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Letter

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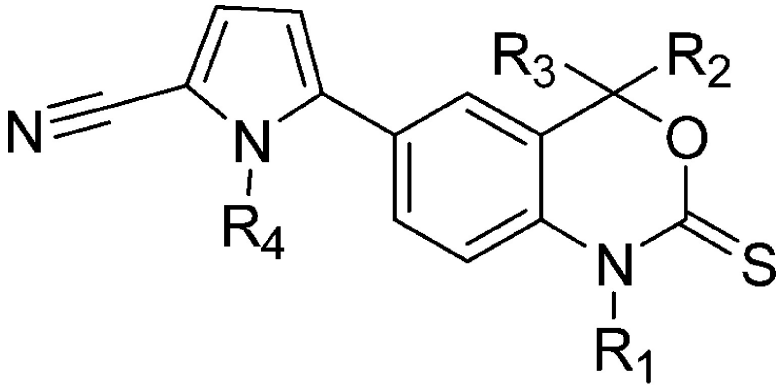

Tanaproget

R_1 = H

R_2, R_3, R_4 = Me

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Synthesis and Structure−Activity Relationship of Novel 6-Aryl-1,4-dihydrobenzo[*d*][1,3]oxazine-2-thiones as Progesterone Receptor Modulators Leading to the Potent and Selective Nonsteroidal Progesterone Receptor Agonist Tanaproget[#]

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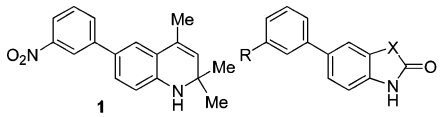
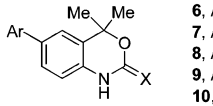
Abstract: Tanaproget represents a potential first-in-class nonsteroidal PR agonist for contraception with improved safety and side effect profiles versus currently available steroidal oral contraceptives. Additional SAR, biological activity, and structural information from a tanaproget/hPR-LBD (hPR-LBD = human progesterone receptor ligand binding domain) cocrystal structure will also be presented.

The progesterone receptor (PR) is a member of the steroid receptor subfamily of the nuclear receptor superfamily, a group of ligand-activated nuclear transcription factors.[1] Progesterone (P4), the endogenous ligand for the PR, is involved in the control of ovulation and preparation of the uterus to support pregnancy. In the clinic, steroidal PR agonists are used mainly in oral contraception (OC) and postmenopausal hormone therapy, typically coadministered with a steroidal estrogen.[2] However, there are steroid-related side effects associated with combined progestin and estrogen contraception that include nausea, headache, weight gain, bloating, breast tenderness, mood changes, and changes in lipids and coagulation factors.[2] More serious risks associated with estrogens in these combinations include stroke, myocardial infarction, and venous thromboembolism.[2] We embarked on a program to identify structurally novel, nonsteroidal PR modulators, which have effects on key target tissues but less impact on other steroid receptors and/or nonclassical-target tissues. In principle, the elimination of steroidal progestins and estrogens from contraceptive regimens will reduce the common side effects and long-term safety concerns associated with these steroidal hormones.

Chart 1. Nonsteroidal PR Scaffolds





The 6-aryldihydroquinolines,[3] such as **1** (Chart 1, IC_{50} = 41 nM, T47D alkaline phosphatase (AP) assay),[4] were previously identified as PR antagonists. In an effort to further explore this simple template, a number of analogues were prepared that replace the dihydroquinoline moiety with other scaffolds. Among the many scaffolds that were examined, ones that maintained PR antagonist potency in the AP assay included 6-arylbenzimidazolones (e.g., **2**, IC_{50} = 73 nM),[5] 6-arylbenzothiazolones (e.g., **3**, IC_{50} = 84 nM),[5] 5-aryl-1,3-dihydroindole-2-ones (e.g., **4**, IC_{50} = 66 nM),[6] and 6-aryl-1,4-dihydrobenzo[*d*][1,3]oxazin-2-ones (e.g., **5**, IC_{50} = 30 nM).[7] The potency exhibited by the 1,4-dihydrobenzo[*d*][1,3]oxazin-2-one spawned particular interest, and hence, further work in this series was carried out.

