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5-(3-Cyclopentyl-2-thioxo-2,3-dihydro-1*H*-benzimidazol-5-yl)-1-methyl-1*H*-pyrrole-2-carbonitrile: A novel, highly potent, selective, and orally active non-steroidal progesterone receptor agonist

Puwen Zhang,[a,*] Eugene Terefenko,[a] Jeffrey Kern,[a] Andrew Fensome,[a] Eugene Trybulski,[a] Ray Unwalla,[a] Jay Wrobel,[a] Susan Lockhead,[b] Yuan Zhu,[c] Jeffrey Cohen,[c] Margaret LaCava,[c] Richard C. Winneker[c] and Zhiming Zhang[c]

[a]*Chemical and Screening Sciences, Wyeth Research, S-2250B, 500 Arcola Road, Collegeville, PA 19426, USA*
[b]*Drug Safety and Metabolism, Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, USA*
[c]*Women's Health and Musculoskeletal Biology, Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, USA*

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Abstract—We have recently discovered 5-(3-cyclopentyl-2-thioxo-2,3-dihydro-1*H*-benzimidazol-5-yl)-1-methyl-1*H*-pyrrole-2-carbonitrile (**14**) as a potent, selective, and orally active non-steroidal progesterone receptor (PR) agonist. Compound **14** and its analog **13** possessed sub-nanomolar in vitro potency (EC_{50} 0.1–0.5 nM) in the T47D alkaline phosphatase assay, similar to that of the steroidal PR agonist medroxyprogesterone acetate (MPA). In contrast to MPA, **14** was highly selective (>500-fold) for the PR over both glucocorticoid and androgen receptors. In the rat uterine decidualization and complement component C3 models, **14** had oral ED_{50} values of 0.02 and 0.003 mg/kg, respectively, and was from 6- to 20-fold more potent than MPA. In the monkey ovulation inhibition model, compound **14** was also highly efficacious and potent with an oral ED_{100} of 0.03 mg/kg.

1. Introduction

Progesterone (P4, **1**) plays a critical role in the regulation of female reproductive function. Synthetic progestins are commonly used in oral contraceptives (OC) and their contraceptive efficacy is achieved via inhibition of ovulation and thickening of the cervical mucus.[1] Progestins can also protect the endometrium from estrogen induced endometrial hyperplasia in women with an intact uterus. This protective function has been used in the estrogen-based hormone therapy in postmenopausal women for the relief of vasomotor symptoms[2,3] and prevention of osteoporosis.[4] Furthermore, progestins are used for the treatment of reproductive disorders such as dysmenorrhea and dysfunctional uterine bleeding.[5] However, combination steroidal progestin and estrogen therapy is often associated with side effects such as mastalgia, nausea, headaches, as well as some metabolic and cardiovascular complications. A tissue selective progesterone receptor (PR) agonist would potentially reduce these side effects. In addition, many of the steroid-based agonists have demonstrated undesirable activities at other steroid receptors such as glucocorticoid receptor (GR) and androgen receptor (AR). Novel non-steroidal PR ligands may offer greater potential for tissue selectivity and receptor selectivity.

In our effort to search for potent and selective non-steroidal PR agonists, we discovered a number of chemical scaffolds,[6–9] such as 6-aryl benzoxazin-2-ones and benzoxazine-2-thiones, that afforded compounds with potent PR agonist activity. Recently, we reported another template, 6-aryl benzimidazolones such as **7**,[10] that demonstrated moderate to potent PR antagonist activity. Unlike the 5-aryl oxindole and 6-aryl benzoxazinone scaffolds,[6] the modification of 6-aryl moiety for the benzimidazolone template did not render PR agonists. Only weak agonist activity for several potent benzimidazolone PR antagonists[10] was observed at a high concentration of 3 μM. After further SAR examination of the benzimidazolone scaffold, gratifyingly, we found

Keywords: Progesterone receptor (PR); PR agonists; PR antagonists; Non-steroidal.
* Corresponding author. Tel.: +1 484 865 3856; fax: +1 484 865 9398; e-mail: Zhangp@wyeth.com

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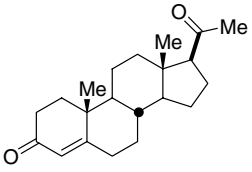
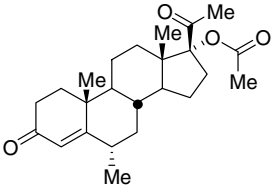
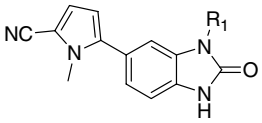
that a number of 6-aryl benzimidazole-2-thiones were potent PR agonists. The synthesis, in vitro SAR, and in vivo activity of novel 6-aryl benzimidazoloe-2-thiones (**8**–**15**) are the subject of this report.



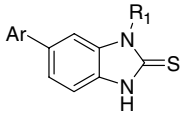
Progesterone (P4, **1**)



Medroxyprogesterone acetate (MPA, **2**)



7f, $R_1 = c\text{-}C_4H_7$
7g, $R_1 = c\text{-}C_5H_9$
7h, $R_1 = 3\text{-}C_5H_{11}$



8-15

2. Synthetic chemistry

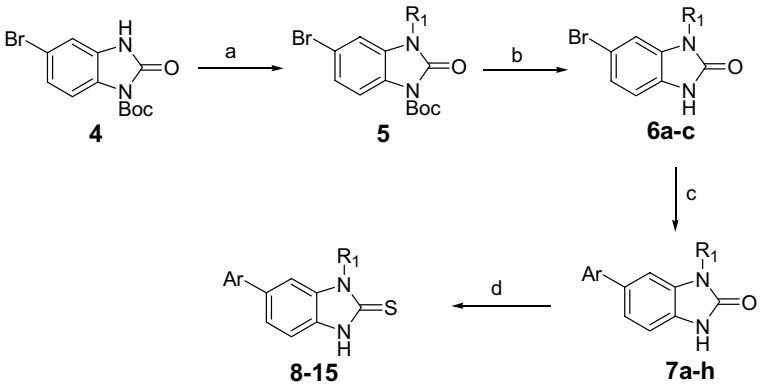
The preparation of the target compounds **8**–**15** as shown in Scheme 1 was described previously.[11,12] In brief, alkylation of the BOC protected benzimidazolones **4**[11] to yield **5** was readily achieved via a Mitsunobu protocol. Removal of protecting groups from benzimidazolone **5** under acidic conditions afforded **6** which was cross-coupled with an appropriate aryl boronic acid to provide the 6-aryl benzimidazolones **7** in good yields. Refluxing a mixture of Lawesson's reagent and benzimidazolones **7** in toluene provided the desired 6-aryl benzimidazole-2-thiones **8**–**15**.

