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Progesterone receptor antagonists with a 3-phenylquinazoline-2,4-dione/2-phenylisoquinoline-1,3-dione skeleton

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ABSTRACT

Novel non-steroidal progesterone receptor antagonists with a 3-phenylquinazoline-2,4-dione/2-phenylisoquinoline-1,3-dione skeleton were developed and their structure–activity relationships were investigated. Among the prepared compounds, 4-(4,4-diethyl-3,4-dihydro-1,3-dioxoquinolin-2(1*H*)-yl)benzonitrile (DEPIQ-4CN) showed the most potent activity, with IC_{50} values of 74–78 nM in alkaline phosphatase activity and reporter gene assays.

1. Introduction

Progesterone [4-pregnen-3,20-dione: P4 (**1**), Fig. 1] plays a critical role in the regulation of female reproductive function, via the nuclear progesterone receptor (PR). PR belongs to the nuclear receptor superfamily of ligand-dependent transcription factors, and undergoes a conformational change upon ligand binding to initiate a cascade of regulatory events related to its target genes.[1] P4 (**1**), the endogenous ligand for the PR, is involved in the regulation of uterine cell proliferation/differentiation, implantation, ovulation, mammary gland growth/differentiation, and preparation of the uterus to support pregnancy.[2]

Based on the diverse physiological effects elicited by P4 (**1**), synthetic agonists (denoted as PR agonists) have been developed, and are widely used in contraception,[3] hormone replacement therapy to reduce estrogen-mediated endometrial cancer risk,[4] and for the treatment of gynecological disorders.[5] PR agonists that have been clinically used generally possess a steroid skeleton. These steroidal PR agonists often cause various side effects due to their cross-binding to other nuclear steroid receptors, including androgen, estrogen, and glucocorticoid receptors.[6] Therefore, non-steroidal PR agonists which are selective to PR are needed. Some non-steroidal PR agonists have been developed, but the diversity of their structures is still limited, and only tanaproget (**2**, Fig. 1) has been successfully tested in a clinical trial for contraception.[7]

On the other hand, progesterone receptor antagonists (denoted as PR antagonists) have also been developed, though their number is quite limited compared with PR agonists. A typical PR antagonist is mifepristone (**3**: RU486, Fig. 1), which is a steroid derivative used to induce abortion in some European countries. Pharmacological data of mifepristone (**3**) have been accumulated, and it has been suggested that mifepristone (**3**) might be effective as a contraceptive agent[8] and for the treatment of endometriosis,[9] uterine leiomyomas,[10] and breast cancer.[2,11,12] However, little pharmacological information is available concerning PR antagonists, and research on PR antagonists for clinical applications is less advanced than that on PR agonists and other steroid hormone agonists/antagonists. This situation seems to be at least partly attributable to the lack of structural diversity of PR antagonists. Therefore, it is of interest to develop novel PR antagonists, especially with non-steroidal scaffolds.[12]

Under such circumstances, we have been engaged in searching for novel scaffolds for PR antagonists, and found that thalidomide metabolites (**5**–**9**)[13,14] exhibit PR antagonistic activity in an established human breast cancer T47D alkaline phosphatase assay (Table 1), which is one of the most commonly used in vitro cell-based functional assays to measure PR agonistic/antagonistic activity. T47D cells express endogenous wild-type human PR,[15] and alkaline phosphatase activity has been established to be induced by PR agonists.[16,17] Our finding that some thalidomide metabolites possess PR antagonistic activity, as well as our previous structural development studies on thalidomide (**4**), including the development of TNF-α production regulators, androgen antagonists, puromycin-sensitive aminopeptidase inhibitors, and α-glucosidase

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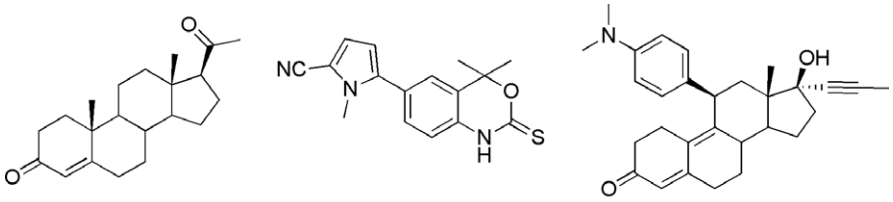


Figure 1. Structures of typical PR agonists [progesterone (**1**, P4) and tanaproget (**2**)] and a PR antagonist [mifepristone (**3**, RU486)].

inhibitors, suggested that novel non-steroidal PR antagonists could be derived from thalidomide (**4**), especially by the use of *N*-phenylphthalimide/*N*-phenylhomophthalimide scaffolds.[18–21] Based on this consideration, Tanatani and we have developed PR antagonists with a 3-phenylquinazoline-2,4-dione skeleton (denoted as PAQ skeleton) or a 2-phenylisoquinoline-1,3-dione skeleton (denoted as PIQ skeleton).[22] In this paper, we present details of the development of novel non-steroidal PR antagonists with PAQ/PIQ scaffolds.

2. Results and discussion

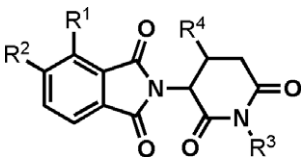
2.1. 3-Phenylquinazoline-2,4-dione (PAQ) derivatives

Our previous structural development studies based on thalidomide (**4**) suggested that a PAQ skeleton affords a superior scaffold structure for the development of various biologically active compounds.[18–21] Based on these results, we first prepared PAQ derivatives as shown in Scheme 1. Briefly, 3-phenylquinazoline-2,4-dione (**10**: PAQ-00) was prepared by condensation of phenyl isocyanate and methyl anthranilate, followed by cyclization of the resulting urea under basic conditions in one pot (Scheme 1a). Alkylated PAQ derivatives (**11**–**14**) were prepared by deprotonation of the amide proton with sodium hydride, followed by alkylation with alkyl halides (Scheme 1b).[23]

The PR antagonistic activity of the prepared compounds (**10**–**14**) was assessed by T47D alkaline phosphatase assay (denoted as AP assay)[15–17] and reporter gene assay (denoted as RG assay) methods. The latter assay method is a well-established assay method in which PR agonistic/antagonistic activities of test compounds can be monitored at the gene expression level. In the reporter gene assay, expression vector genes of PR and luciferase reporter construct containing PR-binding sequence (PR response element: PRE) are co-transfected into CHO (Chinese hamster ovary) cells.[24,25] Treatment of the transfected cells with a PR agonist leads to increased luciferase activity, allowing the identification of both PR agonists and antagonists.[26] The results obtained in AP and RG assays are mutually well correlated, and the results for our compounds are shown in Table 2. None of the compounds prepared (**10**–**14**) showed PR agonistic activity in either assay (data not shown).

As shown in Table 2, PAQ-00 (**10**) did not show PR antagonistic activity in RG assay, but weak PR antagonistic activity was detected in AP assay (34% inhibition at 100 μM). Introduction of a methyl group at the 1-position [MPAQ-00 (**11**)] seems to enhance the activity, resulting in IC_{50} values of 84 and ca. 100 μM in the AP and RG assays, respectively. Introduction of an ethyl group [EPAQ-00 (**12**)] effectively enhanced the activity, and introduction of a larger hydrophobic group further enhanced the activity, that is, the PR antagonistic activities of the compounds increased in the order of: PAQ-00 (**10**, H) < MPAQ-00 (**11**, Me) < EPAQ-00 (**12**, Et) < PPAQ-00 (**13**, Pr) < BnPAQ-00 (**14**, Bn), in both AP and RG assays. Although the number of PAQ derivatives is limited, the results suggest that the bulkiness of the hydrophobic group introduced at the 1-position is critical for the activity.

Table 1
PR antagonistic activity of thalidomide metabolites (**5**–**9**)



Compounds	R^1	R^2	R^3	R^4	% Inhibition[a]
4: Thalidomide	H	H	H	H	Inactive
5: *N*-Hydroxythalidomide	H	H	OH	H	42.5
6: 4,*N*-Dihydroxythalidomide	OH	H	OH	H	30.7
7: 5,*N*-Dihydroxythalidomide	H	OH	OH	H	53.7
8: 5′-Hydroxythalidomide	H	H	H	OH	30.6
9: 5,5′-Dihydroxythalidomide	H	OH	H	OH	29.4

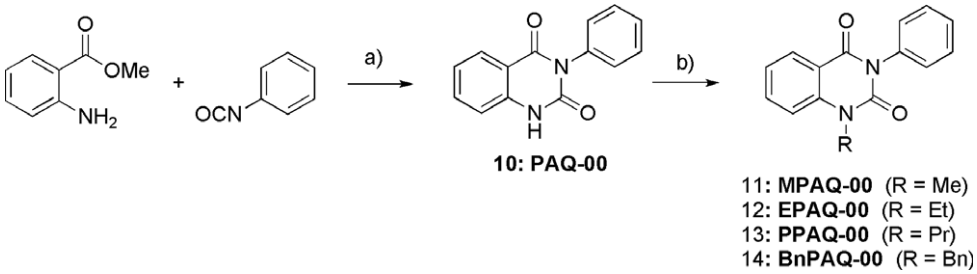
[a] The PR antagonistic activities are shown as percent inhibition of T47D alkaline phosphatase activity induced by 0.3 nM P4 (**1**) with the test compound at the concentration of 100 μM.

2.2. 3-Phenylisoquinoline-2,4-dione (PIQ) derivatives

Based on the above results, we assumed that spatial bulkiness around the 1-position of the PAQ skeleton is critical for potent PR antagonistic activity. This consideration led us design a carba-analog of the PAQ skeleton, that is, PIQ, in which the nitrogen atom at the 1-position is changed to a carbon atom, making it possible to introduce two hydrophobic groups at the corresponding position.

