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Novel pyrrole-containing progesterone receptor modulators

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Abstract—A series of 1,4-dihydro-2*H*-[*d*][3,1]-benzoxazin-2-one and 1,3-dihydro-[3*H*]-indol-2-one containing 6- or 5-, respectively, appended substituted pyrrole moieties were synthesized and evaluated for their ability to modulate the activity of the progesterone receptor (PR). Key structural changes to the pyrrole moieties of these molecules were shown to have a predictive influence as to whether the compounds behaved as PR agonists or antagonists. Compounds with the 5′-cyano-2′-pyrrole moiety (e.g., **32**, **33**, and **38**) were shown to be potent PR agonists (EC_{50}'s of 1.1, 1.8, and 2.8 nM, respectively). Compounds with the 5′-nitro-2′-pyrrole moiety (e.g., **34** and **36**) were shown to be PR antagonists (IC_{50}'s of 180 and 36 nM, respectively).

The progesterone receptor (PR) is a member of the steroid receptor sub-family of the nuclear hormone receptor super-family, a group of ligand dependent nuclear transcription factors.[1] Progesterone (P4, Fig. 1), the endogenous ligand for the PR, is involved in the control of ovulation and preparation of the uterus to support pregnancy. Clinically PR agonists (e.g., Medroxyprogesterone acetate—MPA)[2] are mainly used in contraception and hormone therapy, typically coadministered with an estrogen. One of the main issues with the steroidal PR agonists is that they often bind and modulate the function of other members of the nuclear hormone receptor super-family: for example, the androgen (AR), glucocorticoid (GR), and mineralocorticoid (MR) receptors. In principal a PR antagonist, may also have potential utility as a contraceptive.[3] However, current steroidal PR antagonists, such as Mifepristone (RU-486), are potentially compromised as a clinically useful contraceptive agents due to overt glucocorticoid receptor antagonism.[4]

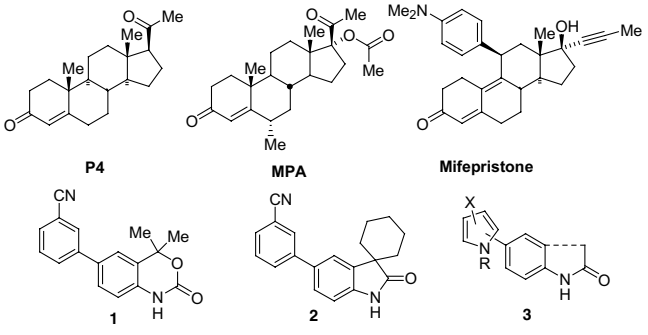

Figure 1. Steroid and nonsteroid PR modulators.

As part of an ongoing Progesterone Receptor Antagonist program, we investigated replacing the cyanophenyl moiety of our potent 1,4-dihydro-2*H*-[*d*][3,1]-benzoxazin-2-one series (e.g., **1**)[5] and 1,3-dihydro-[3*H*]-indol-2-one series (e.g., **2**)[6] with substituted 5-membered, nitrogen-containing heterocyclic ring systems. In particular, when the pyrrole moiety was appended to these scaffolds to afford **3**, we found that the functional activity (agonist vs antagonist) could be altered depending upon the substituent on the pyrrole ring and the substitution pattern of these groups. In this paper, the synthesis of these pyrrole and other heterocyclic-containing PR modulators and their biological activity will be described and compared to that of the cyanophenyl congeners **1** and **2**.

The preparation of the compounds of generic structure **3** is depicted in Schemes 1, 2, 4, and 5. Oxazole and