3. Results and discussion

There have been a number of non-steroidal PR modulators disclosed over the last few years.[13,14] We have recently reported several series of non-steroidal PR modulators such as 5-aryl oxindoles and 6-aryl benzoxazin-2-ones.[15,16] Examination of the SAR results from the oxindole and benzoxazinone scaffolds revealed two PR agonist structural motifs, that is, a pendent 5′-cyanopyrrol-2-yl group and a 2-thiocarbonyl moiety.[6,8,9,15,16] These structural motifs caused the functional activity of 6-aryl benzoxazinones and 5-aryl oxindoles to switch from PR antagonist to PR agonist activity in the alkaline phosphatase assay using the human T47D breast carcinoma cell line.[6,8,9] In addition, incorporation of both structural motifs (5′-cyanopyrrol-2-yl and thiocarbonyl) onto the benzoxazinone template demonstrated synergistic effects on PR agonist activity and led to the discovery of the potent PR agonist, tanaproget.[7,17] In contrast, when placing the 5′-cyanopyrrol-2-yl group onto the benzimidazolone scaffold, the corresponding 6-(5′-cyanopyrrol-2-yl)-benzimidazolones remained as PR antagonists (i.e., the activity did not switch to PR agonism).[10] Intrigued by this finding, we decided to examine the impact of the 2-thiocarbonyl moiety on the SAR of the 6-aryl benzimidazolone template.

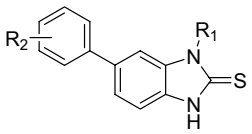
We have previously demonstrated that cyclic butyl, pentyl, and 3-pentyl were among the best substituents at the 1-position for the 6-aryl benzimidazolones.[10] Thus, a number of novel 6-aryl benzimidazole-2-thiones (**8**–**15**) were prepared using these optimized substituents. These compounds were evaluated for PR agonist and antagonist activities in the T47D cell alkaline phosphatase assay.[18,19] The results are listed in Tables 1 and 2.

Table 1 lists the alkaline phosphatase activity data of the 6-phenyl based-benzimidazole-2-thiones. As illustrated, the 2-thiocarbonyl compounds **8**–**10** showed good PR agonist potency compared to the PR antagonist activity exhibited by their corresponding 2-carbonyl benzimidazolones (IC_{50} 3.3–134.7 nM).[10] Interestingly, the 3-pentyl analog **11** exhibited PR antagonist activity with moderate potency. Congener **12** was a potent antagonist at a lower concentration while a weak agonist at a higher concentration. These results indicated that the PR functional activity of benzimidazole-2-thiones was not completely controlled by the 2-thiocarbonyl moiety and dependent on the 1-substituent as well.



Scheme 1. 6-Aryl benzimidazole-2-thiones. Reagents and conditions: (a) R_1OH, DIAD, Ph_3P, THF, rt, 30–80%; (b) CH_2Cl_2, TFA, rt, 80–95%; (c) $ArB(OH)_2$, $Pd(Ph_3P)_4$, K_2CO_3, toluene, EtOH/H_2O, 90 °C, 30–85%; (d) Lawesson's reagent, toluene, reflux, 30–80%.

Table 1. PR agonist and antagonist activity in T47D cell alkaline phosphatase assay



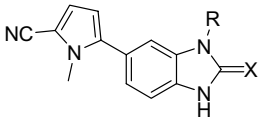
8-12

Compound	R_1	R_2	PR alk. phos. EC_{50}[a] (nM)	PR alk. phos. IC_{50}[b] (nM)
Progesterone (**1**)			0.9	NA[d]
MPA (**2**)			0.1	NA
8	*c*-C_4H_7	3-CN, 5-F	2.7 (60%)[c]	NA
9	*c*-C_5H_9	3-Cl, 4-F	49.3 (55%)	NA
10	*c*-C_5H_9	3-F, 4-F	62.5 (75%)	NA
11	3-C_5H_{11}	3-Cl, 4-F	NA	33.7 (60%)
12	3-C_5H_{11}	3-CN, 5-F	269.8 (70%)	3.6 (50%)

[a] 50% effective concentration of tested compounds on alkaline phosphatase activity in the human T47D breast carcinoma cell line.
[b] 50% inhibitory concentration of tested compounds on 1 nM progesterone induced alkaline phosphatase activity in the human T47D breast carcinoma cell line. Values represent the average of at least duplicate determinations. The standard deviations for these assays were typically ±20% of the mean or less.
[c] Data in parentheses represent compounds' efficacy compared to progesterone (**1**) of 100%.
[d] NA, not active.

The compounds incorporating two PR agonist structural motifs were potent agonists irrespective of the substituent at the 1-position as depicted by 6-(5′-cyano-pyrrol-2-yl)-benzimidazole-2-thiones **13**–**15** (Table 2). In the T47D alkaline phosphatase assay, compounds **13** and **14** had sub-nanomolar in vitro potency (EC_{50} 0.1–0.5 nM), similar to that of the steroidal PR agonist medroxyprogesterone acetate (**2**) and more potent than progesterone (**1**). In comparison with their corresponding 2-carbonyl analogs (**7f**–**h**), compounds **13**–**15** not only switched from PR antagonist to PR agonist they were also more potent in the T47D alkaline phosphatase assay. Consistent with the functional activity, compounds **13**–**15** had higher binding affinity compared to their 2-carbonyl analogs. For example, compound **14** had a binding IC_{50} of 0.4 nM while the IC_{50} of its 2-carbonyl analog **7g** was only 26.2 nM when tested in a PR competition binding assay using cytosol from the human T47D breast carcinoma cell line.[19]

Table 2. PR agonist and antagonist activity in T47D cell alkaline phosphatase assay



13-15

Compound	X	R	PR alk. phos. EC_{50}[a] (nM)	PR alk. phos. IC_{50}[b] (nM)
Progesterone (**1**)			0.9	NA[d]
MPA (2)			0.1	NA
7f	O	*c*-C_4H_7	>3000[c]	10.9
7g	O	*c*-C_5H_9	>3000[c]	5.6
7h	O	3-C_5H_{11}	>3000[c]	11.7
13	S	*c*-C_4H_7	0.5 (70%)[e]	NA
14	S	*c*-C_5H_9	0.1 (75%)	NA
15	S	3-C_5H_{11}	1.0 (70%)	NA

[a] 50% effective concentration of tested compounds on alkaline phosphatase activity in the human T47D breast carcinoma cell line.
[b] 50% inhibitory concentration of tested compounds on 1 nM progesterone induced alkaline phosphatase activity in the human T47D breast carcinoma cell line. Values represent the average of at least duplicate determinations. The standard deviations for these assays were typically ±20% of mean or less.
[c] Compounds showed weak agonist activity in the absence of 1 nM progesterone (agonist mode) at 3000 nM.
[d] NA, not active.
[e] Data in parentheses represent compounds' efficacy compared to progesterone (**1**) of 100%.