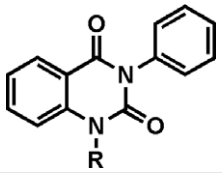
2-Phenylisoquinoline-1,3-dione (PIQ-00, **15**) was prepared by condensation of homophthalic anhydride with aniline.[22] Other PIQ derivatives (**16**–**35**) were prepared by condensation of homophthalic anhydride with various substituted anilines, followed by alkylation at the benzylic position with various alkyl halides in the presence of sodium hydride. All the compounds prepared (**15**–**35**) were assayed for PR agonistic and antagonistic activities by AP and RG assay methods as described above (Tables 3 and 4). None of the compounds prepared (**15**–**35**) showed PR agonistic activity in either of the assay methods (data not shown).

As expected, PIQ-00 (**15**) showed similar features to PAQ-00 (**10**) concerning PR antagonistic activity (Table 3), that is, PIQ-00 (**15**) showed weak PR antagonistic activity in both AP (25% inhibition at 100 μM) and RG (38% inhibition at 100 μM) assays, suggesting that the nitrogen atom at the 1-position of PAQ skeleton can be converted to a carbon atom without affecting the activity (or possibly with a slight enhancement of the activity). Dimethylation of the benzylic carbon of PIQ-00 (**15**), that is, DMPIQ-00 (**16**), dramatically enhanced the PR antagonistic activity, as we had hoped. The PR antagonistic activity of DMPIQ-00 (**16**, IC_{50} = 20 and 26 μM in AP and RG assays, respectively, Table 3) is more potent than that of MPAQ-00 (**11**, IC_{50} = 84 and ca. 100 μM in AP and RG assays, respectively, Table 2), which led us to investigate the effect of the substituents introduced into the *N*-phenyl group of DMPIQ-00 (**16**), that is, DMPIQ derivatives **17**–**25** (Table 3).



Scheme 1. Regents and conditions: (a) triethylamine, THF, 80 °C then NaOH, EtOH, 80 °C; (b) NaH, R1X, DMF, 0 °C to rt.

Table 2
PR-antagonistic activity of PAQ derivatives (**10**–**14**)



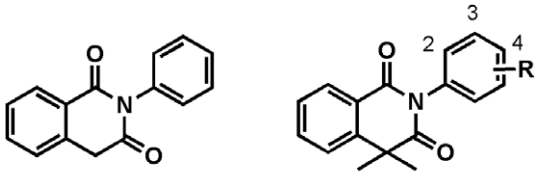
Compound	R	PR antagonistic activity	
		AP assay IC_{50}[a] (μM)	RG assay IC_{50}[b] (μM)
10: PAQ-00	H	(34% inhibition at 100 μM)	Inactive
11: MPAQ-00	Me	84	ca. 100
12: EPAQ-00	Et	30	34
13: PPAQ-00	*n*-Pr	23	18
14: BnPAQ-00	Bn	11	15

[a] Concentration that inhibits 0 3 nM P4 (**1**)-induced alkaline phosphatase activity.
[b] Concentration that inhibits 1 nM P4 (**1**)-induced luciferase activity.

As shown in Table 3, introduction of a substituent into the *N*-phenyl ring generally caused enhancement of the PR antagonistic activity. For fluoro (**17**–**19**) and chloro derivatives (**20**–**22**), introduction of the substituent at the *para*-position seems to be the most effective, because the PR antagonistic activities decreased in the order of: DMPIQ-4F (**19**) > DMPIQ-3F (**18**) =/> DMPIQ-2F (**17**) and DMPIQ-4C (**22**) > DMPIQ-3C (**21**) =/> DMPIQ-2C (**20**). Introduction of a substituent at the *para*-position of the *N*-phenyl ring seems to cause enhancement of PR antagonistic activity regardless of the electronic nature of the introduced substituent, because introduction of both electron-withdrawing groups [fluoro (DMPIQ-4F, **19**), chloro (DMPIQ-4C, **22**), and cyano (DMPIQ-4CN, **23**) groups] and electron-donating groups [methoxy (DMPIQ-4MO, **24**) and methyl (DMPIQ-4M, **25**)] enhanced the activity, though introduction of electron-withdrawing groups seems to be more effective. The PR antagonistic activity of DMPIQ derivatives with a *para*-substituted/non-substituted *N*-phenyl ring increased in the order of: DMPIQ-00 (**16**, H) < DMPIQ-4MO (**24**, OMe) < DMPIQ-4M (**25**, Me) =/< DMPIQ-4F (**19**, F) < DMPIQ-4C (**22**, Cl) =/< DMPIQ-4CN (**23**, CN). Thus, DMPIQ-4C (**22**) and DMPIQ-4CN (**23**), with IC_{50} values of 1.3–3.3 μM in both the AP and RG assay systems, were considered to be superior lead compounds for further structural development studies.

Selecting DMPIQ-4C (**22**) and DMPIQ-4CN (**23**) as the next lead compounds, we investigated the effect of hydrophobic substituents introduced at the benzylic position, that is, DMPIQ-4C derivatives **26**–**30** and DMPIQ-4CN derivatives **31**–**35** (Table 4). As is the case for *N*-alkyl PAQ derivatives (Table 2), the bulkiness of the hydrophobic group(s) introduced at the benzylic position of DMPIQ-4C (**22**) and DMPIQ-4CN (**23**) greatly affected the PR antagonistic activity. Exchange of the two benzylic methyl groups of DMPIQ-4C (**22**) and DMPIQ-4CN (**23**) with two ethyl groups, that is, DE-

Table 3
PR-antagonistic activity of PIQ-QQ (**15**) and its derivatives with a substituent introduced into the *N*-phenyl ring (**16**–**25**)

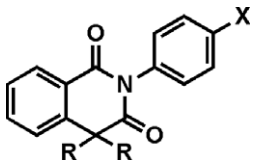


15: PIQ-00 **16-25**

Compound	R	PR antagonistic activity	
		AP assay IC_{50}[a] (μM)	RG assay IC_{50}[b] (μM)
15: PIQ-00	—	(25% inhibition at 100 μM)	(38% inhibition at 100 μM)
16: DMPIQ-00	H	20	26
17: DMPIQ-2F	2-F	13	10
18: DMPIQ-3F	3-F	10	11
19: DMPIQ-4F	4-F	6.8	8.9
20: DMPIQ-2C	2-CI	4.1	4.9
21: DMPIQ3C	3-CI	3.4	5.8
22: DMPIQ-4C	4-CI	1.3	3.3
23: DMPIQ-4CN	4-CN	1.6	1.8
24: DMPIQ-4M0	4-OMe	11	17
25: DMPIQ-4M	4-M	8.1	8.9

[a] Concentration that inhibits 0 3 nM P4 (**1**)-induced alkaline phosphatase activity.
[b] Concentration that inhibits 1 nM P4 (**1**)-induced luciferase activity.

Table 4
PR-antagonistic activity of *para*-chloro (**22** and **26**–**30**) and *para*-cyano-PIQ derivatives (**23** and **31**–**35**) with substituents introduced at the benzylic position



Compound	X	R, R	PR antagonistic activity	
			AP assay IC_{50}[a] (μM)	RG assay IC_{50}[b] (μM)
22: DMPIQ-4C	Cl	Me, Me	1.3	3.3
26: DEPICMC	Cl	Et, Et	0.25	0.28
27: DPPIQ-4C	Cl	*n*-Pr, *n*-Pr	1.1	1.0
28: DAPIQ-4C	Cl	CH_2═$CHCH_2$, CH_2═$CHCH_2$	0.4	0.85
29: C5PIQ-4C	Cl	$-(CH_2)_4-$	1.3	2.1
30: C6PIQ-4C	Cl	$-(CH_2)_5-$	1,1	1.5
23: DMPIQ 4CN	CN	Me, Me	1.6	1.8
31: DEPIQ-4CN	CN	Et, Et	0.074	0.078
32: DPPIQ-4CN	CN	*n*-Pr, *n*-Pr	0.12	0.14
33: DAPIQ-4CN	CN	CH_2═$CHCH_2$, CH_2═$CHCH_2$	0.11	0.15
34: C5PIQ-4CN	CN	$-(CH_2)_4-$	1.2	0.69
35: C6PIQ-4CN	CN	$-(CH_2)_5-$	0.88	0.45

[a] Concentration that inhibits 0.3 nM P4 (**1**)-induced alkaline phosphatase activity.
[b] Concentration thta inhibits 1 nM P4 (**1**)-induced luciferase activity.

PIQ-4C (**26**) and DEPIQ-4CN (**31**), greatly enhanced the activity (IC_{50} values of 74–280 nM). Dehydration of the two methyl groups of DEPIQ-4C (**26**) and DEPIQ-4CN (**31**), that is, DAPIQ-4C (**28**) and DAPIQ-4CN (**33**), slightly decreased the activity. In contrast to the PAQ derivatives, where the *N*-propyl derivative (PPAQ-00, **13**) is a more potent PR antagonist than the *N*-ethyl derivative (EPAQ-00, **12**)], elongation of the two ethyl groups of DEPIQ-4C (**26**) and DEPIQ-4CN (**31**) to *n*-propyl groups, that is, DPPIQ-4C (**27**) and DPPIQ-4CN (**32**) decreased the activity. Further, the PR antagonistic activity elicited by spiro-alkyl derivatives of DEPIQ-4C (**26**) and DEPIQ-4CN (**31**), that is, C5PIQ-4C (**29**), C6PIQ-4C (**30**), C5PIQ-4CN (**34**), and C6PIQ-4CN (**35**) did not exceed those elicited by the corresponding diethyl derivatives. The results suggest critical molecular recognition of the molecular shape around the benzylic position by PR. Thus, DEPIQ-4CN (**31**) was found to be the most potent PR antagonist among the prepared compounds, with IC_{50} values of 74 and 78 nM in the AP and RG assay, respectively.