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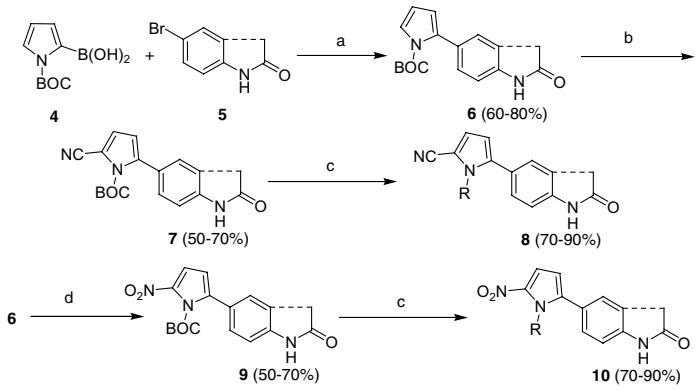
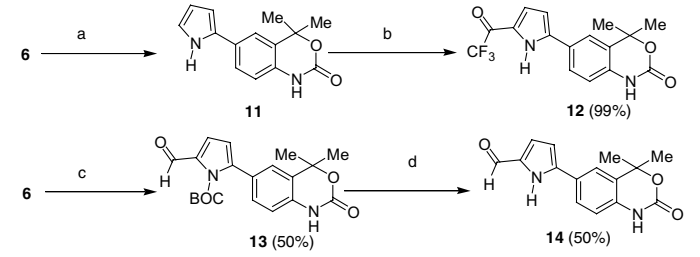
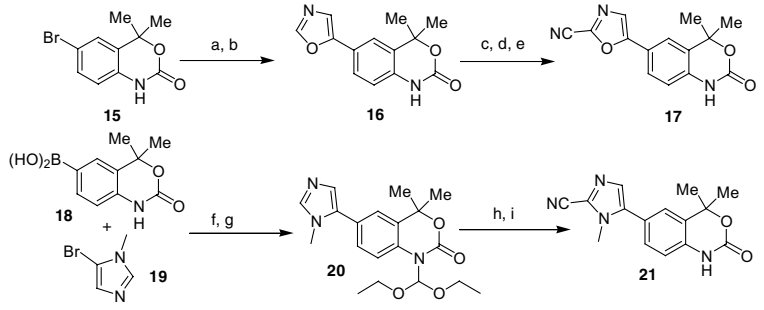


Scheme 1. Reagents and conditions: (a) $Pd(PPh_3)_4$ (cat), K_2CO_3 (aq), DME, 80 °C, 2 h; (b) CSI, THF, −78 °C, 1 h; then DMF, warm to rt; (c) NaOEt, EtOH/THF, 10 min; (for R≠H) then RI (1.0 equiv), K_2CO_3, DMF, rt, 16 h; (d) $AgNO_3$, AcCl, MeCN–CH_2Cl_2 (10:1), −20 °C to rt, 16 h.



Scheme 2. Reagents and conditions: (a) 180 °C; (b) Tf_2O, DCE; (c) $POCl_3$, DMF; (d) 140 °C.



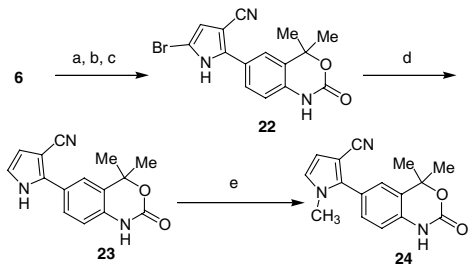
Scheme 3. Reagents and conditions: (a) *n*BuLi/DMF (40%); (b) TOSMIC, K_2CO_3, DCE; (c) *n*BuLi/DMF (38%); (d) $NH_2OH \cdot HCl$; (e) $SOCl_2$; (f) $Pd(PPh_3)_4$ (cat), K_2CO_3 (aq), DME, reflux (40%); (g) $CH(OEt)_3$ (40%); (h) LDA, TsCN; (i) HCl (10%).

imidazole analogs of **3** (compounds **17** and **21**) are depicted in Scheme 3.

According to Scheme 1, Suzuki coupling of Boc-protected pyrrole boronic acid **4** with 6-bromo-1,4-dihydro-2*H*-[*d*][3,1]-benzoxazin-2-one[5] or 5-bromo-1,3-dihydro-[3*H*]-indol-2-one[6] (both represented by **5**) afforded the requisite biaryls **6** in good yield. Cyanation (method b) was cleanly effected with chlorosulfonyl isocyanate (CSI) to provide **7**. Likewise nitration (method d) of **6** could be accomplished readily with silver nitrate to provide **9**. The BOC protecting group of cyanopyrrole **7** or nitropyrrole **9** was removed by brief treatment with ethanolic sodium ethoxide and the free pyrrole could then be selectively alkylated by alkyl halides in DMF under the influence of potassium carbonate to deliver the required substituted pyrroles **8** and **10**.