To understand why subtle structural changes (2-carbonyl in **7f**–**h** vs 2-thiocarbonyl in **13**–**15**) switched PR functional activities, **7f** and **13** were closely examined for their interaction in the PR binding pocket using docking studies.[20] Recently, several co-crystal structures of PR LBD/steroidal and non-steroidal PR agonists have been reported.[7,21,22] Using the PR LBD crystal structure derived from PR LBD/tanaproget (a non-steroidal PR agonist), PR agonist **13** was docked into the PR LBD and was shown to demonstrate the same key interactions within PR binding site as tanaproget (Fig. 1). Similar to tanaproget,[7] the nitrile group of **13** makes important hydrogen bond interactions to residues Gln_{725} from helix-3 and Arg_{766} from helix-5, which are held in position by a water molecule. The NH group of the benzimidazole-2-thione moiety in **13** acts as a hydrogen bond donor by forming an interaction with the side chain oxygen of Asn_{719} within helix-3. Clearly, compound **13** has the same favorable agonist mode interactions with PR as tanaproget and as such it was a potent PR agonist.

In contrast to PR agonists, the co-crystal structure of PR LBD/PR antagonists (in particular non-steroidal

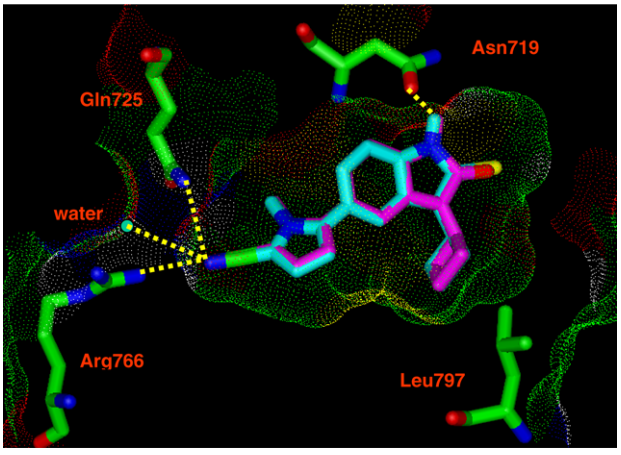


Figure 1. Docked poses of PR antagonist **7f** (magenta) overlaid with agonist **13** (cyan) in PR LBD binding site. (Only key residues and a Connolly surface of the binding site are shown for simplicity. Hydrogen bonds are shown as yellow dotted lines.)

PR antagonists) has not been reported to date. More recently, a co-crystal structure of PR LBD/asoprisnil, a steroidal selective PR modulator (SPRM), in the presence of co-repressor was disclosed.[23] Asoprisnil possesses a large 11-β pendent aryl group (likewise, RU-486 also has a large 11-β pendent aryl moiety) and induced a conformational shift in the helix-12 region and favorably permits co-repressor binding.[23] However, smaller antagonists **7f–h** lack the structural moiety similar to that of 11-β pendent aryl group from asoprisinil and RU-486 and are unlikely to induce helix-12 conformational shift that leads to PR antagonist activity. Considering the close structure similarity between agonists **13–15** versus antagonists **7f–h**, we decided to choose **7f**, the counterpart of agonist **13**, and docked it into the PR LBD binding pocket from PR LBD/tanaproget (Fig. 1). As illustrated in Figure 1, **7f** and **13** overlaid well in the binding pocket and have the same key interactions with PR although **13** has slightly favorable docking score (~0.7 kcal/mol). While we are unable to explain the functional activity switch for **7f** and **13** from docking study with PR LBD, it is possible that non-steroidal PR antagonists such as **7f** have a unique mechanism that induces PR antagonism. Compared to other steroidal receptors such as estrogen receptor (ER), PR has much smaller and less stable dimmer interface[22] that presumably could influence PR functional activity in different ways. Therefore, the PR conformational shifts that may result in the functional activity switch, due to the nature of PR dimmer interface, may be more susceptible to a subtle structural change induced by PR ligands such as non-steroidal PR antagonists **7f–h** versus agonists **13–15**. Further study is needed to shed light on why PR functional activities were switched when there is only a small structural change in these non-steroidal ligands.

Since compound **14** was the most potent analog in the T47D alkaline phosphatase assay it was evaluated for its selectivity over other steroidal receptors and in several animal models. Many steroids such as MPA were known to cross-react with GR and AR.[24] The selectivity of **14** with GR and AR was examined using an HRE-tk-luciferase assay in the human lung carcinoma cell line A549 for GR and in mouse fibroblast cell line L929 for AR.[19] In contrast to MPA, **14** was over 500-fold selective for the PR over both GR and AR (Table 3) suggestive of less potential AR or GR-related side effects.

Excellent oral potency was observed for **14** in the ovariectomized female rat decidualization model (Table 4).[24] In this model it was 20-fold more potent than MPA and over 200-fold than progesterone with both steroids administered by subcutaneous injection. Compound **14** was also tested in an additional in vivo PR agonist model, the adult ovariectomized rat uterine component C3 assay.[25] PR agonists are known to down-regulate the estrogen induced synthesis of complement component C3 in the epithelial cells of the rat uterus. As shown in Table 4, **14** had oral potency of 0.003 mg/kg and was six times more potent than MPA (**2**). The oral activities demonstrated in rat models for **14** were substantiated by its pharmacokinetics profile. In a separate PK study using female rats, compound **14** had moderate clearance (25 mL/min/kg), high volume of distribution (7.6 L/kg), a reasonable terminal half-life (4.4 h), and $AUC_{0-\infty}$ (0.68 μg h/mL) following a single IV bolus injection of 1 mg/kg dose. When administered orally at the dose of 1 mg/kg, its exposure ($AUC_{0-\infty}$) was 0.48 μg h/mL which resulted in an excellent bioavailability of 71%.

