 hr, the medium was aspirated and the cells were fixed for 30 min with 100 μl of 5% formalin (in phosphate-buffered saline). The fixed cells were then washed, and 75 μl of assay buffer (1 mg/ml *p*-nitrophenol phosphate in 1 M diethanolamine, pH 9, 2 mM magnesium chloride) were added. After incubation at 19° for 70 min, the reaction was terminated with the addition of 100 μl of 1 M sodium hydroxide. The absorbance at 405 nm was measured (Biomek).

Receptor binding assays. [^{3}H]Progesterone and [^{3}H]testosterone were purchased from New England Nuclear (Boston, MA). PR (human A subtype) and hAR were each expressed from their respective cDNAs in a baculovirus system and extracted as described previously (20). The receptor preparations were stored at −80° at a typical protein concentration of 10–15 mg/ml.

Stock solutions of all compounds were stored as 5 mM ethanol solutions, and serial dilutions were carried out in 5 mM ethanol. The PR assay buffer consisted of 10% glycerol, 10 mM Tris, 1 mM EDTA, 12 mM monothioglycerol, 2 mM CHAPS, and 1 mM phenylmethylsulfonyl fluoride, pH 7.5 at 4°. The final assay volume was 500 ml, containing 10 mg of protein, 2–4 nM [^{3}H]progesterone, and varying concentrations of competing ligand. Incubations were carried out at 4° for 16 hr. Nonspecific binding was defined as binding remaining in the presence of 500 nM progesterone. At the end of the incubation period, 400 ml of 7.5% (w/v) dextran-coated charcoal suspension in gelatin/phosphate buffer were added. The mixture was vortex-mixed, incubated for 10 min at 4°, and then centrifuged at 800 × *g* for 10 min. The amount of bound [^{3}H]progesterone was determined by liquid scintillation counting of an aliquot (700 μl) of the supernatant fluid using a Wallac Microbeta plate reader counting in the 96-well format, with MultiCalc as the data reduction method.

The AR assay buffer consisted of 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 25 mM sodium phosphate, 10 mM KF, 2 mM dithiothreitol, 2 mM CHAPS, 1.5 mM EDTA, and 10 mM sodium molybdate, pH 7.5. The final volume was 250 μl, containing 50 μg of receptor extract protein, 2–4 nM [^{3}H]testosterone, and varying concentrations of competing ligand at concentrations that ranged from 0 to 10^{-5} M. Incubations were carried out at 4° for 16 hr. Nonspecific binding was defined as binding remaining in the presence of 1000 nM nonradioactive testosterone. At the end of the incubation period, bound ligand and free ligand were separated by the hydroxyapatite method. The amount of bound tritiated hormone was determined by liquid scintillation counting of the hydroxyapatite pellet.

After correction for nonspecific binding, IC_{50} values were determined from logit transformation of the binding curves. The IC_{50} value is defined as the concentration of competing ligand needed to reduce specific binding by 50%. The K_i values were determined by application of the Cheng-Prusoff equation (21) to the IC_{50} values, the labeled ligand concentration, and the K_d of the labeled ligand.

Cytotoxicity assays. Evaluation of compound cytotoxicity was accomplished using the mitochondrial dehydrogenase substrate MTT (22). Cells were incubated in the presence of test compound (0.1, 1.0, or 10 μM) for the same length of time as the gene expression assay for that cell line. The product was quantified by absorbance at 600 nm.

Results

Discovery and chemical characterization of LG100127 and LG100128. In an effort to identify, as potential lead candidates, novel pharmacophores that interact with the hPR, natural product extracts and defined chemicals were tested for biological activity using the co-transfection assay. To accomplish this goal, cells transiently trans-