To confirm that the PR antagonistic activity elicited by DEPIQ-4CN (**31**) is attributable to competitive binding of the compound with P4 (**1**), competitive-binding assay was performed using [^{3}H]P4 and commercially available baculovirus-expressed recombinant human PR (Fig. 2).[17,26–29] As shown in the figure, DEPIQ-4CN (**31**) binds PR competitively with P4 (**1**). The binding selectivity of DEPIQ-4CN (**31**) was also investigated using whole cell extract of T47D cells, which have been established to contain nuclear androgen receptor (AR) and estrogen receptor (ER).[27–29] No competitive binding with [^{3}H]testosterone or [^{3}H]estradiol was detected, suggesting that DEPIQ-4CN (**31**) is a superior PR antagonist that does not cross bind with AR/ER (data not shown). DEPIQ-4CN (**31**) was shown to be effective in vivo as a PR modulation when injected into mice intraperitoneally (details will be published elsewhere).

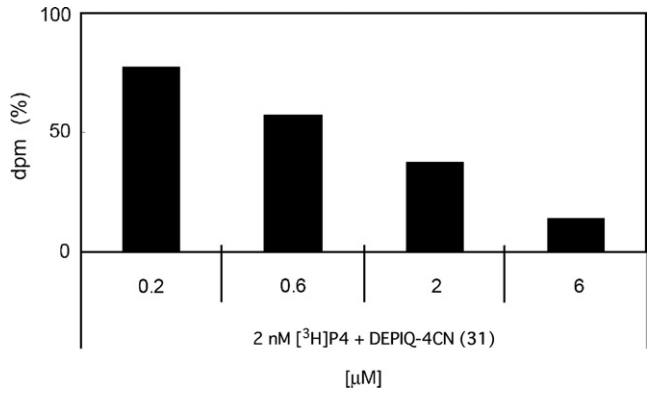


Figure 2. Competitive-binding assay of DEPIQ-4CN (**31**) with [^{3}H]P4.

3. Conclusion

In conclusion, we have developed a novel non-steroidal PR antagonist DEPIQ-4CN (**31**) by utilizing thalidomide (**4**) as a multi-drug-discovery template. Further investigation of the biological activities elicited by DEPIQ-4CN (**31**) is in progress. DEPIQ-4CN (**31**) may be useful as a chemical tool for studies of the clinical application of PR antagonists.

4. Experimental

4.1. General chemical procedures

Melting points were determined on a Yanagimoto hot-stage melting point apparatus and are uncorrected. ^{1}H NMR spectra data were obtained on a JEOL ALPHA500 spectrometer (500 MHz) and a JEOL AL400 spectrometer (400 MHz) and ^{13}C NMR spectra data were obtained on a JEOL ALPHA500 spectrometer (125 MHz). Chemical shifts (δ) for ^{1}H NMR and ^{13}C NMR were given in parts per million (ppm) relative to deuteriochloroform as an internal reference with coupling constants in Hertz. The abbreviations s, d, t, q, br, and m signify singlet, doublet, triplet, quartet, broad, and multiplet, respectively. Fast atom bombardment mass spectra (FAB-MS) and high-resolution mass spectra (HRMS) were measured with a MS-JEOL JMS-HX110 mass spectrometer with *m*-nitrobenzyl alcohol. Routine thin-layer chromatography (TLC) was performed on silica gel 60 F254 plates (Merck, Germany). Flash column chromatography was done using silica gel 60 N spherical (Kanto Chemical Co., Inc., Japan). Elemental analyses were carried out in the Microanalytical Laboratory, Faculty of Pharmaceutical Sciences, University of Tokyo, and were within ±0.4% of theoretical values.

4.1.1. 3-Phenylquinazoline-2,4(1*H*,3*H*)-dione (PAQ-00, 10)

To a solution of phenyl isocyanate (120 μL, 1.10 mmol) and methyl anthranilate (150 μL, 1.16 mmol) in THF (10 mL) was added triethylamine (1 mL, 7.17 mmol) and the reaction mixture was stirred at 80 °C for 30 min. Then the solvent was evaporated, and ethanol (10 mL) and 2 mol/L sodium hydroxide aq (1 mL, 2 mmol) were added. The reaction mixture was stirred at 80 °C for 30 min, neutralized by adding 2 mol/L hydrochloric acid, and extracted with ethyl acetate. The combined organic layer was washed with brine, dried over magnesium sulfate, and evaporated. The residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 1:1–1:2) to give PAQ-00 (**10**) as white crystals (261 mg, 1.10 mmol, quant.). PAQ-00 (**10**) was recrystallized with *n*-hexane–ethyl acetate. Mp 261–263 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.56 (br, 1H), 8.15 (d, J = 7.8 Hz, 1H), 7.59–7.50 (m, 4H), 7.32 (d, J = 7.3 Hz, 2H), 7.24 (t, J = 7.1 Hz, 1H), 6.93 (d, J = 8.3 Hz, 1H); FAB MS: m/z 239 (MH^+); HRMS calcd for $C_{14}H_{11}N_2O_2$ (MH^+) 239.0821; found: 239.0848. Anal. Calcd for $C_{14}H_{10}N_2O_2$: C, 70.58; H, 4.23; N, 11.76. Found: C, 70.82; H, 4.49; N, 11.83.

4.2. General procedure for alkylation of 3-phenylquinazoline-2,4(1*H*,3*H*)-dione (PAQ-00, 10) with alkyl or benzyl halides to give compounds (11–14)

PAQ-00 (**10**) was added to a suspension of sodium hydride (1.1 equiv) in DMF at 0 °C and the mixture was stirred for 15 min, then at room temperature for 15 min. Alkyl iodide or benzyl bromide (1.1 equiv) was added at 0 °C and the reaction mixture was stirred for 15 min and then at room temperature until PAQ-00 was consumed. The reaction mixture was diluted with ethyl acetate, washed with dilute hydrochloric acid and brine, dried over magnesium sulfate, and evaporated. The residue was purified by silica gel column chromatography (*n*-hexane–ethyl acetate).

4.2.1. 1-Methyl-3-phenylquinazoline-2,4(1*H*,3*H*)-dione (MPAQ-00, 11)

Reaction of PAQ-00 (**10**, 50 mg, 0.208 mmol) with methyl iodide gave MPAQ-00 (**11**, 45 mg, 0.177 mmol, 85.0%) as white crystals. MPAQ-00 (**11**) was recrystallized from *n*-hexane–ethyl acetate. Mp 207–208 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (d, J = 7.8 Hz, 1H), 7.74 (d, J = 8.1 Hz, 1H), 7.54–7.43 (m, 3H), 7.32–7.23 (m, 4H), 3.65 (s, 3H); FAB MS: m/z 253 (MH^+); HRMS calcd for $C_{15}H_{13}N_2O_2$ (MH^+) 253.0977; found: 253.0949. Anal. Calcd for $C_{15}H_{12}N_2O_2$: C, 71.42; H, 4.79; N, 11.10. Found: C, 71.39; H, 4.88; N, 11.15.

4.2.2. 1-Ethyl-3-phenylquinazoline-2,4(1*H*,3*H*)-dione (EPAQ-00, 12)

Reaction of PAQ-00 (**10**, 48 mg, 0.201 mmol) with ethyl iodide gave EPAQ-00 (**12**, 38 mg, 0.144 mmol, 71.6%) as white crystals. EPAQ-00 (**12**) was recrystallized from *n*-hexane–ethyl acetate. Mp 181–182 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (d, J = 7.8 Hz, 1H), 7.73 (t, J = 7.8 Hz, 1H), 7.54–7.44 (m, 3H), 7.30–7.27 (m, 4H), 4.24 (q, J = 7.2 Hz, 2H), 1.40 (t, J = 7.1 Hz, 3H); FAB MS: m/z 267 (MH^+); HRMS calcd for $C_{16}H_{15}N_2O_2$ (MH^+) 267.1134; found: 267.1120. Anal. Calcd for $C_{16}H_{14}N_2O_2$: C, 72.16; H, 5.30; N, 10.52. Found: C, 71.91; H, 5.53; N, 10.46.

4.2.3. 3-Phenyl-1-propylquinazoline-2,4(1*H*,3*H*)-dione (PPAQ-00, 13)

Reaction of PAQ-00 (**10**, 46 mg, 0.194 mmol) with propyl iodide gave PPAQ-00 (**13**, 42 mg, 0.150 mmol, 77.3%) as white crystals. PPAQ-00 (**13**) was recrystallized from *n*-hexane–ethyl acetate. Mp 168–169 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (d, J = 7.8 Hz, 1H), 7.72 (t, J = 8.1 Hz, 1H), 7.53–7.44 (m, 3H), 7.30–7.25 (m, 4H), 4.12 (t, J = 7.8 Hz, 2H), 1.83 (sextet, J = 7.8 Hz, 2H), 1.05 (t, J = 7.3 Hz, 3H); FAB MS: m/z 281 (MH^+); HRMS calcd for $C_{17}H_{17}N_2O_2$ (MH^+) 281.1290; found: 281.1278. Anal. Calcd for $C_{17}H_{16}N_2O_2$: C, 72.84; H, 5.75; N, 9.99. Found: C, 72.66; H, 5.93; N, 9.88.

4.2.4. 1-Benzyl-3-phenylquinazoline-2,4(1*H*,3*H*)-dione (BnPAQ-00, 14)

Reaction of PAQ-00 (**10**, 30 mg, 0.126 mmol) with benzyl bromide gave BnPAQ-00 (**14**, 31 mg, 0.0947 mmol, 75.2%) as white crystals. BnPAQ-00 (**14**) was recrystallized from *n*-hexane–ethyl acetate. Mp 147–149 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (d, J = 7.8 Hz, 1H), 7.60 (t, J = 8.8 Hz, 1H), 7.54 (d, J = 7.8 Hz, 2H), 7.47 (d, J = 7.3 Hz, 1H), 7.36–7.20 (m, 9H), 5.41 (s, 2H); FAB MS: m/z 329 (MH^+). HRMS calcd for $C_{21}H_{17}N_2O_2$ (MH^+) 329.1290; found: 329.1293. Anal. Calcd for $C_{21}H_{16}N_2O_2$: C, 76.81; H, 4.91; N, 8.53. Found: C, 76.70; H, 5.09; N, 8.55.