According to Scheme 2, 5′-trifluoroacetyl and 2′-carboxy-aldehyde congeners **12** and **14** could be prepared via electrophilic acylation of compound **6** or its Boc-deprotected analog **11**. The BOC group of **6** or **13** could be removed under thermolysis conditions.

According to Scheme 3, cyano-oxazole **17** could be prepared using a 5-step sequence. Reaction of 6-lithio-1,4-dihydro-2*H*-[*d*][3,1]-benzoxazin-2-one (prepared from bromide **15**) with DMF, followed by reaction with TOSMIC provided oxazole **16**. The 2′-carboxaldehyde was prepared by treating **16** with *n*-butyl lithium



Scheme 4. Reagents and conditions: (a) NBS, THF, −78 °C (95%); (b) CSI, THF, −78 °C, 1 h; then DMF, warm to rt (30%); (c) NaOEt (82%); (d) Zn, NH_4Cl, aq EtOH, 80 °C, 1 h; (e) CH_3I, K_2CO_3, DMF.

followed by DMF. The oxime was then prepared and dehydrated to form target compound **17**.

For the preparation of imidazole **21**, 1,4-dihydro-2*H*-[*d*][3,1]-benzoxazin-2-one, 6-boronic acid **18**[5] was reacted with the 5-bromo-imidazole **19** using standard Suzuki conditions. The NH of the benzoxazin-2-one group was then protected with an acetal group to afford **20**. The 2′-position of the imidazole moiety of **20** was lithiated with LDA and further reacted with tosylcyanide to provide target compound **21**, after removal of the acetal-protecting group.

According to Scheme 4, a bromine group was introduced into the pyrrole 5′-position of compound **6** to serve as a blocking group so that the cyanating reagent (CSI) delivered the CN group to the 3′-position of the pyrrole nucleus. Removal of the BOC group afforded **22**. Reduction of the Br group was effected with zinc to afford target compound **23**. Further methylation provided target compound **24**.

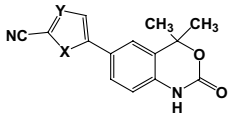
According to Scheme 5, the 1-silyl-pyrrole, 3-boronic acid **25**[7] was coupled with compound **15** using standard Suzuki conditions. The NH of the benzoxazin-2-one group was protected with an acetal-protecting group and desilylated to afford compound **26**.

A bromine group was introduced into the pyrrole 2′-position of compound **26** to serve as a blocking group so that the cyanating reagent (CSI) delivered the CN group to the 5′-position of the pyrrole nucleus to give **27**. Deprotection followed by reduction of the Br group with Zn afforded target compound **28**. Compound **27** was also methylated, and the acetal-protecting group and Br moiety removed to provide compound **29**.

The compounds in Tables 1–5 were evaluated for PR agonist and antagonist activity. The agonist assay measures the compounds' ability to induce alkaline phosphatase in the T47D human breast cancer cell line and the antagonist assay measures the ability to block progesterone induced alkaline phosphatase activity in this cell line. Previously, we showed that compound **1** (Table 1) was a potent progesterone receptor antagonist.[5] We investigated replacing the 6-(cyanophenyl) of compound **1** with 5-membered heterocyclic rings. The thiophene ring has historically been a good replacement for a phenyl ring. This was also true in our series, for 5′-cyano-2′-thiophene **30** retained good PR antagonist potency.[5] Compound **31**, with a 5′-cyano-2′-furan group was also an antagonist, albeit somewhat weaker than **30**.[5]