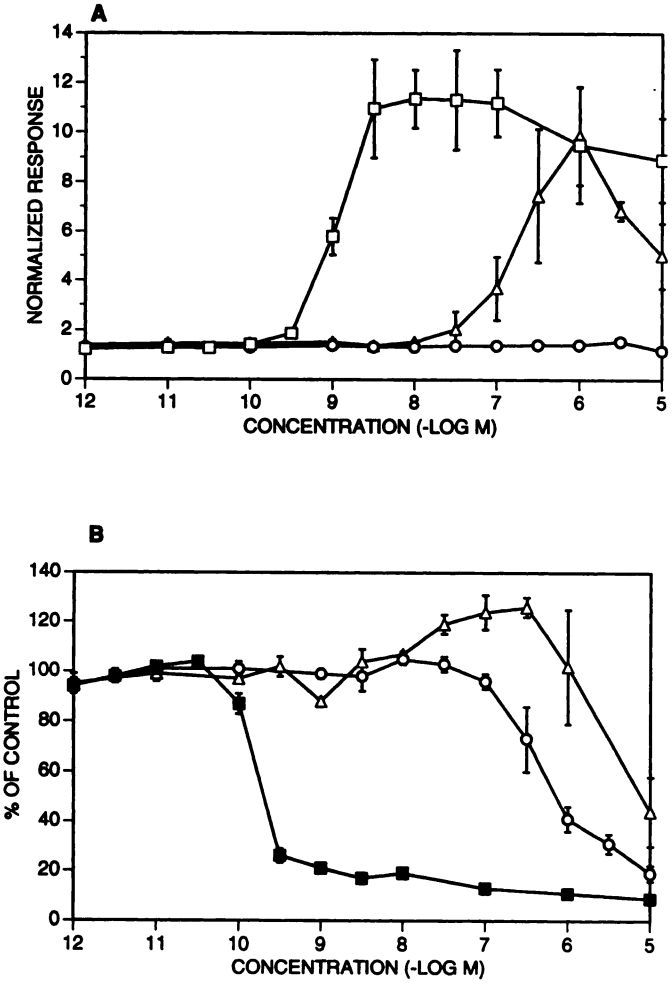


Fig. 3. Modulation of alkaline phosphatase activity in T-47D cells by progesterone agonists and antagonists. A, Human breast carcinoma T-47D cells were added to 96-well plates (1×10^4 cells/well). Three days later, the medium was replaced with medium containing progesterone (□), LG100127 (○), or LG100128 (△). After incubation for 18–20 hr, alkaline phosphatase activity was quantified spectrophotometrically in the presence of a chromogenic substrate. *Ordinate*, relative alkaline phosphatase levels. The results represent the mean ± standard error of six experiments, each having three replicates. B, T-47D cells were prepared as described for A. Cells were incubated in the presence of 1 nM progesterone and RU-486 (■), LG100127 (○), or LG100128 (△). *Ordinate* (percentage of control), alkaline phosphatase expression as a function of that produced by 1 nM progesterone alone. The results represent the mean ± standard error of six experiments, each having three replicates.

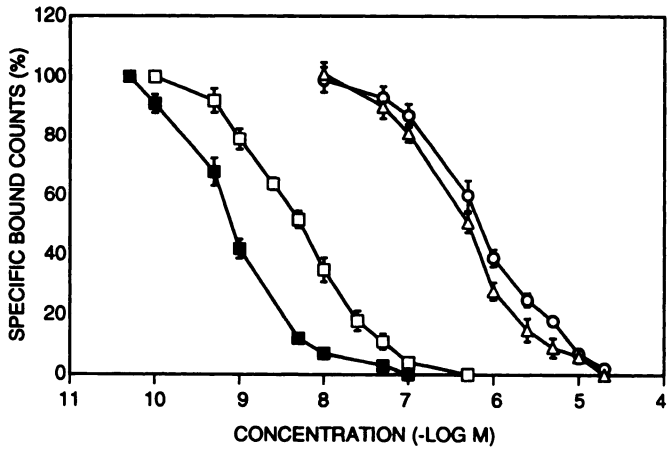


Fig. 4. Displacement of [^{3}H]progesterone from the hPR (A-isoform). Baculovirus-expressed hPR (A-isoform) was prepared as described in Materials and Methods. The receptor preparation was incubated in a buffer containing [^{3}H]progesterone plus test compounds, i.e., RU-486 (■), progesterone (□), LG100127 (○), or LG100128 (△). Nonspecific binding was determined in the presence of 500 nM progesterone. After incubation for 16 hr at 4°, bound and free radioligand were separated via the dextran-coated charcoal method. The results represent the mean ± standard error of three experiments, each carried out in triplicate.

fected with hPR-B1 and appropriate reporters are incubated with an EC_{50} concentration of progesterone plus test sample. PR antagonists block progesterone-stimulated LUC expression, whereas PR agonists further enhance LUC expression. Most samples do not affect progesterone-stimulated gene expression. In some cases, LUC values decrease as a result of toxicity, as demonstrated by concurrent changes in β-Gal expression.