In this respect, exploration of the substituents on the pendent 6-phenyl ring of **5** proved to be particularly fruitful. Halogens, particularly fluorine and chlorine and small electron-withdrawing substituents, particularly cyano, provided significant improvement in in vitro potency and in vivo activity. For instance, the 3′-cyano-4′-fluorophenyl congener (**6**, IC_{50} = 15 nM) showed good potency as an antagonist in its ability to block the response of progesterone in the rat uterine decidualization model (ED_{50} = 0.6 mg/kg, po).[7] The 6-aryl moiety could be replaced by cyano-substituted heterocycles. For instance, the 2′-cyano-4′-furanyl analogue **7** (T47D IC_{50} = 8.0 nM) showed excellent in vitro and oral activity (ED_{50} = 0.9 mg/kg, po, as an antagonist in the rat uterine decidualization model). Furthermore, the compounds demonstrated excellent potency in PR competition binding assays and generally showed exceptional selectivity for the PR over other closely related steroid-hormone receptors such as the androgen (AR), glucocorticoid (GR), and mineralocorticoid receptor (MR).[7]

Through further SAR manipulation, we learned that subtle changes to the structure of the 1,4-dihydrobenzo-[*d*][1,3]oxazin-2-one core could have profound effects on the functional activity of the compound. For instance, changing the C=O of **6** and similar analogues to $CHCH_3$ led to 6-aryl-1,4-dihydrobenzo[*d*][1,3]oxazines (e.g., **8**, EC_{50} = 23 nM), which were now PR agonists in T47D cells.[8] Even more dramatic was the finding that conversion of the 2-carbonyl of benzoxine-based analogues such as **7** to the 2-thiocarbonyl congeners (1,4-dihydrobenzo-[*d*][1,3]oxazine-2-thione, e.g. **9**, EC_{50} = 1.2 nM) resulted in a functional switch from potent PR antagonists to highly potent PR agonists.[9] Other examples, e.g., **10** (EC_{50} = 0.4 nM), were potent agonists in vivo as demonstrated by the robust activity exhibited by **10** in

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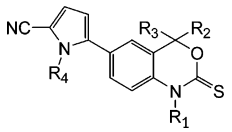
Table 1. T47D Cell Alkaline Phosphatase Assay Data for Compounds **12**–**18**



compd	R_1	R_2	R_3	R_4	T47D EC_{50},[a] nM
12	H	Me	Me	H	0.15
13	H	Me	Me	Me	0.15
14	Me	Me	Me	H	21.1
15	H	Et	Et	Me	0.1
16	H		$-(CH_2)_3-$	Me	0.5
17[b]	H	Me	Et	Me	0.1
18[b]	H	Me	thien-2-yl	Me	0.35

[a] 50% effective concentration of tested compounds on alkaline phosphatase activity in the human T47D breast carcinoma cell line. Values represent the average of at least two determinations. The standard deviations for these assays were typically ±15% of the mean or less. [b] Data obtained from racemic mixture.

the rat uterine decidualization model (ED_{50} = 0.62 mg/kg, po).[10] The decidualization assay was considered an important part of our testing scheme because only progestins are active in this model.[10,11] Compound **10** had high affinity for human progesterone receptor (hPR; IC_{50} = 3.0 nM in the PR competition binding assay) and was more than 1000-fold selective over other steroid receptors (AR and GR).

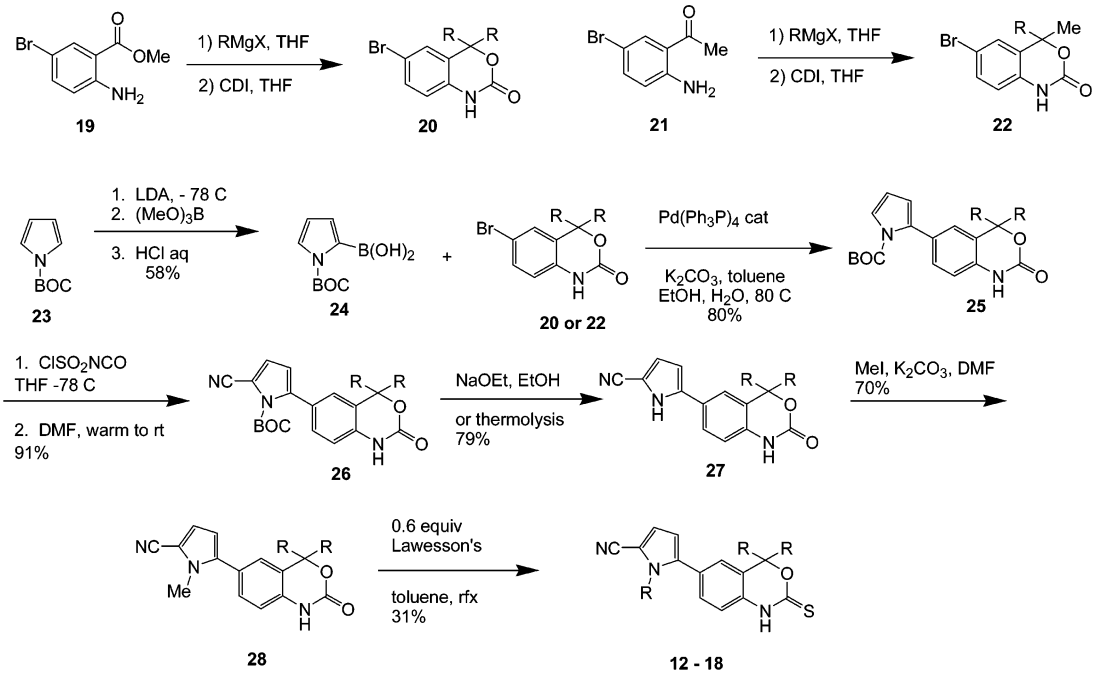
Modification of the 6-aryl moiety of the 1,4-dihydrobenzo[*d*][1,3]oxazin-2-ones also led to conversion of biological function from antagonist to agonist. For instance, replacement of the 6-(3′-chlorophenyl) group of **5** with a 5′-cyano-2′-pyrrole group resulted in compounds (e.g., **11**, EC_{50} = 1.1 nM) with potent agonist activity.[12] We further found that the nature of the substituent on the pyrrole (i.e., nitrile), its position on the pyrrole moiety, and the position of attachment of the pyrrole ring to the benzoxazin-2-one nucleus were important features determining the functional activity of these molecules. Only compounds with the 5′-cyano-2′-pyrrole motif resulted in PR agonist properties.[12]