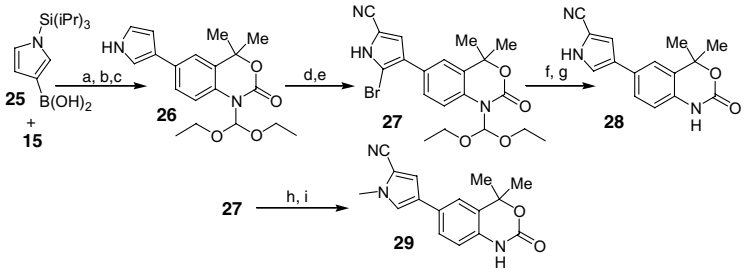
However, quite interestingly, the replacement of the furan oxygen atom of **31** (or thiophene sulfur atom of **30**) with a NCH_3 moiety, to afford 5′-cyano-2′-pyrrole analog **32**, led to a switch in functional activity. Compound **32** was a potent agonist ($EC_{50} = 1.1$ nM) with an

Table 1.



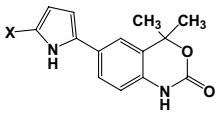
Compound	X	Y	PR alkaline phosphatase[a]	
			IC_{50} (nM)	EC_{50} (nM)
1	See Figure 1		8	∼1000
17	O	N	∼3000	>1000
21	NCH_3	N	>3000	130
30	S	CH	23	∼1000
31	O	CH	65	∼3000
32	NCH_3	CH	>3000	1.1
33	NEt	CH	∼3000	1.8
MPA	See Figure 1			0.5

[a] Values represent the average of at least duplicate determinations. The standard deviation for the assay was typically ±20% of the mean or less.



Scheme 5. Reagents and conditions: (a) $Pd(PPh_3)_4$ (cat), K_2CO_3 (aq), DME, heat (75%); (b) $CH(OEt)_3$, reflux, 16 h (86%); (c) TBAF, THF, 5 min (100%); (d) NBS, THF, −78 °C; (e) CSI, THF, −78 °C, 1 h; then DMF, warm to rt; (f) 1 N HCl, THF, rt, 30 min; (g) Zn, NH_4Cl, aq EtOH, 80 °C, 1 h; (h) MeI, DMF, K_2CO_3, 16 h; (i) NH_4Cl, aq EtOH, 80 °C, 10 min, then Zn, 1 h.

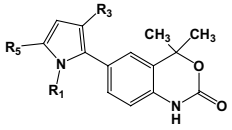
Table 2.



Compound	X	PR alkaline phosphatase[a]	
		IC_{50} (nM)	EC_{50} (nM)
11	H	~3000	>3000
12	CF_3CO	>3000	~1000
14	CHO	>3000	~1000
34	NO_2	180	~1000
35	CN	>3000	2.1

[a] Values represent the average of at least duplicate determinations. The standard deviation for the assay was typically ±20% of the mean or less.

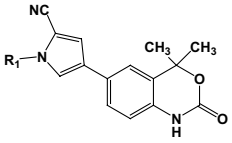
Table 3.



Compound	R_1	R_3	R_5	PR alkaline phosphatase[a]	
				IC_{50} (nM)	EC_{50} (nM)
23	H	CN	H	>3000	>1000
24	CH_3	CN	H	~3000	>1000
32	CH_3	H	CN	>3000	1.1
35	H	H	CN	>3000	2.1

[a] Values represent the average of at least duplicate determinations. The standard deviation for the assay was typically ±20% of the mean or less.

Table 4.

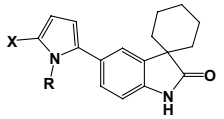


Compound	R_1	PR alkaline phosphatase[a]	
		IC_{50} (nM)	EC_{50} (nM)
28	H	~2000	>1000
29	CH_3	41	>1000

[a] Values represent the average of at least duplicate determinations. The standard deviation for the assay was typically ±20% of the mean or less.

activity approaching that of steroids such as MPA. The *N*-ethyl analog **33** was also a potent agonist. The corresponding 2′-cyano-5′-imidazole analog **21**, also was an agonist; however it was over 100-fold weaker than pyrrole **32**. The 2′-cyano-5′-oxazole congener **17** was only a weak modulator. Since the 5′-cyano-2′-pyrrole moiety showed such a pronounced and different effect than we have seen previously with the benzoxazin-2-one series, we decided to investigate the SAR of this moiety in more depth.