4.3. General procedure for preparation of PIQ-00 (15) and its derivatives (16–35)

A mixture of substituted or non-substituted aniline and homophthalic anhydride was stirred without solvent at 200 °C. The reaction mixture was dissolved in ethyl acetate, and the solution was washed with dilute hydrochloric acid, saturated aqueous sodium bicarbonate, water and brine, dried over magnesium sulfate, and evaporated. The residue was purified by silica gel column chromatography (*n*-hexane–ethyl acetate) to give PIQ-00 (**15**) or its derivatives with a substituted *N*-phenyl moiety. These PIQ derivatives were added to a suspension of sodium hydride (2.2 equiv) in DMF at 0 °C and the mixture was stirred for 15 min and then at room temperature for 15 min. Alkyl iodide or allyl bromide (2.2 equiv) or alkyl diiodide (1.1 equiv) was added at 0 °C and the reaction mixture was stirred for 15 min and at room temperature. The reaction mixture was diluted with ethyl acetate, washed with dilute hydrochloric acid and brine, dried over magnesium sulfate, and evaporated. The residue was purified by silica gel column chromatography (*n*-hexane–ethyl acetate).

4.3.1. 2-Phenylisoquinoline-1,3(2*H*,4*H*)-dione (PIQ-00, 15)

Reaction of aniline (216 mg, 2.32 mmol) with homophthalic anhydride (355 mg, 2.19 mmol) gave PIQ-00 (**15**, 299 mg, 1.19 mmol, 53.8%) as a light yellow powder. PIQ-00 (**15**) was recrystallized from *n*-hexane–chloroform. Mp 184–186 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25 (d, J = 7.7 Hz, 1H), 7.65 (t, J = 7.5 Hz, 1H), 7.51 (t, J = 7.7 Hz, 1H), 7.49–7.43 (m, 3H), 7.35 (d, J = 7.7 Hz, 1H), 7.21 (d, J = 7.7 Hz, 2H), 4.23 (s, 2H). MS m/z 238 (MH^+); HRMS calcd for $C_{15}H_{12}NO_2$ (MH^+) 238.0868; found: 238.0841. Anal. calcd for $C_{15}H_{11}NO_2$: C, 75.94; H, 4.67; N, 5.90. Found: C, 75.71; H, 4.92; N, 5.91.

4.3.2. 4,4-Dimethyl-2-phenylisoquinoline-1,3(2*H*,4*H*)-dione (DMPIQ-00, 16)

Reaction of PIQ-00 (**15**, 145 mg, 0.612 mmol) with methyl iodide gave DMPIQ-00 (**16**, 70 mg, 0.264 mmol, 43.1%) as a light yellow powder. DMPIQ-00 (**16**) was recrystallized from *n*-hexane–chloroform. Mp 139–141 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.27 (d, J = 7.7 Hz, 1H), 7.69 (t, J = 7.6 Hz, 1H), 7.56–7.42 (m, 5 H), 7.19 (d, J = 7.3 Hz, 2H), 1.74 (s, 6H); MS m/z 266 (MH^+); HRMS calcd for $C_{17}H_{16}NO_2$ (MH^+) 266.1181; found: 266.1172. Anal. Calcd for $C_{17}H_{15}NO_2$: C, 76.96; H, 5.70; N, 5.28. Found: C, 77.08; H, 5.79; N, 5.06.

4.3.3. 2-(2-Fluorophenyl)-4,4-dimethylisoquinoline-1,3(2*H*,4*H*)-dione (DMPIQ-2F, 17)

Reaction of *o*-fluoroaniline (207 mg, 1.86 mmol) with homophthalic anhydride (295 mg, 1.82 mmol) gave 2-(2-fluorophenyl)isoquinoline-1,3(2*H*,4*H*)-dione (PIQ-2F, 283 mg, 1.11 mmol, 61.0%) as a light yellow powder, which was recrystallized from *n*-hexane–

chloroform. Mp 158–159 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (d, J = 7.7 Hz, 1H), 7.66 (t, J = 6.8 Hz, 1H), 7.49 (t, J = 7.5 Hz, 1H), 7.46–7.43 (m, 2H), 7.36 (d, J = 7.7 Hz, 1H), 7.29–7.23 (m, 2H), 4.28 (d, J = 22.7 Hz, 1H), 4.22 (d, J = 22.7 Hz, 1H). MS m/z 256 (MH^+); HRMS calcd for $C_{15}H_{11}FNO_2$ (MH^+) 256.0774; found: 256.0785. Anal. Calcd for $C_{15}H_{10}FNO_2$: C, 70.58; H, 3.95; N, 5.49. Found: C, 70.30; H, 4.21; N, 5.32. Reaction of PIQ-2F (189 mg, 0.739 mmol) with methyl iodide gave DMPIQ-2F (**17**, 107 mg, 0.376 mmol, 50.9%) as a colorless oil, which later became a white solid. DMPIQ-2F (**17**) was recrystallized from *n*-hexane–chloroform. Mp 98–100 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.27 (d, J = 7.3 Hz, 1H), 7.70 (t, J = 7.7 Hz, 1H), 7.55 (d, J = 7.7 Hz, 1H), 7.48 (t, J = 7.7 Hz, 1H), 7.49–7.43 (m, 2H), 7.29–7.21 (m, 2H), 1.76 (s, 3H), 1.74 (s, 3H); MS m/z 284 (MH^+); HRMS calcd for $C_{17}H_{15}FNO_2$ (MH^+) 284.1087; found: 284.1098. Anal. Calcd for $C_{17}H_{14}FNO_2$: C, 72.07; H, 4.98; N, 4.94. Found: C, 72.11; H, 4.78; N, 5.16.

4.3.4. 2-(3-Fluorophenyl)-4,4-dimethylisoquinoline-1,3(2*H*,4*H*)-dione (DMPIQ-3F, 18)

Reaction of *m*-fluoroaniline (216 mg, 1.94 mmol) with homophthalic anhydride (294 mg, 1.81 mmol) gave 2-(3-fluorophenyl)isoquinoline-1,3(2*H*,4*H*)-dione (PIQ-3F, 289 mg, 1.13 mmol, 62.4%) as a light yellow powder which was recrystallized from *n*-hexane–chloroform. Mp 125–128 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (d, J = 8.1 Hz, 1H), 7.66 (t, J = 7.2 Hz, 1H), 7.49–7.47 (m, 2H), 7.36 (d, J = 7.7 Hz, 1H), 7.16 (dt, J = 8.6, 2.6 Hz, 1H), 7.01 (d, J = 7.7 Hz, 1H), 6.96 (td, J = 9.0, 2.1 Hz, 1H), 4.23 (s, 2H). MS m/z 256 (MH^+); HRMS calcd for $C_{15}H_{11}FNO_2$ (MH^+) 256.0774; found: 256.0814. Anal. Calcd for $C_{15}H_{10}FNO_2$: C, 70.58; H, 3.95; N, 5.49. Found: C, 70.67; H, 4.17; N, 5.46. Reaction of PIQ-3F (272 mg, 1.07 mmol) with methyl iodide gave DMPIQ-3F (**18**, 194 mg, 0.684 mmol, 63.9%) as white crystals. DMPIQ-3F (**18**) was recrystallized from *n*-hexane–ethyl acetate. Mp 113–114 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (d, J = 7.7 Hz, 1H), 7.70 (t, J = 7.7 Hz, 1H), 7.54 (d, J = 7.7 Hz, 1H), 7.49–7.44 (m, 2H), 7.15 (dt, J = 7.9, 2.1 Hz, 1H), 7.00 (d, J = 7.7 Hz, 1H), 6.95 (d, J = 9.0 Hz, 1H), 1.74 (s, 6H); MS m/z 284 (MH^+); HRMS calcd for $C_{17}H_{15}FNO_2$ (MH^+) 284.1087; found: 284.1060. Anal. Calcd for $C_{17}H_{14}FNO_2$: C, 72.07; H, 4.98; N, 4.94. Found: C, 72.20; H, 4.89; N, 5.24.

4.3.5. 2-(4-Fluorophenyl)-4,4-dimethylisoquinoline-1,3(2*H*,4*H*)-dione (DMPIQ-4F, 19)

Reaction of *p*-fluoroaniline (212 mg, 1.89 mmol) with homophthalic anhydride (303 mg, 1.87 mmol) gave 2-(4-fluorophenyl)isoquinoline-1,3(2*H*,4*H*)-dione (PIQ-4F, 181 mg, 0.706 mmol, 37.8%) as a light yellow powder which was recrystallized from *n*-hexane–chloroform. Mp 198–200 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (d, J = 7.6 Hz, 1H), 7.66 (t, J = 7.5 Hz, 1H), 7.49 (t, J = 7.6 Hz, 1H), 7.36 (d, J = 7.6 Hz, 1H), 7.20–7.18 (m, 4H), 4.23 (s, 2H); MS m/z 256 (MH^+); HRMS calcd for $C_{15}H_{11}FNO_2$ (MH^+) 256.0774; found: 256.0791. Anal. Calcd for $C_{15}H_{10}FNO_2$: C, 70.58; H, 3.95; N, 5.49. Found: C, 70.49; H, 4.22; N, 5.33. Reaction of PIQ-4F (187 mg, 0.739 mmol) with methyl iodide gave DMPIQ-4F (**19**, 98 mg, 0.345 mmol, 57.9%) as light yellow crystals. DMPIQ-4F (**19**) was recrystallized from *n*-hexane–chloroform. Mp 161–162 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (d, J = 7.7 Hz, 1H), 7.70 (t, J = 7.3 Hz, 1H), 7.54 (d, J = 7.7 Hz, 1H), 7.48 (t, J = 7.7 Hz, 1H), 7.19–7.17 (m, 4H), 1.74 (s, 6H); MS m/z 284 (MH^+); HRMS calcd for $C_{17}H_{15}FNO_2$ (MH^+) 284.1087; found: 284.1101. Anal. Calcd for $C_{17}H_{14}FNO_2$: C, 72.07; H, 4.98; N, 4.94. Found: C, 72.22; H, 5.18; N, 4.78.