Combining the 5′-cyano-2′-pyrrole moiety with the 1,4-dihydrobenzo[*d*][1,3]oxazine-2-thione template proved to be additive, as described in Table 1.

The molecules were prepared according to Scheme 1. The symmetrical bromide coupling partners **20** (used in the preparation of **12**–**16**) were prepared by treatment of the anthranilate **19** with the appropriate Grignard agent. In the case of the unsymmetrical racemates **22** (used in the preparation of **17** and **18**), these were prepared by reaction of the methyl ketone **21** with either ethylmagnesium bromide or 2-thienyllithium. Reaction of the pyrrole **23** with LDA and trimethyl borate afforded the boronic acid **24** after aqueous workup, which was then coupled under standard conditions with the appropriate bromide **20** or **22** to afford the pyrroles **25**. The 5-cyano group was installed by treatment with chlorosulfonyl isocyanate, followed by a DMF quench. Deprotection with NaOEt in ethanol then afforded the pyrrole **27**. Alkylation of **27** (MeI, K_2CO_3, DMF), followed by thionation with Lawesson's reagent in toluene, gave target molecules **13** and **15**–**18**. Compound **12** was prepared by thionation of **27**, while compound **14** was prepared by alkylation of the BOC derivative **26** (MeI, K_2CO_3, DMF), followed by deprotection (thermolysis at 160 °C or NaOEt/ EtOH) and finally reaction with Lawesson's reagent.

As anticipated, the combination of these two structural features, the 5-cyanopyrrole and benzoxazin-2-thione, did yield a synergistic increase in potency. The unsubstituted **12** (R_1, R_4 = H, R_2, R_3 = Me) proved to be very potent in the T47D alkaline phosphatase assay (EC_{50} = 0.15 nM). Alkylation of the pyrrole nitrogen leading to **13** (R_4 = Me) maintained this potency, while methylation of the benzoxazin- 2-thione led to **14**, which lost approximately 200-fold in potency (EC_{50} = 21 nM). The small symmetrical substituents (R_2, R_3 = Me **15** and R_2 = Me, R_3 = Et **17**) typically maintained the same low nanomolar potency (T47D EC_{50} = 0.1 nM for both

Scheme 1. Synthesis of Pyrollobenzoxazin-2-thiones



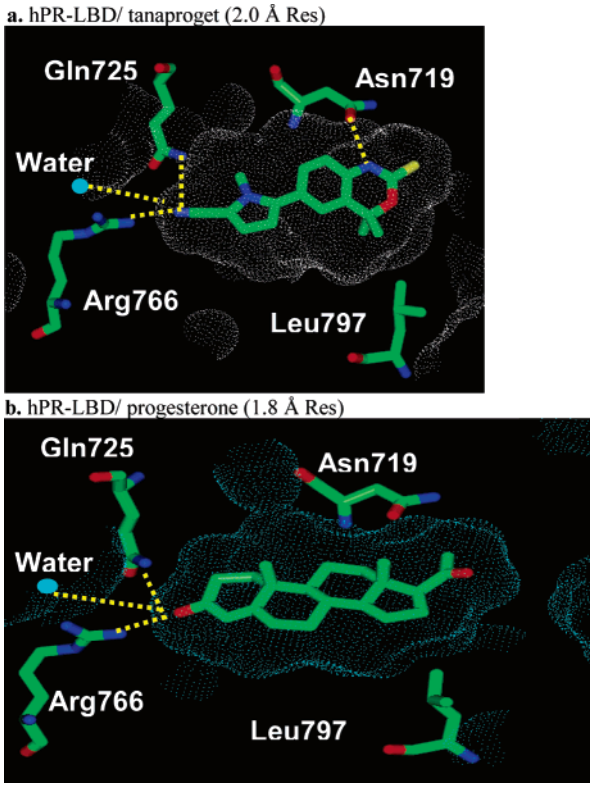


Figure 1. Binding pocket of (a) PR complexed with tanaproget (colored by atom type) and (b) PR complexed with progesterone (colored by atom type). Only key residues, including a Connolly surface of the binding site, are shown for simplicity. Hydrogen bonds to key residues are shown as yellow dotted lines.

