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1,5-Dihydro-benzo[*e*][1,4]oxazepin-2(*1H*)-ones containing a 7-(5′-cyanopyrrol-2-yl) group as nonsteroidal progesterone receptor modulators ☆

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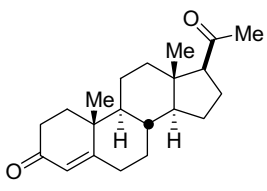
ABSTRACT

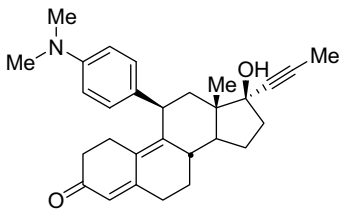
A series of novel 7-(5′-cyanopyrrol-2-yl) substituted benzo[1,4]oxazepin-2-ones were prepared and tested for their progesterone receptor (PR) agonist or antagonist activity in the alkaline phosphatase assay using the human T47D breast carcinoma cell line. Both PR agonists and antagonists were achieved with an appropriate choice of 5-substitution. Several analogs were potent PR agonists (e.g., **12** and **13**) or PR antagonists (e.g., **18**) with good selectivity over other steroid receptors.

The progesterone receptor (PR) is a member of the superfamily of ligand-dependent transcription factors.[1] Progesterone (**1**) is an endogenous hormone which plays an important role in female reproduction. In the clinic, steroidal PR agonists have been used widely in contraception and postmenopausal hormone therapy, often in combination with an estrogen.[2] Mifepristone (**2**), a steroidal PR antagonist, demonstrated potential utility in female contraception,[3] and for the treatment of various gynecological and obstetric diseases including hormone dependent cancers and non-malignant chronic conditions such as fibroids and endometriosis.[4–7] However, steroidal PR agonists and antagonists, albeit efficacious in their respective indications, have side effects due to the interactions with other steroidal receptors such as the glucocorticoid receptor (GR), and potential effects of steroidal metabolites. In an effort to mitigate some of these undesirable effects associated with steroidal compounds, we have previously reported several series of non-steroidal PR modulators including 6-aryl benzoxazinones and benzoxazine-2-thiones.[8–10] We found in the 6-aryl-benzoxazinone series that replacement of the 6-phenyl moiety with a 6-(5′-cyanopyrrol-2-yl) group (e.g., **3**)[8] caused the functional activity of this series to switch from PR antagonism to PR agonism in the PR alkaline phosphatase assay using the human T47D breast carcinoma cell line.

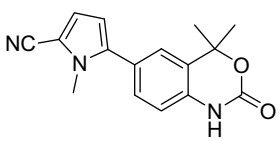
Intrigued by the impact of the 6-(5′-cyanopyrrol-2-yl) group on PR functional activity of the 6-aryl benzoxazinone series, we decided to expand the six-membered ring benzoxazinone core and examine the SAR of 7-(5′-cyanopyrrol-2-yl)benzo[1,4]oxazepin-2-ones (**4–24**)[11] at the PR. Herein, we report the synthesis and the SAR of 7-(5′-cyanopyrrol-2-yl)benzo[1,4]oxazepin-2-ones as PR modulators.



Progesterone, 1



Mifepristone, 2



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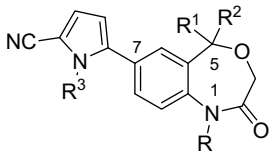