Table 5.



Compound	R	X	PR alkaline phosphatase[a]	
			IC_{50} (nM)	EC_{50} (nM)
2	See Figure 1		6.6	~1000
36	H	NO_2	36	>1000
37	H	CN	>3000	60
38	CH_3	CN	>3000	2.8

[a] Values represent the average of at least duplicate determinations. The standard deviation for the assay was typically ±20% of the mean or less.

According to Table 2, the 5′-cyano-2′-pyrrole **35**, which differs from **32** in that it has an NH instead of an NCH_3, was also a potent agonist. Replacing the CN of **35** with H (compound **11**), CHO (compound **12**), $COCF_3$ (compound **14**), or NO_2 (compound **34**) did not afford compounds with good agonist activity. The nitro analog **34** was a moderately active antagonist with some agonist activity at approximately 1 μM, however the others were very weak modulators.

Moving the nitrile of **32** or **35** from the 5′-position to the 3′-position as shown by compounds **23** and **24** in Table 3 led to only weak modulators.

The attachment position of the pyrrole to the benzoxazin-2-one scaffold from the 2′-position of **32** and **35** to the 3′-position (Table 4) was also examined. The NCH_3 congener (**29**) was active as an antagonist with an IC_{50} of 41 nM, while the NH analog (**28**) was only a very weak modulator. Thus, the nature of the substituent on the pyrrole (i.e., nitrile) as well as its position on the pyrrole moiety and the position of attachment of the pyrrole ring to the benzoxazin-2-one nucleus are important features determining the functional activity of these molecules.

The benzoxazin-2-one (e.g., **1**) and the indol-2-one (e.g., **2**) series has generally shown parallel SAR.[5,6] With this in mind, we prepared several analogs in the indol-2-one series with various substituted pyrrole groups appended to the indol-2-one 5-position. The results are displayed in Table 5. Indeed, the 5′-nitro-2′-pyrrole analog **36** was a respectable antagonist, consistent with its benzoxazine-2-one counterpart **34**. Also, quite in line with the benzoxazine-2-one SAR, the indole-2-one-containing 5′-cyano-2′-pyrrole analogs **37** and **38** were agonists, with the NCH_3 analog **38** showing strong potency ($EC_{50} = 2.8$ nM).

Table 6. T47D whole cell binding[a]

Compound	**1**	**2**	**29**	**32**	**33**	**35**	**38**	**P4**	**Mif**[b]
IC_{50} (nM)	18	25	88	4.9	9.4	29	3.4	3.4	0.7

[a] Values represent the average of at least duplicate determinations. The standard deviation for the assay was typically ±20% of the mean or less.
[b] Mifepristone.

Selected compounds were tested for their ability to displace tritiated progesterone in a T47D whole cell binding assay (Table 6). Both the antagonists (**1**, **2**, and **29**) and agonists (**32**, **33**, **35**, and **38**) showed competitive binding activity in the nanomolar range. The nonsteroidal PR agonists tended to be more potent than the nonsteroidal PR antagonists in this assay.

The most potent compound, **32** was profiled for its selectivity against other closely related steroid hormone receptors.[8] This compound showed no discernible androgen receptor (AR) or glucocorticoid receptor (GR) agonist activity at 10 μM or below. It showed weak AR antagonist activity ($IC_{50} > 1000$ nM), and weak GR antagonist activity (IC_{50} of ~2000 nM).

Several of these pyrrole-containing PR modulators have shown oral activity in the rat decidualization assay.[9] This assay, when run in the agonist mode, measures the ability of a test compound to induce a decidual response (i.e., stromal cell proliferation and differentiation) of the endometrium. When run in the antagonist mode this assay measures the ability of a test compound to block the progesterone induced decidual response. The compounds herein showed in vivo functional activity consistent with the functional activity they displayed in vitro. For instance, cyanopyrrole agonists **32**[10] and **33** showed statistically significant ($p<0.05$) levels of activity when administered orally at 10 mg/kg (50% and 30% response, respectively, relative to the positive control progesterone). In contrast, nitropyrrole antagonist **36** showed 50% inhibition ($p<0.05$) of progesterone-induced stimulation when administered orally at 3 mg/kg. Typically the potent agonists, such as **32** and **33** exhibited efficacies in the in vitro T47D assay of greater than 80% (relative to P4). These compounds have extremely weak antagonist potency and efficacy at high concentration (>3000 μM). Likewise, the antagonist efficacy of **36** was 75% (relative to Mifepristone) and showed weak efficacy as agonist only at high concentrations. It is therefore unlikely that these levels of antagonism for **32** and **33** and agonism for **36** would influence their in vivo activity.