In the course of screening natural product extracts, a sample (an organic extract of *Cymopolia barbata*) was identified that possessed agonist activity in the progesterone-stimulated LUC expression assay. The calcareous green alga *C. barbata* Lamouroux (Dasycladaceae) is found in the tropical waters of the Caribbean and was collected in the Florida Keys. After collection, the alga was kept frozen until extracted. An initial extract of the thawed alga was prepared by homogenization in an industrial Waring blender with a mixture of methanol and methylene chloride (1:1), followed by filtration and concentration *in vacuo*. This mixture was partitioned between water and methylene chloride, and the resulting organic phase was concentrated *in vacuo* to obtain the crude organic extract. In subsequent experiments, the extract had agonist activity in the absence of progesterone. If hPR-B1 was omitted from the transfection, no biological activity was present, suggesting that the activity of LG100128 was mediated through the receptor. The agonist activity was attributed to a single fraction obtained by reversed-phase high performance liquid chromatography on octadecylsilyl silica. However, proton NMR spectroscopy of the active fraction indicated that it was a mixture of two compounds. After acetylation, normal phase high performance liquid chromatography yielded two pure compounds. Upon deacetylation, the two compounds, LG100127 and LG100128, were present in a ratio of 4:1. Comparison of the spectral data (^{1}H and ^{13}C NMR spectroscopy and mass spectrometry) with the reported values demonstrated that they were diastereomers of cyclocymopol monomethyl ether, as illustrated in Fig. 1. The chemistry of *C. barbata*, including the identification of cyclocymopol monomethyl ethers, has been reported previously (23, 24).

Biological actions of LG100127 and LG100128. Using CV-1 cells co-transfected with plasmids encoding hPR-B1 and the β-Gal and MTV-LUC reporters, compounds were evaluated in the absence and presence of progesterone (see Materials and Methods). Mean data from separate experiments are illustrated in Fig. 2. Both progesterone and LG100128 caused concentration-dependent increases in LUC expression (Fig. 2A; Table 1). LG100128 had an efficacy similar to that of progesterone, with a potency (EC_{50} = 35 nM) within 2 log units of that of progesterone (EC_{50} = 0.67 nM). The other diastereomer, LG100127, did not possess intrinsic hPR agonist activity.

Antagonist effects on hPR-B1 were determined by evaluating compounds in the presence of progesterone at its EC_{50} concentration (Fig. 2B; Table 1)). Either RU-486 or LG100127 blocked 1 nM progesterone-induced LUC expression. LG100127 (IC_{50} = 549 nM) was 3700-fold less potent than RU-486 as a PR antagonist (IC_{50} = 0.15 nM). LG100128 did not block the effects of progesterone. In contrast to LG100127 or RU-486, LG100128 further enhanced LUC expression, as would be expected for a PR agonist. The agonist effects of LG100128 could be blocked by LG100127 (data not shown).

The co-transfection assay employed in Fig. 2 used a monkey fibroblast cell line transfected with amino-terminally modified hPR-B. We wanted to further characterize the biological properties of these novel compounds in a human cell line expressing endogenous wild-type PR. The T-47D cell line was chosen because these cells are derived from a human breast cancer and because they express both endogenous wild-type hPR isoforms (PR-A and PR-B). Incubation of T-47D cells with PR agonists induces expression of alkaline phosphatase. As found with the co-transfection assay, LG100128 mimicked the actions of progesterone and enhanced alkaline phosphatase activity in a concentration-dependent manner (Fig. 3A). The efficacy and potency values for LG100128 in the T-47D cell assay were similar to those in the co-transfection assay (Table 1). The agonist activity of LG100128 (EC_{50} = 353 nM) was blocked in a concentration-dependent manner by RU-486 (IC_{50} = 0.6 nM) (data not shown).

In the T-47D cell assay, LG100127 was devoid of agonist properties (Fig. 3A) but prevented 1 nM progesterone-induced enhancement of alkaline phosphatase expression (Fig. 3B). The potency values for LG100127 and RU-486 were similar in the two assays (Table 1). LG100127 was 1000-fold less potent than RU-486 in the T-47D cell assay. The values from the co-transfection and T-47D cell assays, therefore, were comparable for both the steroidal and nonsteroidal compounds.

The MTT assay is a sensitive indicator of cell viability. In the present studies, compounds were evaluated for cytotoxicity with both cell lines, using the same incubation times as in the gene expression assays (CV-1 cells, 40 hr; T-47D cells, 20 hr). Neither of the cyclocymopols reduced cell viability (data not shown).

An advantage of the co-transfection assay (or other functional assays) as a screening tool is that compounds may be identified that act at any point from receptor occupation to gene expression. Once an active sample is found (concentration-dependent changes in LUC expression with no change in β-Gal expression), studies are initiated to determine the mechanism of action. Because all ligands that selectively modulate receptor activity bind to the receptor, the ability of the cymopols to interact with the active site was determined. Fig. 4 illustrates the binding curves obtained with progesterone, RU-486, and the cyclocymopols using baculovirus-expressed hPR-A. Progesterone and RU-486 had K_d values of 3.5 ± 0.2 and 0.58 ± 0.03 nM, respectively. Both LG100127 and LG100128 displaced [^{3}H]progesterone in a competitive manner (Hill coefficients not significantly different from unity), with K_d values of 490 ± 30 and 344 ± 23 nM, respectively. In addition, there was no significant difference in the affinities of the cymopols when recombinant hPR-B or T-47D cell extract was used as the source of receptor protein (data not shown).