Table 3. Relative potencies of P4 (**1**), MPA (**2**), and **14** at AR and GR

Compound	PR EC_{50} (nM)	AR EC_{50} (nM)	AR IC_{50} (nM)	GR EC_{50} (nM)	GR IC_{50} (nM)
1[a]	0.9	ND[c]	37 (46%)	ND	>1000
2[a]	0.1	6.1 (159%)	ND	10 (157%)	ND
14[b]	0.1	>10,000	~1000	>10,000	99

[a] Data from Ref. 6.
[b] Experimental values represent the average of at least duplicate determinations. The standard deviation for these assays was typically ±15% of mean or less.
[c] ND, not determined.

Table 4. Oral activities of P4 (**1**), MPA (**2**), and **14** in rat decidualization and complement component C3 model

Compound	**1**	**2**	**14**
Decidualization ED_{50}[a] (mg/kg)	5.62[b]	0.40[b]	0.02
Component C3 ED_{50}[a] (mg/kg)	ND[c]	0.03[b]	0.003

[a] Experimental values represent the average of at least duplicate determinations. The standard deviation for the decidualization and C3 assays was typically ±15% of mean or less.
[b] Dosed via subcutaneous injection.
[c] ND, not determined.

Compound **14** was further evaluated for ovulation inhibition by treating cynomolgus monkeys once daily throughout a normal menstrual cycle. In this model, **14** inhibited ovulation-induced progesterone levels with an ED_{100} of 0.03 mg/kg when dosed orally, which was similar to that of tanaproget (ED_{100} 0.01 mg/kg, po). In comparison, single subcutaneous injection of MPA at the dose of 15 mg/kg[26] also completely blocked progesterone level in cynomolgus monkey for a prolonged time. Levonorgestrel, another steroidal PR ligand evaluated in this model, achieved complete blockage of progesterone level at 0.9 μg/kg via intramuscular injection.[27] Limited data have been reported in this model for non-steroidal PR ligands. However, a tetrahydropyridazine[27] was described to completely block progesterone level at 0.9 mg/kg when dosed intramuscularly. While comparison of these PR ligands is difficult because of different routes of administration, **14** was clearly a potent and orally active non-steroidal PR agonist in the cynomolgus monkeys.

In conclusion, the SAR examination of 6-aryl benzimidazole-2-thiones has led to discovery of novel potent PR agonists. Among the benzimidazole-2-thiones prepared, compound **14** was the most potent analog and, in contrast to MPA, highly selective (>500-fold) for the PR over both glucocorticoid and androgen receptors. In the rat uterine decidualization and complement component C3 models, **14** was from 6- to 20-fold more potent than MPA. In the monkey ovulation inhibition model,

compound **14** was also highly efficacious and potent. Taken together, compound **14** is potentially useful as a novel contraceptive and for the treatment of reproductive disorders.

4. Experimental

^{1}H NMR spectra were recorded on a Bruker DPX300, Varian INOVA 400, or Varian INOVA 500 instrument. Chemical shifts are reported in δ values (parts per million, ppm) relative to an internal standard of tetramethylsilane in $CDCl_3$ or DMSO-d_6. Electrospray (ESI) mass spectra were recorded using a Hewlett-Packard 5989B MS engine or Waters Alliance-ZMD mass spectrometer. Electron Impact ionization (EI, EE = 70 eV) mass spectra were recorded on a Finnigan Trace mass spectrometer. Elemental analyses were carried out on a modified PerkinElmer model 2400 series II CHN analyzer or sent to Robertson Microlit. Analytical thin-layer chromatography (TLC) was carried out on pre-coated plates (silica gel, 60 F-254), and spots were visualized with UV light and stained in iodine. Preparative HPLC purifications were performed on a preparative Gilson HPLC system using a CombiPrep Pro C18 column with acetonitrile (0.1% TFA) and water (0.1% TFA) as solvents at a flow rate of 20 mL/min. Solvents were purchased as anhydrous grade and were used without further purification.

4.1. 6-Bromo-1-cyclobutyl-1,3-dihydro-2*H*-benzimidazol-2-one (6a)

A mixture of 5-bromo-2-oxo-2,3-dihydro-benzimidazole-1-carboxylic acid *tert*-butyl ester[11] (10 g, 31.9 mmol), cyclobutanol (4.9 mL, 48.4 mmol), and triphenylphosphine (16.7 g, 48.4 mmol) in anhydrous THF (320 mL) was treated with a solution of diethylazodicarboxylate (10 mL, 48.4 mmol) in THF (60 mL) in a dropwise manner at room temperature under nitrogen. After stirred for 30 min, to the reaction mixture was added ethyl acetate (100 mL). The organic layer was separated and aqueous layer was extracted with ethyl acetate (2× 50 mL). The combined organic layers were washed with a saturated aqueous sodium bicarbonate solution (100 mL), dried with anhydrous sodium sulfate, concentrated, and purified via a silica gel column (5% ethyl acetate in hexane) to give *tert*-butyl 5-bromo-3-cyclobutyl-2-oxo-2,3-dihydro-1*H*-benzo[d]imidazole-1-carboxylate (2.29 g, 20%) as a clear oil. The oil was taken up in dichloromethane (40 mL) and treated with trifluoroacetic acid (2.4 mL, 31 mmol). After stirred for 30 min, the solvent was removed under a reduced pressure and the residue was taken up in ethyl acetate (50 mL) and washed with saturated aqueous sodium bicarbonate solution (50 mL). The organic layer was separated, dried over anhydrous sodium sulfate, and concentrated to give **6a** (1.5 g, 88%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 11.02 (s, 1H, D_2O exchangeable), 7.48 (d, 1H, J = 1.77 Hz), 7.14 (dd, 1H, J = 8.25, 1.80 Hz), 6.91 (d, 1H, J = 8.24 Hz), 4.77 (m, 1H), 2.8 (m, 2 H), 2.23 (m, 2H), 1.79 (m, 2H). MS (ESI) m/z 267 ([M+H]$^+$).