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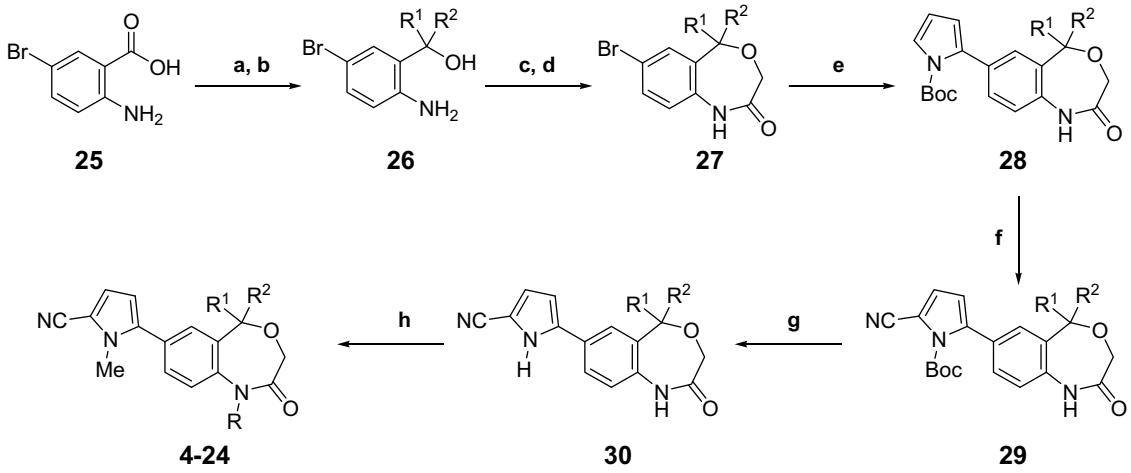


Scheme 1. Synthesis of 7-(5′-cyanopyrrol-2-yl)benzo[1,4]oxazepin-2-ones. Reagents and conditions: (a) R^1Li, THF, −78 °C to 0 °C, N_2, 20–70%; (b) R^2Li, THF, 0 °C, N_2; or LAH, THF, −78 °C, N_2; 40–80%; (c) $ClCH_2COCl$, Et_3N, THF, 0 °C to rt, 40–70%; (d) NaH, THF, 0 °C, N_2, 50–85%; (e) N-Boc-pyrrole-2-boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, glyme/H_2O, 80 °C, N_2, 50–80%; (f) CSI, THF, −78 °C, DMF, N_2, 30–70%; (g) neat, 180 °C, 60–90%; (h) MeI, K_2CO_3, DMF, rt, N_2, 40–80%.

Target compounds **4–24** (Scheme 1) were prepared as follows: addition of an appropriate organolithium reagent to anthranilic acid **25** resulted in the corresponding ketone, which was treated with the second organolithium species or reduced with lithium aluminum hydride to give carbinols **26**. Acetylation of carbinols **26** with chloroacetyl chloride followed by a ring closure furnished the core structure benzoxazepinones **27**. The Boc-pyrrol-2-yl moiety was installed at the 7-position via a Suzuki coupling protocol using a palladium catalyst to afford **28**.

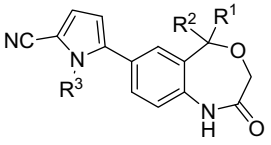
Cyanation of pyrrole at the 5′-position to yield **29** was achieved by treatment of **28** with chlorosulfonyl isocyanate (CSI), followed by addition of DMF at low temperature. Removal of the BOC group by heating **29** gave **30**, which was alkylated with methyl iodide to deliver the target compounds **4–24**.

We have previously reported a number of cyanopyrrole-containing non-steroidal PR modulators such as 6-aryl benzoxazin-2-ones, 6-aryl benzimidazolones, and 5-aryl oxindoles.[8,12,13] Among the analogs reported in the 6-aryl-benzoxazinone series, replacement of the 6-phenyl moiety with a 6-(5′-cyanopyrrol-2-yl) group caused the functional activity of this series to switch from PR antagonism to PR agonism.[8] Interestingly, in the benzimidazolone series, the cyanopyrrole moiety did not change function in this way.[13] PR functional activities of cyanopyrrole-containing oxindoles were instead controlled by 3,3-substitutions.[12] These results suggested that the dominance of the cyanopyrrole motif on PR functional activity was template-dependent. To examine the SAR of 7-(5′-cyanopyrrol-2-yl)benzoxazepinones, numerous analogs were prepared and tested in the T47D PR alkaline phosphatase functional assay. The results are shown in Table 1.