15 and **17**). However the larger substituents, R_2/R_3 = spirocyclobutyl **16** and R_2 = Me, R_3 = 2-thienyl **18**, did lose between 3- and 5-fold in potency (EC_{50} = 0.5 and 0.35 nM for **16** and **18**, respectively).

Compound **13**, tanaproget, was chosen for further characterization. In the alkaline phosphatase assay in T47D cells, tanaproget had an EC_{50} of 0.15 nM, comparable to steroidal PR agonists such as medroxyprogesterone acetate (MPA, EC_{50} = 0.12 nM). Tanaproget showed high affinity for the human PR with an IC_{50} of 1.6 nM in the T47D cell cytosol competition-binding assay (MPA IC_{50} = 10.8 nM). Tanaproget did not show any ER, AR, or GR agonist activity when tested at concentrations up to 10 μM in appropriate cell-based reporter assays. Tanaproget demonstrated relatively weak antagonist activity for the GR (IC_{50} = 40 nM, GRE-luciferase reporter assay in A549 lung carcinoma cell line) and AR (IC_{50} = 131 nM, ARE-luciferase reporter assay in L929 cells) with a greater than 250-fold functional selectivity for PR versus these other closely related steroid receptors. Tanaproget did not demonstrate any significant anti-glucocorticoid activity in vivo at doses up to 30 mg/kg, po.

The in vivo progestational potency and efficacy of tanaproget were evaluated in the rat uterine decidualization model, with the reference steroid MPA, by gavage, in 2% Tween-80/0.5% methylcellulose. In this model, tanaproget had a mean ED_{50} of 0.01 mg/kg, approximately 40-fold more potent than MPA. The rat plasma kinetics of tanaproget are in accord with the rodent efficacy data (female Sprague-Dawley rats: 1 mg/kg iv, $AUC_{0-\infty} = 3.42 \pm 0.33$ μg·h/mL, $t_{1/2} = 3.83 \pm 0.25$ h; 1 mg/kg po, $AUC_{0-\infty} = 1.06 \pm 0.35$ μg·h/mL, $f = 31\%$).

To investigate the binding mode of tanaproget, a cocrystal structure was obtained with the hPR ligand binding domain (LBD) (Figure 1a).[13] In comparison with the hPR-LBD/progesterone structure previously published by Sigler's group (Figure 1b), no overall gross changes to the tertiary structure of the complex is apparent.[14] Tanaproget occupies the same binding site as does the steroidal ligand. In the progesterone cocrystal structure, the 3-keto function binds in a three-center hydrogen-bonding network with Gln-725, Arg-766, and a water molecule. Interestingly it is the nitrile function of tanaproget that makes these same key H-bonding contacts with Gln-725 (3.0 Å) and Arg-766 (2.8 Å), and not the thione moiety, as may have been predicted. The benzoxazinone core of tanaproget lies just above the plane of the C and D rings of progesterone, with the 3,3-dimethyl groups lying in a lipophilic pocket. Most interestingly, the benzoxazin-2-thione *N*-H is able to form a hydrogen bond (2.8 Å) with Asn-719, an interaction that is not available to progesterone. The presence of a hydrogen bond between the tanaproget *N*-H and Asn-719 provides an explanation of why the *N*-methyl derivative **14** loses so much potency relative to the parent **12** because **14** is no longer an H-bond donor.

In summary, we have presented data that demonstrate the synergistic combination of two PR agonist moieties: the 5-cyanopyrrole and benzoxazin-2-thione groups. This combination of functionalities has given rise to a PR agonist, tanaproget, which has in vitro potency competitive with the best of the steroidal progestins but with superior selectivity over the other members of the steroid receptor family. This high potency also translates to the in vivo situation. In a rat model of progestational activity, the uterine decidual model, tanaproget is 40 times more potent than medroxyprogesterone acetate. We have also presented the first cocrystal structure of a nonsteroidal PR agonist (tanaproget) with the human PR ligand binding domain. This structure has revealed the unique binding mode of this molecule. Tanaproget is currently being evaluated in the clinic for contraception.

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Supporting Information Available: Full experimental information for the preparation of tanaproget **13** and analytical characterization of **12** and **14** − **18**. This material is available free of charge via the Internet at http://pubs.acs.org.

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