4.3.6. 2-(2-Chlorophenyl)-4,4-dimethylisoquinoline-1,3(2*H*,4*H*)-dione (DMPIQ-2C, 20)

Reaction of *o*-chloroaniline (287 mg, 2.25 mmol) with homophthalic anhydride (302 mg, 1.86 mmol) gave 2-(2-chlorophenyl)isoquinoline-1,3(2*H*,4*H*)-dione (PIQ-2C, 351 mg, 1.29 mmol, 69.5%) which was recrystallized from *n*-hexane–chloroform. Mp 106–109 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (d, J = 7.7 Hz, 1H), 7.66 (t, J = 7.5 Hz, 1H), 7.56 (m, 1H), 7.50 (t, J = 7.7 Hz, 1H), 7.44–7.41 (m, 2H), 7.37 (d, J = 7.7 Hz, 1H), 7.27 (m, 1H), 4.29 (d, J = 22.7 Hz, 1H), 4.22 (d, J = 22.2 Hz, 1H); MS m/z 272 (MH^+); HRMS calcd for $C_{15}H_{11}ClNO_2$ (MH^+) 272.0478; found: 272.0484. Anal. Calcd for $C_{15}H_{10}ClNO_2$: C, 66.31; H, 3.71; N, 5.16. Found: C, 66.29; H, 3.88; N, 5.13. Reaction of PIQ-2C (114 mg, 0.418 mmol) with methyl iodide gave DMPIQ-2C (**20**, 26 mg, 0.0871 mmol, 20.8%) as a colorless oil, which later became a white solid. DMPIQ-2C (**20**) was recrystallized from *n*-hexane–ethyl acetate. Mp 123–125 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.27 (d, J = 8.1 Hz, 1H), 7.71 (t, J = 7.7 Hz, 1H), 7.57–7.55 (m, 2H), 7.48 (t, J = 7.3 Hz, 1H), 7.42–7.40 (m, 2H), 7.27–7.25 (m, 1H), 1.77 (s, 3H), 1.76 (s, 3H); MS m/z 300 (MH^+); HRMS calcd for $C_{17}H_{15}ClNO_2$ (MH^+) 300.0791; found: 300.0823. Anal. Calcd for $C_{17}H_{14}ClNO_2$: C, 68.12; H, 4.71; N, 4.67. Found: C, 67.90; H, 4.86; N, 4.61.

4.3.7. 2-(3-Chlorophenyl)-4,4-dimethylisoquinoline-1,3(2*H*,4*H*)-dione (DMPIQ-3C, 21)

Reaction of *m*-chloroaniline (237 mg, 1.86 mmol) with homophthalic anhydride (269 mg, 1.66 mmol) gave 2-(3-chlorophenyl)isoquinoline-1,3(2*H*,4*H*)-dione (PIQ-3C, 284 mg, 1.05 mmol, 63.4%) as a white powder, which was recrystallized from *n*-hexane–chloroform. Mp 154–155 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (d, J = 8.1 Hz, 1H), 7.66 (t, J = 7.5 Hz, 1H), 7.49 (t, J = 7.7 Hz, 1H), 7.46–7.43 (m, 2H), 7.36 (d, J = 7.7 Hz, 1H), 7.26–7.24 (m, 1H), 7.13–7.11 (m, 1H), 4.23 (s, 2H); MS m/z 272 (MH^+); HRMS calcd for $C_{15}H_{11}ClNO_2$ (MH^+) 272.0478; found: 272.0501. Anal. Calcd for $C_{15}H_{10}ClNO_2$: C, 66.31; H, 3.71; N, 5.16. Found: C, 66.14; H, 3.99; N, 5.15. Reaction of PIQ-3C (166 mg, 0.612 mmol) with methyl iodide gave DMPIQ-3C (**21**, 45 mg, 0.148 mmol, 24.3%) as a colorless oil, which later became a white solid. DMPIQ-3C (**21**) was recrystallized from *n*-hexane–chloroform. Mp 139–142 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (d, J = 8.1 Hz, 1H), 7.70 (t, J = 7.7 Hz, 1H), 7.54 (d, J = 7.7 Hz, 1H), 7.48 (t, J = 7.5 Hz, 1H), 7.45–7.43 (m, 2H), 7.24–7.22 (m, 1H), 7.11–7.09 (m, 1H), 1.74 (s, 6H); MS m/z 300 (MH^+); HRMS calcd for $C_{17}H_{15}ClNO_2$ (MH^+) 300.0791; found: 300.0746. Anal. Calcd for $C_{17}H_{14}ClNO_2$: C, 68.12; H, 4.71; N, 4.67. Found: C, 67.87; H, 4.89; N, 4.63.

4.3.8. 2-(4-Chlorophenyl)-4,4-dimethylisoquinoline-1,3(2*H*,4*H*)-dione (DMPIQ-4C, 22)

Reaction of *p*-chloroaniline (268 mg, 2.10 mmol) with homophthalic anhydride (312 mg, 1.92 mmol) gave 2-(4-chlorophenyl)isoquinoline-1,3(2*H*,4*H*)-dione (PIQ-4C, 331 mg, 1.22 mmol, 63.5%) as a white powder, which was recrystallized from *n*-hexane–chloroform. Mp 173–175 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (d, J = 7.3 Hz, 1H), 7.66 (t, J = 6.9 Hz, 1H), 7.51–7.47 (m, 3H), 7.36 (d, J = 7.7 Hz, 1H), 7.15 (d, J = 6.4 Hz, 2H), 4.23 (s, 2H); MS m/z 272 (MH^+); HRMS calcd for $C_{15}H_{11}ClNO_2$ (MH^+) 272.0478; found: 272.0500. Anal. Calcd for $C_{15}H_{10}ClNO_2$: C, 66.31; H, 3.71; N, 5.16. Found: C, 66.12; H, 3.77; N, 5.17. Reaction of PIQ-4C (148 mg, 0.545 mmol) with methyl iodide gave DMPIQ-4C (**22**, 61 mg, 0.202 mmol, 37.1%) as white crystals. DMPIQ-4C (**22**) was recrystallized from *n*-hexane–chloroform. Mp 171–172 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (d, J = 8.1 Hz, 1H), 7.70 (t, J = 7.7 Hz, 1H), 7.54 (d, J = 7.7 Hz, 1H), 7.49–7.46 (m, 3H), 7.14 (d, J = 9.0 Hz, 2H), 1.73 (s, 6H); MS m/z 300 (MH^+); HRMS calcd for $C_{17}H_{15}ClNO_2$ (MH^+) 300.0791; found: 300.0754. Anal. Calcd for $C_{17}H_{14}ClNO_2$: C, 68.12; H, 4.71; N, 4.67. Found: C, 67.74; H, 4.73; N, 4.67.

4.3.9. 4-(3,4-Dihydro-4,4-dimethyl-1,3-dioxoisoquinolin-2(1*H*)-yl)benzonitrile (DMPIQ-4CN, 23)

Reaction of 4-aminobenzonitrile (203 mg, 1.72 mmol) with homophthalic anhydride (303 mg, 1.87 mmol) gave 4-(3,4-dihy-

dro-1,3-dioxoisoquinolin-2(1*H*)-yl)benzonitrile (PIQ-4CN, 253 mg, 0.966 mmol, 56.2%) as a yellow powder, which was recrystallized from *n*-hexane–ethyl acetate. Mp 169–170 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (d, J = 8.1 Hz, 1H), 7.81 (d, J = 8.1 Hz, 2H), 7.68 (t, J = 7.5 Hz, 1H), 7.51 (t, J = 7.7 Hz, 1H), 7.38–7.35 (m, 3H), 4.25 (s, 2H); MS m/z 263 (MH^+); HRMS calcd for $C_{16}H_{11}N_2O_2$ (MH^+) 263.0821; found: 263.0858. Anal. Calcd for $C_{16}H_{10}N_2O_2$: C, 73.27; H, 3.84; N, 10.68. Found: C, 73.15; H, 4.10; N, 10.50. Reaction of PIQ-4CN (156 mg, 0.594 mmol) with methyl iodide gave DMPIQ-4CN (**23**, 101 mg, 0.349 mmol, 58.7%) as white crystals. DMPIQ-4CN (**23**) was recrystallized from *n*-hexane–ethyl acetate. Mp 197–198 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25 (d, J = 8.1 Hz, 1H), 7.80 (t, J = 8.1 Hz, 2H), 7.72 (t, J = 7.7 Hz, 1H), 7.56 (d, J = 8.1 Hz, 1H), 7.49 (t, J = 7.1 Hz, 1H), 7.34 (d, J = 8.6 Hz, 2H), 1.75 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.71, 163.90, 150.0, 139.7, 134.8, 133.0, 129.8, 129.4, 127.7, 125.3, 123.5, 118.2, 112.6, 44.3, 29.2; MS m/z 291 (MH^+); HRMS calcd for $C_{18}H_{15}N_2O_2$ (MH^+) 291.1134; found: 291.1096. Anal. Calcd for $C_{18}H_{14}N_2O_2$: C, 74.47; H, 4.86; N, 9.65. Found: C, 74.50; H, 5.08; N, 9.68.