4.2. 3-(3-Cyclobutyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)-5-fluorobenzonitrile (7a)

A mixture of 6-bromo-1-cyclobutyl-1,3-dihydro-2*H*-benzimidazol-2-one (**6a**) (1.0 g, 3.7 mmol), 3-cyano-5-fluorophenyl boronic acid (0.92 g, 5.6 mmol), and sodium carbonate (0.78 g, 7.4 mmol) in DME (60 mL) and water (30 mL) was subject to a blanket of nitrogen for 15 min at 50 °C. Tetrakis(triphenylphosphine)-palladium (0) (0.43 g, 0.37 mmol) was added and the reaction mixture was heated to 85 °C for 1 h. The reaction mixture was then cooled to room temperature and ethyl acetate (100 mL) added. The organic layer was separated and aqueous layer extracted with ethyl acetate (2× 50 mL). The combined organic layers were washed with a saturated aqueous ammonium chloride solution (2× 100 mL), dried over magnesium sulfate, and concentrated. The residue was purified via a silica gel column (40% ethyl acetate in hexane) to give **7a** (0.34 g, 31%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 11.01 (s, 1H), 8.10 (m, 1H), 7.97 (m, 1H), 7.77 (m, 1H), 7.68 (d, 1H, J = 8.0, 1.6 Hz), 7.43 (dd, 1H, J = 8.0, 1.6 Hz), 7.06 (d, 1H, J = 8.0 Hz), 4.89 (m, 1H), 2.94 (m, 2H), 2.26 (m, 2H), 1.87 (m, 1H), 1.78 (m, 1H). MS (ESI) m/z 308 ([M+H]$^+$). HRMS: Calcd for $C_{18}H_{14}FN_3O+H^+$, 308.11937. Found (ESI [M+H]$^+$): 308.12050.

4.3. 3-(3-Cyclobutyl-2-thioxo-2,3-dihydro-1*H*-benzimidazol-5-yl)-5-fluorobenzonitrile (8)

A mixture of 3-(3-cyclobutyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)-5-fluorobenzonitrile (**7a**) (80 mg, 0.26 mmol) and Lawesson's reagent (110 mg, 0.27 mmol) in anhydrous toluene was heated at reflux under nitrogen for 18 h. The solvent was removed in vacuo and residue was purified on a silica gel column (30% ethyl acetate in hexane) to afford **8** as an off-white solid (28 mg, 33%). ^{1}H NMR (DMSO-d_6) δ 12.94 (s, 1H, D_2O exchangeable), 8.19 (s, 1H), 8.04–8.16 (m, 1H), 7.97 (s, 1H), 7.82–7.86 (m, 1H), 7.61 (dd, 1H, J = 8.27, 1.55 Hz), 7.28 (d, 1H, J = 8.28 Hz), 5.62–5.69 (m, 1H), 3.09–3.19 (m, 2H), 2.28–2.35 (m, 2H), 2.01–2.11 (m, 1H), 1.82–1.92 (m, 1H). MS (ESI) m/z 324 ([M+H]$^+$). Anal. Calcd for $C_{18}H_{14}FN_3S{\cdot}0.1H_2O$: C, 66.48; H, 4.4; N, 12.92. Found: C, 66.81; H, 4.33; N, 12.57.

The following compounds (**9**–**12**) were prepared by following the procedures of compound **8**.

4.4. 6-Bromo-1-cyclopentyl-1,3-dihydro-2*H*-benzimidazol-2-one (6b)

The title compound was prepared from 5-bromo-2-oxo-2,3-dihydro-benzimidazole-1-carboxylic acid *tert*-butyl ester and cyclopentanol as an off-white solid (2.1 g, 85%). ^{1}H NMR (DMSO-d_6) δ 11.0 (s, 1H), 7.32 (d, 1H, J = 1.79 Hz), 7.13 (dd, 1 H, J = 8.22, 1.87 Hz), 6.92 (d, 1H, J = 8.25 Hz), 4.68 (m, 1H), 2.0 (m, 2H), 1.88 (m, 4H), 1.64 (m, 2H). MS (ESI) m/z 281 ([M+H]$^+$).

4.5. 6-(3-Chloro-4-fluorophenyl)-1-cyclopentyl-1,3-dihydro-2*H*-benzimidazol-2-one (7b)

The title compound was prepared from **6b** and 3-chloro-4-fluorophenyl boronic acid as a white solid (0.67 g, 44%). ^{1}H NMR (DMSO-d_6) δ 10.93 (s, 1H, D_2O exchangeable), 7.87 (dd, 1H, J = 7.15, 2.34 Hz), 7.64–7.68 (m, 1H), 7.47 (t, 1H, J = 9.10 Hz), 7.41 (d, 1H, J = 1.55 Hz), 7.28 (dd, 1H, J = 8.06, 1.69 Hz), 7.04 (d, 1H, J = 8.06 Hz), 4.72–4.82 (m, 1H), 2.09–2.18 (m, 2H), 1.83–1.92 (m, 4H), 1.60–1.73 (m, 2H). MS (ESI) m/z 331 ([M+H]$^+$).

4.6. 6-(3-Chloro-4-fluorophenyl)-1-cyclopentyl-1,3-dihydro-2*H*-benzimidazole-2-thione (9)

The title compound was prepared from **7b** and Lawesson's reagent as an off-white solid (89 mg, 46%). ^{1}H NMR (DMSO-d_6) δ 12.88 (s, 1H, D_2O exchangeable), 7.91 (dd, 1H, J = 7.02, 2.34 Hz), 7.68–7.72 (m, 1H), 7.45–7.56 (m, 3H), 7.27 (d, 1H, J = 8.31 Hz), 5.48–5.56 (m, 1H), 2.22–2.33 (m, 2H), 1.92–2.06 (m, 4H), 1.67–1.78 (m, 2H). MS (ESI) m/z 347 ([M+H]$^+$).

4.7. 1-Cyclopentyl-6-(3,4-difluorophenyl)-1,3-dihydro-2*H*-benzimidazol-2-one (7c)

The title compound was prepared from **6b** and 3,4-difluorophenyl boronic acid as a white solid (0.87 g, 54%). ^{1}H NMR (DMSO-d_6) δ 10.93 (s, 1H, D_2O exchangeable), 7.75 (m, 1H), 7.5 (m, 2H), 7.40 (d, 1H, J = 1.56 Hz), 7.29 (dd, 1H, J = 8.06, 1.69 Hz), 7.04 (d, 1H, J = 8.06 Hz), 4.78 (m, 1H), 2.13 (m, 2H), 1.91 (m, 4H), 1.65 (m, 2H). MS (ESI) m/z 315 ([M+H]$^+$).