Cross-reactivity of the cymopols was evaluated by testing the compounds for activity with a variety of related and unrelated receptors. Using the co-transfection assay, the compounds were not active (<50% efficacy) with hGR, hER, hRAR, hRXR, and hVDR.

With the hAR, neither compound had agonist properties. Both cyclocymopols, however, blocked the ability of an EC_{50} concentration (5 nM) of dihydrotestosterone to enhance AR-mediated LUC expression (Table 1). For LG100127, the IC_{50} and efficacy values were 449 nM and 79%, respectively. Similarly, LG100128 had potency and efficacy values of 238 nM and 89%, respectively. As with the PR, both compounds displaced [^{3}H]dihydrotestosterone from recombinant hAR, with K_d values of 490 ± 30 and 343 ± 23 nM for LG100127 and LG100128, respectively (data not shown).

Discussion

Using the hPR-B1 co-transfection assay, two new modulators of the hPR were identified. These natural products, cyclocymopol monomethyl ethers from the marine alga *C. barbata*, represent the first examples of nonsteroidal compounds to exhibit such activity. All other known modulators of PR, including the natural ligand progesterone and the synthetic antagonist RU-486, are steroids and hence share the steroidal cyclic carbon skeleton (25). In contrast, the carbon skeleton common to cyclocymopol monomethyl ethers is composed of a monoterpene linked to an aromatic moiety via a one-carbon methylene bridge (23, 24). The two cyclocymopols differ only in the configuration at C3, which, interestingly, leads them to exhibit opposite interactions with the receptor. LG100128 mimicked the actions of progesterone in a RU-486-reversible manner. In contrast, LG100127 was devoid of intrinsic agonist activity with this receptor but blocked the actions of progesterone. The biological properties of the diastereomers appear to be mediated through a competitive interaction with the hPR, although the precise site within the hormone binding domain has not been elucidated. As expected, based upon its low potency, LG100127 was not active in rodent models of antiprogestin action.[3] None of the related compounds isolated from the complex mixture of secondary metabolites in the alga, notably including cymopol, which has a monocyclic structure with an acyclic terpenoid fragment, was active in the co-transfection assay.

A drawback to the chronic use of steroidal antiprogestins such as RU-486 is cross-reactivity with the GR (2, 10, 11). The cyclocymopols, however, do not possess functional cross-reactivity with the GR or many other receptors (hER, hRAR-α, hRXR-α, or hVDR). There is, however, cross-reactivity with the hAR. The interactions of these compounds with the hAR appear to be different from those with the hPR, because both diastereomers are antagonists of hAR in the co-transfection assay. Additional evidence for differences in the actions of the diastereomers at the two receptors is derived from synthetic analogue studies that demonstrate distinct relative patterns of activity with the two receptors.[4] In practice, however, cross-reactivity with the hAR is of less

[3] M.-W. Wang, unpublished observations.
[4] L. Hamann, L. Farmer, M. Johnson, A. Davtian, M. Goldman, and T. Jones, unpublished observations.

consequence than interaction with the hGR, because most clinical uses of PR antagonists are in women, where androgens typically play a smaller role than glucocorticoids.

In these studies, the potency values for LG100127 and LG100128 in the T-47D cell assay were very similar to the K_d values in the PR binding assays, suggesting that the functional activities of the compounds were due to direct binding to the hPR. In contrast, LG100128 was approximately 10-fold more potent in the co-transfection assay using the modified hPR. This effect has been observed many times in our studies with structurally distinct molecules. However, the primary purpose of the co-transfection assay is to identify new, biologically active molecules and, based upon characterizaton of reference compounds as well as parallel testing of representative screening compounds in other assays, we believe that the high-throughput hPR assay is capable of identifying active compounds, including those with relatively low affinities.

In summary, these studies demonstrate that the co-transfection assay is an effective tool for the identification of novel ligands for intracellular receptors. By testing screening samples in the presence of an EC_{50} concentration of reference agonist, either agonist or antagonist activities can be observed. An added advantage of the co-transfection assay over binding or endogenous gene expression assays (such as the T-47D cell assay) is that monitoring of β-Gal expression in the cells can be used to rapidly rule out nonspecific or cytotoxic compounds that might appear to reduce agonist-stimulated gene expression. Furthermore, novel compounds acting throughout the cascade of events involved in PR-mediated gene expression can be identified.

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