4.3.10. 2-(4-Methoxyphenyl)-4,4-dimethylisoquinoline-1,3(2*H*,4*H*)-dione (DMPIQ-4MO, 24)

Reaction of *p*-anisidine (201 mg, 1.63 mmol) with homophthalic anhydride (289 mg, 1.78 mmol) gave 2-(4-methoxyphenyl)isoquinoline-1,3(2*H*,4*H*)-dione (PIQ-4MO, 220 mg, 0.823 mmol, 50.5%) as white crystals, which were recrystallized from *n*-hexane–ethyl acetate. Mp 179–180 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, J = 7.7 Hz, 1H), 7.64 (t, J = 7.5 Hz, 1H), 7.48 (t, J = 7.3 Hz, 1H), 7.34 (d, J = 7.7 Hz, 1H), 7.12 (d, J = 8.8 Hz, 2H), 7.02 (d, J = 8.8 Hz, 2H), 4.22 (s, 2H), 3.85 (s, 3H); MS m/z 268 (MH^+); HRMS calcd for $C_{16}H_{14}NO_3$ (MH^+) 268.0974; found: 268.0951. Anal. Calcd for $C_{16}H_{13}NO_3$: C, 71.90; H, 4.90; N, 5.24. Found: C, 71.96; H, 4.92; N, 5.21. Reaction of PIQ-4MO (150 mg, 0.560 mmol) with methyl iodide gave DMPIQ-4MO (**24**, 97 mg, 0.329 mmol, 58.7%) as white crystals. DMPIQ-4MO (**24**) was recrystallized from *n*-hexane–ethyl acetate. Mp 166–167 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (d, J = 7.8 Hz, 1H), 7.68 (t, J = 6.8 Hz, 1H), 7.53 (d, J = 7.8 Hz, 1H), 7.46 (t, J = 7.8 Hz, 1H), 7.11 (d, J = 8.8 Hz, 2H), 7.01 (d, J = 8.8 Hz, 2H), 3.85 (s, 3H), 1.75 (s, 6H); MS m/z 296 (MH^+); HRMS calcd for $C_{18}H_{18}NO_3$ (MH^+) 296.1287. Found: 296.1308. Anal. Calcd for $C_{18}H_{17}NO_3$: C, 73.20; H, 5.80; N, 4.74. Found: C, 72.97; H, 5.94; N, 4.70.

4.3.11. 4,4-Dimethyl-2-*p*-tolylisoquinoline-1,3(2*H*,4*H*)-dione (DMPIQ-4M, 25)

Reaction of *p*-toluidine (210 mg, 1.96 mmol) with homophthalic anhydride (332 mg, 2.05 mmol) gave 2-*p*-tolylisoquinoline-1,3(2*H*,4*H*)-dione (PIQ-4M, 238 mg, 0.949 mmol, 48.4%) as white crystals, which were recrystallized from *n*-hexane–ethyl acetate. Mp 154–156 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (d, J = 7.7 Hz, 1H), 7.64 (t, J = 7.7 Hz, 1H), 7.48 (t, J = 7.3 Hz, 1H), 7.34 (t, J = 7.7 Hz, 1H), 7.31 (d, J = 8.1 Hz, 2H), 7.09 (d, J = 8.4 Hz, 2H), 4.22 (s, 2H), 2.42 (s, 3H); MS m/z 252 (MH^+); HRMS calcd for $C_{16}H_{14}NO_2$ (MH^+) 252.1025; found: 252.1000. Anal. Calcd for $C_{16}H_{13}NO_2$: C, 76.96; H, 5.70; N, 5.28. Found: C, 77.08; H, 5.79; N, 5.06. Reaction of PIQ-4M (149 mg, 0.593 mmol) with methyl iodide gave DMPIQ-4M (**25**, 127 mg, 0.455 mmol, 76.7%) as white crystals. DMPIQ-4M (**25**) was recrystallized from *n*-hexane–ethyl acetate. Mp 158–159 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (d, J = 7.8 Hz, 1H), 7.68 (t, J = 7.5 Hz, 2H), 7.53 (d, J = 6.8 Hz, 1H), 7.46 (t, J = 7.5 Hz, 1H), 7.30 (t, J = 8.3 Hz, 2H), 7.07 (d, J = 8.3 Hz, 2H), 2.41 (s, 3H), 1.75 (s, 6H); MS m/z 280 (MH^+); HRMS calcd for $C_{18}H_{18}NO_2$ (MH^+) 280.1338; found: 280.1330. Anal. Calcd for $C_{18}H_{17}NO_2$: C, 77.40; H, 6.13; N, 5.01. Found: C, 77.27; H, 6.30; N, 4.99.

4.3.12. 2-(4-Chlorophenyl)-4,4-diethylisoquinoline-1,3(2*H*,4*H*)-dione (DEPIQ-4C, 26)

Reaction of 2-(4-chlorophenyl)isoquinoline-1,3(2*H*,4*H*)-dione PIQ-4C (241 mg, 0.887 mmol) with ethyl iodide gave DEPIQ-4C (**26**, 150 mg, 0.457 mmol, 51.6%) as white crystals. DEPIQ-4C (**26**) was recrystallized from *n*-hexane–ethyl acetate. Mp 130–132 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.29 (d, J = 7.7 Hz, 1H), 7.73 (t, J = 7.7 Hz, 1H), 7.50–7.46 (m, 3H), 7.44 (d, J = 8.1 Hz, 1H), 7.11 (d, J = 9.0 Hz, 2H), 2.38 (qd, J = 13.7, 7.3 Hz, 2H), 1.97 (qd, J = 13.5, 7.5 Hz, 2H), 0.66 (t, J = 7.3 Hz, 6H); MS m/z 328 (MH^+); HRMS calcd for $C_{19}H_{19}ClNO_2$ (MH^+) 328.1104; found: 328.1141. Anal. Calcd for $C_{19}H_{18}ClNO_2$: C, 69.62; H, 5.53; N, 4.27. Found: C, 69.43; H, 5.51; N, 4.15.

4.3.13. 2-(4-Chlorophenyl)-4,4-dipropylisoquinoline-1,3(2*H*,4*H*)-dione (DPPIQ-4C, 27)

Reaction of 2-(4-chlorophenyl)isoquinoline-1,3(2*H*,4*H*)-dione PIQ-4C (255 mg, 0.939 mmol) with propyl iodide gave DPPIQ-4C (**27**, 39 mg, 0.110 mmol, 11.7%) as white crystals. DPPIQ-4C (**27**) was recrystallized from *n*-hexane–ethyl acetate. Mp 148–150 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (d, J = 7.7 Hz, 1H), 7.72 (t, J = 7.7 Hz, 1H), 7.48–7.45 (m, 4H), 7.10 (d, J = 8.6 Hz, 2H), 2.32 (dt, J = 12.6, 4.6 Hz, 2H), 1.89 (dt, J = 12.6, 4.0 Hz, 2H), 1.13–1.06 (m, 2H), 0.93–0.85 (m, 2H), 0.79 (t, J = 7.3 Hz, 6H); MS m/z 356 (MH^+); HRMS calcd for $C_{21}H_{23}ClNO_2$ (MH^+) 356.1417; found: 356.1391. Anal. Calcd for $C_{21}H_{22}ClNO_2$: C, 70.88; H, 6.23; N, 3.94. Found: C, 70.67; H, 6.11; N, 3.88.

4.3.14. 4,4-Diallyl-2-(4-chlorophenyl)isoquinoline-1,3(2*H*,4*H*)-dione (DAPIQ-4C, 28)

Reaction of 2-(4-chlorophenyl)isoquinoline-1,3(2*H*,4*H*)-dione PIQ-4C (245 mg, 0.901 mmol) with allyl bromide gave DAPIQ-4C (**28**, 212 mg, 0.604 mmol, 67.0%) as white crystals. DAPIQ-4C (**28**) was recrystallized from *n*-hexane–ethyl acetate. Mp 109–110 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (d, J = 7.7 Hz, 1H), 7.74 (t, J = 7.7 Hz, 1H), 7.54 (d, J = 8.1 Hz, 1H), 7.48 (t, J = 7.5 Hz, 1H), 7.44 (d, J = 8.6 Hz, 2H), 7.04 (d, J = 8.6 Hz, 2H), 5.37–5.28 (m, 2H), 5.00 (d, J = 24.8 Hz, 2H), 4.98 (d, J = 18.0 Hz, 2H), 3.07 (dd, J = 13.3, 8.6 Hz, 2H), 2.74 (dd, J = 13.5, 6.2 Hz, 2H); MS m/z 352 (MH^+); HRMS calcd for $C_{21}H_{19}ClNO_2$ (MH^+) 352.1104; found: 352.1064. Anal. Calcd for $C_{21}H_{22}ClNO_2$: C, 71.69; H, 5.16; N, 3.98. Found: C, 71.44; H, 5.34; N, 3.78.

4.3.15. 4,4-Spiropentyl-2-(4-chlorophenyl)isoquinoline-1,3(2*H*,4*H*)-dione (C5PIQ-4C, 29)

Reaction of 2-(4-chlorophenyl)isoquinoline-1,3(2*H*,4*H*)-dione PIQ-4C (408 mg, 1.50 mmol) with 1,4-diiodobutane gave C5PIQ-4C (**29**, 229 mg, 0.702 mmol, 46.8%) as white crystals. C5PIQ-4C (**29**) was recrystallized from *n*-hexane–ethyl acetate. Mp 181–182 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.22 (d, J = 8.1 Hz, 1H), 7.69 (t, J = 8.3 Hz, 1H), 7.48–7.43 (m, 4H), 7.14 (d, J = 8.6 Hz, 2H), 2.63–2.59 (m, 2H), 2.13–2.06 (m, 4H), 2.03–1.97 (m, 2H); MS m/z 326 (MH^+); HRMS calcd for $C_{19}H_{17}ClNO_2$ (MH^+) 326.0948; found: 326.0905. Anal. Calcd for $C_{19}H_{16}ClNO_2$: C, 70.05; H, 4.95; N, 4.30. Found: C, 69.95; H, 5.12; N, 4.31.