4.8. 1-Cyclopentyl-6-(3,4-difluorophenyl)-1,3-dihydro-2*H*-benzimidazole-2-thione (10)

The title compound was prepared from **7c** and Lawesson's reagent as an off-white solid (0.12 g, 67%). ^{1}H NMR (DMSO-d_6) δ 12.88 (s, 1H, D_2O exchangeable), 7.81 (m, 1H), 7.47–7.56 (m, 4H), 7.27 (d, 1H, J = 8.18 Hz), 5.53 (m, 1H), 2.23 (m, 2H), 1.97 (m, 4H), 1.73 (m, 2H). MS (ESI) m/z 331 ([M+H]$^+$). Anal. Calcd for $C_{18}H_{16}F_2N_2S$: C, 65.44; H, 4.88; N, 8.48. Found: C, 65.07; H, 4.98; N, 8.30.

4.9. 6-Bromo-1-(1-ethylpropyl)-1,3-dihydro-2*H*-benzimidazol-2-one (6c)

The title compound was prepared from 5-bromo-2-oxo-2,3-dihydro-benzimidazole-1-carboxylic acid *tert*-butyl ester and 3-pentanol as a white solid (1.9 g, 73%). ^{1}H NMR (DMSO-d_6) δ 11.03 (s, 1H, D_2O exchangeable), 7.39 (d, 1H, J = 1.80 Hz), 7.12 (dd, 1H, J = 8.24, 1.81 Hz), 6.93 (d, 1H, J = 8.24 Hz), 4.05 (m, 1H), 1.90–2.02 (m, 2 H), 1.72–1.83 (m, 2H), 0.72 (t, 6H, J = 7.36 Hz). MS (ESI) m/z 283 ([M+H]$^+$).

4.10. 6-(3-Chloro-4-fluorophenyl)-1-(1-ethylpropyl)-1,3-dihydro-2*H*-benzimidazol-2-one (7d)

The title compound was prepared from **6c** and 3-chloro-4-fluorophenyl boronic acid as a white solid (0.77 g, 64%). ^{1}H NMR (DMSO-d_6) δ 10.92 (s, 1H, D_2O exchangeable), 7.88 (dd, 1H, J = 7.15, 2.34 Hz), 7.67 (m, 1H), 7.48 (m, 2H), 7.28 (dd, 1H, J = 8.19, 1.69 Hz), 7.04 (d, 1H, J = 8.06 Hz), 4.25 (m, 1H), 2.08–2.18 (m, 2H), 1.73–1.82 (m, 2H), 0.76 (t, 6H, J = 7.40 Hz). MS (ESI) m/z 333 ([M+H]$^+$).

4.11. 6-(3-Chloro-4-fluorophenyl)-1-(1-ethylpropyl)-1,3-dihydro-2*H*-benzimidazole-2-thione (11)

The title compound was prepared from **7d** and Lawesson's reagent as an off-white solid (0.11 g, 55%). ^{1}H NMR (DMSO-d_6) δ 12.86 (s, 1H, D_2O exchangeable), 7.93 (d, 1H, J = 5.20 Hz), 7.72 (m, 2H), 7.48 (m, 2H), 8.18 (d, 1H, J = 8.18 Hz), 5.17 (m, 1H), 2.12–2.21 (m, 2H), 1.90–1.99 (m, 2H), 0.74 (t, 6H, J = 7.27 Hz). MS (ESI) m/z 349 ([M+H]$^+$). Anal. Calcd for $C_{18}H_{18}ClFN_2S$: C, 61.97; H, 5.20; N, 8.03. Found: C, 61.76; H, 5.09; N, 7.84.

4.12. 3-[3-(1-Ethylpropyl)-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl]-5-fluorobenzonitrile (7e)

The title compound was prepared from **6c** and 3-cyano-5-fluorophenyl boronic acid as a white solid (0.55 g, 44%). ^{1}H NMR (DMSO-d_6) δ 11.04 (s, 1H, D_2O exchangeable), 8.10 (s, 1H), 7.95 (m, 1H), 7.77 (m, 1H), 7.62 (s, 1H), 7.42 (dd, 1H, J = 8.13, 1.51 Hz), 7.07 (d, 1H, J = 8.15 Hz), 4.16 (m, 1H), 2.05–2.15 (m, 2 H), 1.72–1.81 (m, 2H), 0.76 (t, 6H, J = 7.32 Hz). MS (ESI) m/z 324 ([M+H]$^+$). Anal. Calcd for $C_{19}H_{18}FN_3O{\cdot}0.2EtOH$: C, 70.1; H, 5.76; N, 12.64. Found: C, 70.28; H, 5.73; N, 12.39.

4.13. 3-[3-(1-Ethylpropyl)-2-thioxo-2,3-dihydro-1*H*-benzimidazol-5-yl]-5-fluorobenzonitrile (12)

The title compound was prepared from **7e** and Lawesson's reagent as an off-white solid (0.13 g, 46%). ^{1}H NMR (DMSO-d_6) δ 12.91 (s, 1H, D_2O exchangeable), 8.14 (s, 1H), 8.00 (d, 1H, J = 10.50 Hz), 7.85 (m, 2H), 7.61 (dd, 1H, J = 8.32, 1.15 Hz), 7.29 (d, 1H, J = 8.20 Hz), 5.17 (m, 1H), 2.12–2.21 (m, 2H), 1.91–2.03 (m, 2H), 0.72 (t, 6H, J = 7.31 Hz). MS (ESI) m/z 340 ([M+H]$^+$). Anal. Calcd for $C_{19}H_{18}FN_3S{\cdot}0.5H_2O$: C, 65.49; H, 5.5; N, 12.06. Found: C, 65.17; H, 5.2; N, 11.76.