As illustrated in Table 1, the size of the R^1/R^2 substituents at the 5-position had a marked impact on the type of functional activity as well as the potency of 7-(5′-cyanopyrrol-2-yl)benzoxazepinones. Compounds **4** and **5** with a single methyl group at the 5-position were much less potent compared to their 5,5-dimethyl disubstituted congeners (**6** and **7**) (Table 1).

Increasing the size of 5,5-substitution to a spiral cyclohexyl moiety (**8**) caused significant reduction in potency. Replacing one of the methyl groups in compounds **6** and **7** (EC_{50} = 3.3 nM) with a thienyl or furyl ring (**9–13**, EC_{50} = 0.4–1.1 nM) resulted in greater than threefold improvement in potency and gave numerous PR agonists with potency comparable to that of steroidal progesterone (**1**). It was noted that *N*-methyl substitution on

Table 1
PR-induced alkaline phosphatase activity and competition binding of 7-(5′-cyanopyrrol-2-yl)benzoxazepinones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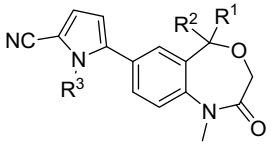
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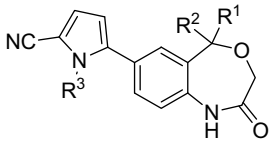
Compound	R^1	R^2	R^3	EC_{50}[a] (nM)	IC_{50}[b] (nM)	Binding IC_{50}[c] (nM)
1				0.9		
2					0.2	
3				1.1		
4	Me	H	H	245.8		
5	Me	H	Me	137.7		
6	Me	Me	H	3.3		10.7
7	Me	Me	Me	3.3		
8	$-C_5H_{10}-$		H	30.5		
9	Me	Thien-2-yl	H	0.7		3.8
10	Me	Thien-2-yl	Me	0.8		2.4
11	Me	Thien-3-yl	H	1.1		
12	Me	Thien-3-yl	Me	0.8		3.4
13	Me	Fur-2-yl	Me	0.4		
14	Et	Thien-2-yl	Me		14.6	75
15	*n*-Propyl	Thien-2-yl	Me		56.6	
16	*i*-Propyl	Thien-2-yl	Me		36.5	
17	Thien-2-yl	Thien-2-yl	Me		11.3	60
18	Fur-2-yl	Fur-2-yl	Me		6.6	28.4
19	Phenyl	Phenyl	Me		14.3	36
20	5′-Cl-thien-2-yl-	5′-Cl-thien-2-yl-	Me		197.4	
21	3′-Cl-phenyl	3′-Cl-phenyl	Me		175.1	
22	Me	Thien-2-yl	Me	37.5		
23	Et	Thien-2-yl	Me		119.8	
24	Phenyl	Phenyl	Me		500.0	

[a] Fifty percent effective concentration of tested compounds on alkaline phosphatase activity in the human T47D breast carcinoma cell line.

[b] Fifty percent 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 deviation for the assays was typically ±20% of the mean or less.

[c] Value was 50% inhibitory concentration of tested compounds on 1 nM ^{3}H-progesterone binding at PR using human T47D breast carcinoma cell line cytosol. The standard deviations for these assays were typically ±20% of mean or less. Blanks in EC_{50} and IC_{50} columns indicate that values were not measured.

Table 2
PR alkaline phosphatase activity of 7-(5′-cyanopyrrol-2-yl) benzoxazepinone stereoisomers



Compound	R^1	R^2	R^3	EC_{50}[a] (nM)	IC_{50}[b] (nM)
11	Me	Thien-3-yl	H	1.1	
(*S*)-11[*]	Me	Thien-3-yl	H	110.0	
(*R*)-11[*]	Me	Thien-3-yl	H	0.5	
14	Et	Thien-2-yl	Me		14.6
(*S*)-14[*]	Et	Thien-2-yl	Me		17.1
(*R*)-14[*]	Et	Thien-2-yl	Me		10.0

[*] Absolute stereochemistry was not determined. The absolute stereochemistry was arbitrarily assigned for both pair of enantiomers.
[a] Fifty percent effective concentration of tested compounds on alkaline phosphatase activity in the human T47D breast carcinoma cell line.
[b] Fifty percent 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 deviation for the assays was typically ±20% of the mean or less.