In conclusion, we found for the compounds evaluated herein, that the nature of the substituent on the pyrrole (i.e., nitrile) as well as its position on the pyrrole moiety and the position of attachment of the pyrrole ring to the scaffold were important features determining the functional activity of these molecules. Compounds containing the 5′-cyano-2′-pyrrole group (**32**, **33**, **35**, **38**) where shown to possess agonist properties in vitro and/or in vivo, while other analogs with different heterocycles, substituents, or substitution patterns were generally antagonists or weak modulators.

Acknowledgement

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References and notes

1. Mangelsdorf, D. J.; Thummel, C.; Beato, M.; Herrlich, P.; Schütz, G.; Umesono, K.; Blumberg, B.; Kastner, P.; Mark, M.; Chambon, P.; Evans, R. M. *Cell* **1995**, *83*, 835.
2. (a) Jeppsson, S. *Acta Obstet. Gynecol. Scand. Suppl.* **1981**, *101*, 7; (b) O'Connell, B. J. *Curr. Opin. Pediatr.* **1995**, *7*, 371.
3. (a) Ulmann, A.; Peyron, R.; Silvestre, L. *Ann. N.Y. Acad. Sci.* **1995**, *761*, 248; (b) Kekkonen, R.; Lahteenmaki, P.; Luukkainen, T.; Tuominen, J. *Fertil. Steril.* **1993**, *60*, 610; (c) Chwalisz, K; Stockemann, K. World patent application WO 96/19997, 1996.
4. Lázár, G.; Lázár, G., Jr.; Husztik, E.; Duda, E.; Agarwal, K. K. *Ann. N.Y. Acad. Sci.* **1995**, *277*, 248.
5. Zhang, P.; Terefenko, E. A.; Fensome, A.; Wrobel, J.; Winneker, R.; Lundeen, S.; Marschke, K. B.; Zhang, Z. *J. Med. Chem.* **2002**, *45*, 4379.
6. Fensome, A.; Bender, R.; Cohen, J.; Collins, M. A.; Mackner, V. A.; Miller, L. L.; Ullrich, J. W.; Winneker, R.; Wrobel, J.; Zhang, P.; Zhang, Z.; Zhu, Y. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3487.
7. Alvarez, A.; Guzman, A.; Ruiz, A.; Velarde, E.; Muchowski, J. M. *J. Org. Chem.* **1992**, *57*, 1653.
8. Zhang, Z.; Lundeen, S. G.; Zhu, Y.; Caver, J. M.; Winneker, R. C. *Steroids* **2000**, *65*, 637.
9. Zhang, Z.; Funk, C.; Glasser, S. R.; Mulholland, J. *Endocrinology* **1994**, *135*, 1256.
10. Analytical data for 5-(4,4-dimethyl-2-oxo-1,4-dihydro-2*H*-3,1-benzoxazin-6-yl)-1-methyl-1*H*-pyrrole-2 carbonitrile (**32**): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.64 (s, 6H), 3.71 (s, 3H), 6.33 (d, 1H, $J = 4.1$ Hz), 6.98 (d, 1H, $J = 8.0$ Hz), 7.03 (d, 1H, $J = 4.1$ Hz), 7.39 (m, 2H), 10.39 (s, 1H). MS (APCI (−)) m/z 280 $(M-H)^-$. Anal. Calcd for $C_{16}H_{15}N_3O_2$, C, 68.31; H, 5.37; N, 14.94. Found, C, 68.41; H, 5.51; N, 14.56.