4.14. 5-(3-Cyclopentyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)-1-methyl-1*H*-pyrrole-2-carbonitrile (7g)

To a stirred solution of *N*-methyl-2-cyanopyrrole (0.25 g, 2.32 mmol) and triisopropylborate (0.55 mL, 2.38 mmol) in THF (5 mL) at 0 °C under nitrogen was added lithium diisopropyl amide (2.0 M in THF, 1.5 mL, 3 mmol) in a dropwise manner. After stirred for 1 h, the solution was treated with 6-bromo-1-cyclopenyl-1,3-dihydro-2*H*-benzimidazol-2-one (**6b**, 0.33 g, 1.16 mmol), glyme (5 mL), sodium carbonate (0.37 g, 3.48 mmol) dissolved in water (2 mL), and tetrakis(triphenylphosphine) palladium (0) (0.07 g, 0.06 mmol). The resulting mixture was heated at 70 °C for 3 h and cooled to ambient temperature. A saturated ammonium

chloride aqueous solution (50 mL) and ethyl acetate (100 mL) was added. The organic layer was separated, dried over magnesium sulfate, and concentrated. The residue was purified via a silica gel column (50% ethyl acetate in hexane) to afford **7g** as an off-white solid (0.12 g, 34%). ^{1}H NMR (DMSO-d_6) δ 11.02 (s, 1H), 7.23 (d, 1H, J = 1.17 Hz), 7.06–7.12 (m, 2H), 7.03 (d, 1H, J = 4.03 Hz), 6.31 (d, 1H, J = 4.03 Hz), 4.72 (q, 1H, J = 8.6 Hz), 3.72 (s, 3H), 2.05–2.10 (m, 2H), 1.85–1.93 (m, 4H), 1.62–1.66 (m, 2H). MS (ESI) m/z 307 ([M+H]$^+$). HRMS: Calcd for $C_{18}H_{18}N_4O + H^+$, 307.15534. Found (ESI_FT [M+H]$^+$): 307.15508.

4.15. 5-(3-Cyclopentyl-2-thioxo-2,3-dihydro-1*H*-benzimidazol-5-yl)-1-methyl-1*H*-pyrrole-2-carbonitrile (14)

To a stirred solution of 5-(3-cyclopentyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)-1-methyl-1*H*-pyrrole-2-carbonitrile (0.10 g, 0.33 mmol) in toluene (7 mL) was added Lawesson's reagent (0.19 g, 0.47 mmol) and the resulting solution was heated to 100 °C overnight. The solution was allowed to cool, partitioned between saturated ammonium chloride solution (50 mL) and ethyl acetate (80 mL). The organic layer was separated, dried over magnesium sulfate, and concentrated. The residue was purified via a silica gel column using a 10–20% ethyl acetate in hexane gradient to afford **14** as a yellowish solid (0.035 g, 33%). ^{1}H NMR (DMSO-d_6) δ 12.95 (s, 1H), 7.44 (s, 1H), 7.28–7.32 (m, 2H), 7.05 (d, 1H, J = 4.03 Hz), 6.39 (d, 1H, J = 4.03 Hz), 5.54 (q, 1H, J = 9.0 Hz), 3.73 (s, 3H), 2.10–2.19 (m, 2H), 1.95–1.99 (m, 4H), 1.70–1.73 (m, 2H). MS (ESI) m/z 323 ([M+H]$^+$). HRMS: Calcd for $C_{18}H_{18}N_4S + H^+$, 323.13249. Found (ESI_FT [M+H]$^+$): 323.13252. Anal. Calcd for $C_{18}H_{18}N_4S$: C, 67.05; H, 5.63; N, 17.38. Found: C, 66.71; H, 5.43; N, 17.29.

The following compounds (**13** and **15**) were prepared by following the procedures of compound **14**.

4.16. 5-(3-Cyclobutyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)-1-methyl-1*H*-pyrrole-2-carbonitrile (7f)

The title compound was prepared from **6a** and *N*-methyl-2-cyanopyrrole as an off-white solid (0.23g, 44%). ^{1}H NMR (DMSO-d_6) δ 11.02 (s, 1H, D_2O exchangeable), 7.40 (s, 1H), 7.02–7.12 (m, 3H), 6.32 (dd, 1H, J = 4.03 Hz), 4.82–4.88 (m, 1H), 3.73 (s, 3H), 2.81–2.91 (m, 2H), 2.40–2.49 (m, 2H), 1.82–1.92 (m, 1H), 1.72–1.81 (m, 1H). MS (ESI) m/z 293 ([M+H]$^+$). Anal. Calcd for $C_{17}H_{16}N_4O{\cdot}0.5H_2O$: C, 67.76; H, 5.69; N, 18.59. Found: C, 67.82; H, 5.42; N, 18.21.

4.17. 5-(3-Cyclobutyl-2-thioxo-2,3-dihydro-1*H*-benzimidazol-5-yl)-1-methyl-1*H*-pyrrole-2-carbonitrile (13)

The title compound was prepared from **7f** and Lawesson's reagent a yellowish solid (0.11 g, 67%). ^{1}H NMR (DMSO-d_6) δ 12.95 (s, 1H, D_2O exchangeable), 7.79 (d, 1H, J = 0.91 Hz), 7.27–7.34 (m, 2H), 7.06 (d, 1H, J = 4.03 Hz), 6.41 (d, 1H, J = 4.03 Hz), 5.62–5.71 (m, 1H), 3.75 (s, 3H), 2.91–3.03 (m, 2H), 2.38–2.49 (m, 2H), 1.98–2.07 (m, 1H), 1.82–1.91 (m, 1H). MS (ESI) m/z 309 ([M+H]$^+$).

4.18. 5-[3-(1-Ethylpropyl)-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl]-1-methyl-1*H*-pyrrole-2-carbonitrile (7h)

The title compound was prepared from **6c** and *N*-methyl-2-cyanopyrrole as an off-white solid (0.36 g, 48%). ^{1}H NMR (DMSO-d_6) δ 11.00 (s, 1H), 7.30 (s, 1H), 7.06–7.11 (m, 2H), 7.01 (d, 1H, J = 4.03 Hz), 6.30 (d, 1H, J = 4.03 Hz), 4.02–4.13 (m, 1H), 3.71 (s, 3H), 1.97–2.05 (m, 2H), 1.74–1.81 (m, 2H), 0.73 (t, 6H, J = 7.41 Hz). MS (ESI) m/z 309 ([M+H]$^+$). HRMS: Calcd for $C_{18}H_{20}N_4O + H^+$, 309.17099. Found (ESI_FT [M+H]$^+$): 309.17076.