4.3.16. 4,4-Spirohexyl-2-(4-chlorophenyl)isoquinoline-1,3(2*H*,4*H*)-dione (C6PIQ-4C, 30)

Reaction of 2-(4-chlorophenyl)isoquinoline-1,3(2*H*,4*H*)-dione PIQ-4C (400 mg, 1.47 mmol) with 1,5-diiodipentane gave C6PIQ-4C (**30**, 144 mg, 0.423 mmol, 28.8%) as white crystals. C6PIQ-4C (**30**) was recrystallized from *n*-hexane–ethyl acetate. Mp 154–156 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 (d, J = 7.7 Hz, 1H), 7.68 (t, J = 6.8 Hz, 1H), 7.63 (d, J = 7.7 Hz, 1H), 7.48–7.45 (m, 3H), 7.13

(d, J = 8.6 Hz, 2H), 2.19–2.17 (m, 2H), 2.05–1.94 (m, 4H), 1.83–1.80 (m, 2H), 1.74–1.71 (m, 1H), 1.39–1.34 (m, 1H); MS m/z 340 (MH^+); HRMS calcd for $C_{20}H_{19}ClNO_2$ (MH^+) 340.1104; found: 340.1108. Anal. Calcd for $C_{20}H_{18}ClNO_2$: C, 70.69; H, 5.34; N, 4.12. Found: C, 70.49; H, 5.37; N, 3.99.

4.3.17. 4-(4,4-Diethyl-3,4-dihydro-1,3-dioxoisoquinolin-2(1*H*)-yl) benzonitrile (DEPIQ-4CN, 31)

Reaction of 4-(3,4-dihydro-1,3-dioxoisoquinolin-2(1*H*)-yl)benzonitrile (PIQ-4CN, 177 mg, 0.676 mmol) with ethyl iodide gave DEPIQ-4CN (**31**, 85 mg, 0.267 mmol, 39.5%) as a colorless oil, which later became a white solid. DEPIQ-4CN (**31**) was recrystallized from *n*-hexane–ethyl acetate. Mp 149–150 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.28 (d, J = 7.7 Hz, 1H), 7.80 (d, J = 8.5 Hz, 2H), 7.75 (t, J = 7.7 Hz, 1H), 7.50 (t, J = 7.3 Hz, 1H), 7.46 (d, J = 8.1 Hz, 1H), 7.31 (d, J = 8.5 Hz, 2H), 2.39 (qd, J = 13.7, 7.7 Hz, 2H), 1.99 (qd, J = 13.7, 7.3 Hz, 2H), 0.68 (t, J = 7.5 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.7, 164.1, 141.9, 139.8, 134.9, 133.1, 129.8, 128.9, 127.5, 126.3, 125.3, 118.2, 112.6, 54.6, 36.1, 9.4; MS m/z 319 (MH^+); HRMS calcd for $C_{20}H_{19}N_2O_2$ (MH^+) 319.1447; found: 319.1454. Anal. Calcd for $C_{20}H_{18}N_2O_2$: C, 75.45; H, 5.70; N, 8.80. Found: C, 75.23; H, 5.85; N, 8.80.

4.3.18. 4-(3,4-Dihydro-1,3-dioxo-4,4-dipropylisoquinolin-2(1*H*)-yl)benzonitrile (DPPIQ-4CN, 32)

Reaction of 4-(3,4-dihydro-1,3-dioxoisoquinolin-2(1*H*)-yl)benzonitrile (PIQ-4CN, 153 mg, 0.584 mmol) with propyl iodide gave DPPIQ-4CN (**32**, 161 mg, 0.464 mmol, 79.5%) as white crystals. DPPIQ-4CN (**32**) was recrystallized from *n*-hexane–ethyl acetate. Mp 147 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (d, J = 7.7 Hz, 1H), 7.80 (d, J = 8.1 Hz, 2H), 7.74 (t, J = 7.7 Hz, 1H), 7.50–7.46 (m, 2H), 7.31 (d, J = 8.6 Hz, 2H), 2.32 (dt, J = 12.6 Hz, 4.6 Hz, 2H), 1.91 (dt, J = 12.7 Hz, 4.1 Hz, 2H), 1.13–1.06 (m, 2H), 0.93–0.84 (m, 2H), 0.80 (t, J = 7.1 Hz, 6H); MS m/z 347 (MH^+); HRMS calcd for $C_{22}H_{23}N_2O_2$ (MH^+) 347.1760; found: 347.1800. Anal. Calcd for $C_{22}H_{22}N_2O_2$: C, 76.28; H, 6.40; N, 8.09. Found: C, 76.28; H, 6.51; N, 8.01.

4.3.19. 4-(4,4-Diallyl-3,4-dihydro-1,3-dioxoisoquinolin-2(1*H*)-yl)benzonitrile (DAPIQ-4CN, 33)

Reaction of 4-(3,4-dihydro-1,3-dioxoisoquinolin-2(1*H*)-yl)benzonitrile (PIQ-4CN, 162 mg, 0.619 mmol) with allyl bromide gave DAPIQ-4CN (**33**, 112 mg, 0.328 mmol, 53.0%) as white crystals. DAPIQ-4CN (**33**) was recrystallized from *n*-hexane–ethyl acetate. Mp 131–133 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (d, J = 7.7 Hz, 1H), 7.78–7.75 (m, 3H), 7.56 (d, J = 7.7 Hz, 1H), 7.50 (t, J = 7.7 Hz, 1H), 7.24 (d, J = 8.6 Hz, 2H), 5.37–5.28 (m, 2H), 5.02 (d, J = 21.0 Hz, 2H), 4.99 (d, J = 14.1 Hz, 2H), 3.07 (dd, J = 13.7 Hz, 8.6 Hz, 2H), 2.76 (dd, J = 13.5 Hz, 6.2 Hz, 2H); MS m/z 343 (MH^+); HRMS calcd for $C_{22}H_{19}N_2O_2$ (MH^+) 343.1447; found: 343.1489. Anal. Calcd for $C_{22}H_{18}N_2O_2$: C, 77.17; H, 5.30; N, 8.18. Found: C, 77.14; H, 5.48; N, 8.13.

4.3.20. 4-(4,4-Spiropentyl-3,4-dihydro-1,3-dioxoisoquinolin-2(1*H*)-yl)benzonitrile (C5PIQ-4CN, 34)

Reaction of 4-(3,4-dihydro-1,3-dioxoisoquinolin-2(1*H*)-yl)benzonitrile (PIQ-4CN, 153 mg, 0.583 mmol) with 1,4-diiodobutane gave C5PIQ-4CN (**34**, 85 mg, 0.269 mmol, 46.2%) as white crystals. C5PIQ-4CN (**34**) was recrystallized from *n*-hexane–ethyl acetate. Mp 221–223 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.22 (d, J = 8.1 Hz, 1H), 7.80 (d, J = 8.1 Hz, 2H), 7.71 (t, J = 7.7 Hz, 1H), 7.48–7.45 (m, 2H), 7.34 (d, J = 8.5 Hz, 2H), 2.64–2.58 (m, 2H), 2.17–2.07 (m, 4H), 2.05–1.99 (m, 2H); MS m/z 317 (MH^+); HRMS calcd for $C_{20}H_{17}N_2O_2$ (MH^+) 317.1290; found: 317.1315. Anal. Calcd for $C_{20}H_{16}N_2O_2$: C, 75.93; H, 5.10; N, 8.86. Found: C, 75.80; H, 5.30; N, 8.78.

4.3.21. 4-(4,4-Spirohexyl-3,4-dihydro-1,3-dioxoisoquinolin-2(1*H*)-yl)benzonitrile (C6PIQ-4CN, 35)

Reaction of 4-(3,4-dihydro-1,3-dioxoisoquinolin-2(1*H*)-yl)benzonitrile (PIQ-4CN, 156 mg, 0.595 mmol) with 1,5-diiodipentane gave C6PIQ-4CN (**35**, 147 mg, 0.445 mmol, 74.7%) as a white powder. C6PIQ-4CN (**35**) was recrystallized from *n*-hexane–ethyl acetate. Mp 173–174 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 (d, J = 7.7 Hz, 1H), 7.80 (d, J = 8.1 Hz, 2H), 7.71 (t, J = 8.6 Hz, 1H), 7.65 (d, J = 7.7 Hz, 1H), 7.48 (t, J = 7.5 Hz, 1H), 7.33 (d, J = 8.5 Hz, 2H), 2.16–2.00 (m, 2H), 2.05–1.94 (m, 4H), 1.85–1.80 (m, 1H), 1.77–1.72 (m, 2H), 1.42–1.35 (m, 1H); MS m/z 331 (MH^+); HRMS calcd for $C_{21}H_{19}N_2O_2$ (MH^+) 331.1447; found: 331.1468. Anal. Calcd for $C_{21}H_{18}N_2O_2$: C, 76.34; H, 5.49; N, 8.48. Found: C, 76.30; H, 5.67; N, 8.51.

4.4. T47D alkaline phosphatase assay

T47D alkaline phosphatase assay was performed as described in the literature. T47D human breast-carcinoma cell line was purchased from Dainippon Sumitomo Pharma Co., Ltd, T47D cells were cultured in RPMI 1640 medium (Wako Pure Chemical Industries, Ltd) with 10% (v/v) fetal bovine serum (GIBCO) and Penicillin–Streptomycin Mixed Solution (Nacalai tesque). Cells were plated in 96-well plates at 1×10^4 cells/well and incubated overnight (37 °C, 5% CO_2). The next day, cells were treated with fresh media containing either test compound or test compound plus progesterone (0.3 nM), and incubated for 2 days. The medium was aspirated and the cells were fixed with 100 μL of 1.8% formalin (in PBS). The fixed cells were washed with PBS and 75 μL of assay buffer [1 mg/mL *p*-nitrophenol phosphate (Sigma) in diethanolamine water solution, pH 9.0, 2 mM $MgCl_2$] was added. Following incubation at room temperature with shielding from light for 2 h, the reaction was terminated by the addition of 100 μL of 1 M NaOH. The absorbance at 405 nm was measured (Tosoh, Japan). All data points were measured in triplicate.