the pyrrole ring did not have any significant impact on either potency or functional activity compared to their unsubstituted analogs (e.g., **6**, **9**, **11** vs **7**, **10**, **12**) and all analogs (**4–13**) are PR agonists. Surprisingly, substitution of an ethyl group (**14**) for the methyl moiety (**10**) switched PR agonism to PR antagonism, but with reduced potency in the functional assay. Increasing size from ethyl to propyl groups (**15** and **16**) maintained PR antagonist activity with slightly reduced potency. 5,5-Diaryl analogs **17**–**19** also showed good PR antagonist potency with an IC_{50} ranging from 6.6 to 14.3 nM. A greater than 10-fold loss of potency was observed when chlorine was added to the 5-aryl substituents (**20, 21**) suggesting a size limitation in this region of the molecule (or an unfavorable electronic effect due to chlorine). Methyl substitution at the 1-position amide nitrogen of the benzoxazepinone (**22**–**24**) significantly decreased potency but did not change the mode of PR functional activity.

The binding affinities of several potent analogs were determined at PR from human T47D breast carcinoma cell line cytosol using [^{3}H]-progesterone as the radio-labeled competitor. In general, these compounds demonstrated good binding affinities to PR. Consistent with their potency in the functional assay, the PR agonists were more potent binders than the antagonists.

The impact of chirality at the 5-position on PR activity was examined by chiral resolution of compounds **11** and **14**. As illustrated in Table 2, both enantiomers retained the same functional activity as their respective racemate. However, for PR agonist **11**, the eutomer (EC_{50} = 0.5 nM) is two orders of magnitude more potent than its distomer (EC_{50} = 110.0 nM) suggesting a clear stereochemical preference for PR agonism at the binding site. In contrast, both enantiomers of antagonist **14** showed similar PR antagonist potency indicating little impact of chirality on **14**'s action at PR.

Compounds **12, 13, 17**, and **18** were evaluated for their selectivity against other steroidal receptors by using a Gal4-DNA binding domain (DBD)-hormone receptor ligand binding domain (LBD) one-hybrid assay for each receptor (Table 3).[14] When tested in the agonist mode, these compounds did not show any significant activity at estrogen (ER), androgen (AR), glucocorticoid (GR), and mineralcorticoid (MR) receptors. When tested in the antagonist mode, compounds **12** and **13** did not show any significant activity at ER. Although **12** and **13** showed moderate antagonist potency at AR, GR, and MR, they were still over 100-fold functionally selective against these receptors.

Table 3
Antagonist cross-activities (IC_{50} nM) of **2**, **12**, **13**, **17**, and **18**

Compound	PR[a]	ER[b]	AR[b]	GR[b]	MR[b]
2	0.2	5000	6.9	0.6	590
12	0.8[c]	>10,000	325	127	440
13	0.4[c]	>10,000	215	120	155
17	11.3	>10,000	775	>3000	2300
18	6.6	>10,000	740	>3000	2800

[a] Fifty percent 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.
[b] Experimental values represented the average of at least duplicate determinations. The standard deviation for these assays was typically ±30% of mean or less. See Ref. 12 for details.
[c] EC_{50} values.

For PR antagonists **17** and **18**, no significant activity was observed at ER and GR in contrast to the mifepristone (**2**) which demonstrated equal potency at PR and GR. Against AR and GR, both **17** and **18** showed better selectivity compared to that of mifepristone.

In summary, we have examined a class of novel 7-(5′-cyanopyrrol-2-yl)benzoxazepinones as PR modulators. The SAR reveals that the size of substituents at the 5-position has a profound impact on the PR functional activity and potency. With an appropriate choice of 5-substitution, both PR agonists and antagonists can be achieved from the 7-(5′-cyanopyrrol-2-yl)benzoxazepinone scaffold. The SAR effort has led to a number of potent PR agonists and antagonists with good to excellent selectivity against other steroidal receptors.

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