4.19. 5-[3-(1-Ethylpropyl)-2-thioxo-2,3-dihydro-1*H*-benzimidazol-5-yl]-1-methyl-1*H*-pyrrole-2-carbonitrile (15)

The title compound was prepared from **7h** and Lawesson's reagent a yellowish solid (0.088g, 63%). ^{1}H NMR (DMSO-d_6) δ 12.92 (s, 1H), 7.60 (s, 1H), 7.29 (s, 2H), 7.05 (d, 1H, J = 4.03 Hz), 6.36 (d, 1H, J = 4.03 Hz), 5.10–5.20 (m, 1H), 3.72 (s, 3H), 2.02–2.10 (m, 2H), 1.89–1.95 (m, 2H), 0.71 (t, 6H, J = 7.41 Hz). MS (ESI) m/z 325 ([M+H]$^+$). HRMS: Calcd for $C_{18}H_{20}N_4S + H^+$, 325.14814. Found (ESI_FT [M+H]$^+$): 325.14805.

4.20. Rat decidualization assay

4.20.1. Reagents. Test compounds were dissolved in 100% ethanol and mixed with corn oil (vehicle). Stock solutions of the test compounds in oil (Mazola™) were then prepared by heating (~80 °C) the mixture to evaporate ethanol. Test compounds were subsequently diluted with 100% corn oil or 10% ethanol in corn oil prior to the treatment of animals. No difference in decidual response was found when these two vehicles were compared.

4.20.2. Animals. Ovariectomized mature female Sprague–Dawley rats (~60-day-old and 230 g) were obtained from Taconic (Taconic Farms, NY) following surgery. Ovariectomy was performed at least 10 days prior to treatment to reduce circulating sex steroids. Animals were housed under 12 h light/dark cycle and given standard rat chow and water ad libitum.

4.20.3. Treatment. Rats were weighed and randomly assigned to groups of 4 or 5 before treatment. Test compounds in 0.2 mL vehicle were administered by subcutaneous injection in the nape of the neck or by gavage using 0.5 mL. The animals were treated once daily for seven days. For testing antiprogestins, animals were given the test compounds and an EC_{50} dose of progesterone (5.6 mg/kg) during the first 3 days of treatment. Following decidual stimulation, animals continued to receive progesterone until necropsy 4 days later.

4.20.4. Dosing. Doses were prepared based upon mg/kg mean group body weight. In all studies, a control group receiving vehicle was included. Determination of dose–

response curves was carried out using doses with half-log increases (e.g., 0.1, 0.3, 1.0, 3.0 mg/kg...).

4.20.5. Decidual induction. Approximately 24 h after the third injection, decidualization was induced in one of the uterine horns by scratching the antimesometrial luminal epithelium with a blunt 21 G needle. The contralateral horn was not scratched and served as an unstimulated control. Approximately 24 h following the final treatment, rats were sacrificed by CO_2 asphyxiation and body weight measured. Uteri were removed and trimmed of fat. Decidualized (D-horn) and control (C-horn) uterine horns were weighed separately.

4.20.6. Analysis of results. The increase in weight of the decidualized uterine horn was calculated by D-horn/C-horn and logarithmic transformation was used to maximize normality and homogeneity of variance. The Huber M-estimator was used to down weight the outlying transformed observations for both dose–response curve fitting and one-way analysis of variance. JMP software (SAS Institute, Inc.) was used for both one-way ANOVA and non-linear dose–response analyses.

4.21. Rat uterine C3 model

4.21.1. Reagents. Stock solutions of the test compounds are prepared in 100% ethanol or 100% DMSO if they are not soluble in ethanol. The compounds are prepared for dosing in 10% ethanol in corn oil (Mazola™) vehicle.

4.21.2. Animals. Ovariectomized-female, 60-day-old Sprague–Dawley rats are obtained following surgery (Harlan). The surgeries are to be done a minimum of 8 days prior to the first treatment. The animals are housed under 12 h light/dark cycle. In some of the assay validation studies, the animals were fed standard rat chow and water ad libitum, while in others the animals were fed the casein-based Laboratory Rodent Diet 5K96 (Purina) and water ad libitum. All future studies will be run using the 5K96 diet.

4.21.3. Treatment. Upon arrival the rats are randomized, placed in groups of six, and given a minimum of 72 h to acclimate to the surroundings. They are then treated once a day for two days with the compound(s) of interest or vehicle (vehicle control group). Administration of the compound is either by subcutaneous injection of 0.2 mL in the nape of the neck or orally by gavage of 0.5 mL. On the second day, the animals are co-treated with 17α-ethinyl estradiol (EE) or vehicle (vehicle control group), orally by gavage, 0.5 mL per dose dosing: two control groups are included in all analyses: a vehicle group and an EE group. Doses are prepared based on mg/kg mean group body weight. Initial screening of test compounds is done at three doses (e.g., 0.03, 0.3, 3 mg/kg body weight). Dose–response curves may be run on active compounds using doses with half-log increments over a dose range determined from the initial screening data. Approximately 24 h following the final treatment the animals are killed by CO_2 asphyxiation and the body weight and uterine wet weight determined.

4.21.4. Assays uterine complement component C3. Following euthanasia, the uteri are removed from the animals, stripped of remaining fat and mesentary, and weighed. The uteri are snap-frozen on dry ice in groups of two. Total RNA is extracted using TRIzol reagent (Gibco-BRL). RNA samples are run on a 1% agarose/formaldehyde gel. The nucleic acids are then transferred to a nylon membrane overnight by capillary action. The nucleic acids are crosslinked to the membrane using UV light, the 28S rRNA is quantitated using the IP Lab Gel software (Signal Analytics Corp.), and then the blot is hybridized with a cDNA probe for complement component C3. Following hybridization, the blot is exposed to a phosphorscreen. Quantitation of C3 message is performed using a phosphorimager (Molecular Dynamics).

4.21.5. Analysis of results. Results are reported as the ratio C3/28S. These ratios are transformed by logarithms to normalize the data. The Huber M-estimator is used to down weight the outlying transformed observations. The JMP software (SAS Institute, Inc.) is used to analyze the transformed and weighted data for both the one-way ANOVA and the non-linear dose–response curves. The IC_{50} values with 95% confidence intervals are calculated using a four parameter logistic model that calculates minimum, maximum, slope, and IC_{50}.

4.22. Primate ovulation inhibition assay

The ovulation inhibition assay in cynomolgus monkeys was carried out as described[27] with modifications. Briefly, animals were treated with vehicle (2% Tween 80/0.5% methylcellulose) or test compounds orally from day 2 to 28 of the menstrual cycle. Blood samples were taken every Monday, Wednesday, and Friday until next menses for progesterone measurement. The occurrence of ovulation was inferred from the pattern of progesterone during the menstrual cycles.

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