4.5. Reporter gene assay

Reporter gene assays using hPR were carried out with CHO (Chinese hamster ovary) cells. CHO cells were cultured in RPMI 1640 medium (Wako Pure Chemical Industries, Ltd.) with 10% (v/v) fetal bovine serum (Gibco) and Penicillin–Streptomycin Mixed Solution (Nacalai tesque). Twenty-one microliters of transfection reagent FuGENE® 6 Transfection Reagent (Roche) was diluted with 350 μL Opti-MEM (Gibco), 3.5 μL 1.00 γ/λ pBind-hPR-LBD and 3.5 μL 1.00 γ/λ p5G-Luc were diluted with 350 μL Opti-MEM, and the transfection reagent FuGENE 6 and plasmid DNA were combined and incubated at room temperature for 30 min. To CHO cells incubated with FuGENE 6 and plasmid DNA, RPMI1640 medium with 10% FBS was added (14 mL) to give a concentration of 1×10^5 cells/mL. The cells were plated in a 96-well plate at 200 μL/well and incubated for 24 h (37 °C, 5% CO_2). The medium was replaced with fresh medium containing either test compound or test compound plus progesterone (1 nM), and incubation was continued for 24 h. The medium was aspirated and 50 μL of PicaGene LT2.0 Luminescene Kit (Toyo Ink Manufacturing Company, Ltd) was added. Incubation was continued at room temperature with shielding from light for 15 min, then the LUC was measured (Tosoh, Japan). All data points were measured in triplicate.

4.6. Progesterone receptor-binding assay

PR-binding assay was performed using baculovirus-expressed hPR purchased from Panvera. Incubation buffer was prepared as follows, 20 mM Tris–HCl (pH 8.0), 0.3 M NaCl, 1 mM EDTA (pH

8.0), 10 mM 2-mercaptoethanol, and 0.2 mM PMSF. To 450 μL of progesterone receptor solution, 25 μL of DMSO solution of test compound and 25 μL of DMSO solution of [1,2,6,7-^{3}H]progesterone (GE Healthcare) were added. The reaction mixture was incubated for 1 h at 4 °C. After incubation, 200 μL of 0.1 % (w/v) dextran-coated charcoal (Sigma) solution was added to the incubation mixture. Then, the mixture was centrifuged at 10,000 rpm for 10 min. The radioactivity of 400 μL of the supernatant was measured in 4 mL of cocktail with a liquid scintillation counter (Beckman LS 6500 LL). All experiments were performed in duplicate.

4.7. Estrogen receptor-binding assay and androgen receptor-binding assay

ER- and AR-binding assays were performed using whole cell extracts of T47D cells, which express not only PR, but also ER and AR. Collected T47D cells were sonicated in a buffer consisting of 20 mM Hepes (pH 7.6), 0.3 M NaCl, 1 mM PMSF, 0.5 μg/mL aprotinin, 0.5 μg/mL leupeptin, 0.7 μg/mL pepstatin A. After centrifugation at 36,000 rpm for 1 h at 4 °C, the supernatant containing ER and AR was collected and measured for protein concentration. ER-binding assays were performed as follows. To 294 μL of T47D cytosol (1.0 mg protein/mL), 3 μL of [6,7-^{3}H]estradiol [final concentration of 1 nM (GE Healthcare)] and DEPIQ-4CN (**31**, final concentration of 1 μM), DMSO, or 11β-estradiol (final concentration of 100 nM) were added. The reaction mixtures were incubated overnight at 4 °C. After incubation, 120 μL of 0.3% (w/v) dextran-coated charcoal solution was added to the incubation mixture. Then, the mixture was centrifuged at 10,000 rpm for 10 min. The radioactivity of 250 μL of the supernatant was measured in 4 mL of cocktail with a liquid scintillation counter. AR-binding assay was performed as follows. To 294 μL of T47D cytosol (1.4 mg protein/mL), 3 μL of [1,2,6,7-^{3}H]testosterone [final concentration of 1 nM (GE Healthcare)] and DEPIQ-4CN (**31**, final concentration of 1 μM), DMSO, or testosterone (final concentration of 100 nM) were added. The reaction mixture was incubated overnight at 4 °C. After incubation, 120 μL of 1.0 % (w/v) dextran-coated charcoal solution was added to the incubation mixture. Then, the mixture was centrifuged at 10,000 rpm for 10 min. The radioactivity of 250 μL of the supernatant was measured in 4 mL of cocktail with a liquid scintillation counter. All experiments were performed in duplicate.

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References and notes

1. Mangelsdorf, D. J.; Thummel, C.; Beato, M.; Herrlich, P.; Schtüz, G.; Umesono, K.; Blumberg, B.; Kastner, P.; Mark, M.; Chambon, P.; Evans, M. *Cell* **1995**, *83*, 835–883.
2. Adashi, E. Y.; Rock, J. A.; Rosenwaks.Reproductive Endocrinology, Surgery, and Technology. 1996, pp 493–504, Chapter 24.
3. Edgren, R. A.; Sturtevant, F. M. *Am. J. Obstet. Gynecol.* **1976**, *125*, 1029–1038.
4. Trogerson, D. J.; Bell-Syer, S. E. M. *JAMA* **2001**, *285*, 2891–2897.
5. Vercellini, P.; De Giorgi, O.; Oldani, S.; Cortesi, I.; Panazza, S.; Crosgnani, P. G. *Am. J. Obstet. Gynecol.* **1996**, *175*, 396–401.
6. Winneker, R. C.; Fensome, A.; Wrobel, J. E.; Zhang, A.; Zhang, P. *Semin. Reprod. Med.* **2005**, *23*, 46–57.
7. Fensome, A.; Bender, R.; Chopra, R.; Cohen, J.; Collins, M. A.; Hudak, V.; Malakian, K.; Lockhead, S.; Olland, A.; Svenson, K.; Terefenko, E. A.; Unwalla, R. J.; Wilhelm, J. M.; Wolfrom, S.; Zhu, Y.; Zhang, Z.; Zhang, P.; Winneker, R. C.; Wrobel, J. *J. Med. Chem.* **2005**, *48*, 5092–5095.
8. Brown, A.; Cheng, L.; Lin, S.; Baird, D. T. *J. Clin. Endocrinol. Metabol.* **2002**, *87*, 63–70.
9. Kettel, L. M.; Murphy, A. A.; Mortola, J. F. *Fertil. Steril.* **1991**, *56*, 402–407.
10. Murphy, A. A.; Kettel, L. M.; Morales, A. J.; Roberts, V. J.; Yen, S. S. C. *J. Clin. Endocrinol. Metab.* **1993**, *76*, 513–517.
11. Kettel, L. M. *Clin. Obstet. Gynecol.* **1995**, *38*, 921–934.
12. Chabbert-Buffet, N.; Meduri, G.; Bouchard, P.; Spitz, I. M. *Hum. Reprod. Update* **2005**, *11*, 293–307.
13. Nakamura, T.; Noguchi, T.; Kobayashi, H.; Miyachi, H.; Hashimoto, Y. *Chem. Pharm. Bull.* **2006**, *54*, 1709–1714.
14. Nakamura, T.; Noguchi, T.; Miyachi, H.; Hashimoto, Y. *Chem. Pharm. Bull.* **2007**, *55*, 651–654.
15. Lorenzo, D. D.; Albertini, A.; Zava, D. *Cancer Res.* **1991**, *51*, 4470–4475.
16. Lorenzo, D. D.; Gianni, M.; Savoldi, G. F.; Ferrari, F.; Albertini, A.; Garattini, E. *Biochem. Biophys. Res. Commun.* **1993**, *192*, 1066–1072.
17. Hamann, L. G.; Farmer, L. J.; Johnson, M. G.; Bender, S. L.; Mais, D. E.; Wang, M-W. M.-W.; Crombie, D.; Goldman, M. E.; Jones, T. K. *J. Med. Chem.* **1996**, *39*, 1778–1789.
18. Hashimoto, Y. *Curr. Med. Chem.* **1998**, *5*, 163–178.
19. Hashimoto, Y. *Bioorg. Med. Chem.* **2002**, *10*, 461–479.
20. Hashimoto, Y. *Mini-Rev. Med. Chem.* **2002**, *2*, 543–551.
21. Hashimoto, Y.; Tanatani, A.; Nagasawa, K.; Miyachi, H. *Drugs Future* **2004**, *29*, 383–391.
22. (a) Nakagawa, A.; Tanatani, A.; Miyachi, H.; Hashimoto, Y. *Drugs Future* **2007**, 92; (b) Tanatani, A.; Miyachi, H.; Nakagaea, A.; Hashimoto, Y. *Jpn. Kokai Tokkyo Koho*, JP 2007 204371.
23. Kakuta, H.; Tanatani, A.; Nagasawa, K.; Hashimoto, Y. *Chem. Pharm. Bull.* **2003**, *51*, 1273–1282.
24. Toya, T.; Fukasawa, H.; Masui, A.; Endo, Y. *Biochem. Biophys. Res. Commun.* **2002**, *292*, 1087–1091.
25. Takahashi, B.; Ohta, K.; Kawachi, E.; Fukasawa, H.; Hashimoto, Y.; Kagechika, H. *J. Med. Chem.* **2002**, *45*, 3327–3330.
26. Pathirana, C.; Stein, R. B.; Berger, T. S.; Fenical, W.; Ianiro, T.; Mais, D. E.; Torres, A.; Goldman, M. E. *Mol. Pharmocol.* **1995**, *47*, 630–635.
27. Zhang, Z.; Lundeen, S. G.; Zhu, Y.; Carver, J. M.; Winneker, R. C. *Steroids* **2000**, *65*, 637–643.
28. Palmer, S.; Campen, C. A.; Allan, G. F.; Rybczynski, P.; Haynes-Johnson, D.; Hutchins, A.; Kraft, P.; Kiddoe, M.; Lai, M-T. M.-T.; Lombardi, E.; Pedersen, P.; Hodgen, G.; Combs, D. W. *J. Steroid Biochem. Mol. Biol.* **2000**, *75*, 33–42.
29. Tabata, y.; Iizuka, T.; Kashiwa, J.; Takei-Masuda, N.; Shinei, R.; Kurihara, K.-i.; Okonogi, T.; Hoshiko, S.; Kurata, Y. *Eur. J. Pharmacol.* **2001**, *430*, 159–165.