Bioorganic & Medicinal Chemistry 18 (2010) 4255–4268

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Aromatic β-amino-ketone derivatives as novel selective non-steroidal progesterone receptor antagonists

Yongli Du [a,†], Qunyi Li [b,†], Bing Xiong [a], Xin Hui [b], Xin Wang [a], Yang Feng [b], Tao Meng [a], Dingyu Hu [a], Datong Zhang [c], Mingwei Wang [a,b,*], Jingkang Shen [a,*]

[a] State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Shanghai 201203, PR China
[b] The National Center for Drug Screening, 189 Guo Shou Jing Road, Shanghai 201203, PR China
[c] School of Chemical Engineering, Shandong Institute of Light Industry, Jinan, Shandong 250353, PR China

ARTICLE INFO

Article history:
Received 23 March 2010
Revised 28 April 2010
Accepted 28 April 2010
Available online 24 May 2010

Keywords:
Progesterone receptor (PR)
Progesterone receptor antagonist
Non-steroidal progesterone receptor antagonist
Aromatic β-amino-ketone
Mannich reaction

ABSTRACT

A novel class of non-steroidal progesterone receptor antagonists with aromatic β-amino-ketone scaffold have been synthesized and characterized with high binding affinity and great selectivity for the cognate receptors. Among them, compound **22** was shown to be the most potent progesterone receptor antagonist in cotransfection assay and a murine model of ligand-induced decidualization.

1. Introduction

Progesterone receptor (PR) is a member of the nuclear hormone receptor super family which is a group of ligand-dependent transcription factors.[1] Small molecule antagonists to PR have important roles in healthcare, including the potential use as therapeutic agents for treatment of leiomyomas,[2] endometriosis,[3] breast cancer,[4] and meningiomas,[5] as well as application in fertility control.[6] However, the most well-known PR antagonist, namely, mifepristone (RU486), also possesses potent activity at other steroid receptors such as glucocorticoid receptor (GR), which limits its broad clinical utility, especially for chronic administration. Non-steroidal PR antagonists with distinct structural features may therefore circumvent the liabilities manifested by their steroidal counterparts.

During the past decade, several classes of non-steroidal PR antagonists have been described in the literature, and their pharmacological properties characterized.[7,8] Some of the most frequently reported structural features of non-steroidal PR antagonists included 6-aryl-1,3-dihydrobenzoimidazol-2-ones,[9,10] 5-Aryl-1,3-dihydro-indol-2-ones,[11] 6-aryl-1,4-dihydrobenzo[*d*][1,3]oxazin-2-ones,[12] benzoxazin-2-ones,[12] 3-aryl-1,2-diazepines,[13] 5-aryl inden-1-ols[14], and 5-aryl indanones,[14] as exemplified by compounds **1**–**7** shown in Figure 1.

In our pursuit to discover non-steroidal modulators to nuclear hormone receptors, a series of β-amino-ketone analogues were identified as androgen receptor (AR) modulators through a high-throughput screening campaign described in a recent report.[15] During the process of modifying the β-amino-ketone derivatives (**8**, Fig. 2) against AR, compound **9** (Fig. 2) was shown to possess moderate PR (IC_{50} = 180 nM) and high potent AR binding affinities (IC_{50} = 2.9 nM).[15] Although it is well documented that the steroidal ligands of nuclear hormone receptors have a common fused four-ring system, little is known relative to shared non-steroidal scaffolds for different nuclear hormone receptors. Thus, the finding that β-amino-ketone derivatives affect both AR and PR activities instigated us to further study this novel non-steroidal template that led to the discovery of a series of PR antagonists with high potency and selectivity. In this report we describe in detail the syntheses and structure–activity relationship (SAR) analyses of these novel non-steroidal PR antagonists.

2. Chemistry

The syntheses of aromatic β-amino-ketone analogues **13**–**55** were accomplished through Mannich reactions, and the mixture

* Corresponding authors. Tel.: +86 21 50806600 5407; fax: +86 21 50807088 (J.S.), tel.: +86 21 50800598; fax: +86 21 50800721 (M.W.).
E-mail addresses: wangmw@mail.shcnc.ac.cn (M. Wang), jkshen@mail.shcnc.ac.cn (J. Shen).
† These two authors contributed equally to this work.

0968-0896/$ - see front matter © 2010 Published by Elsevier Ltd.
doi:10.1016/j.bmc.2010.04.092

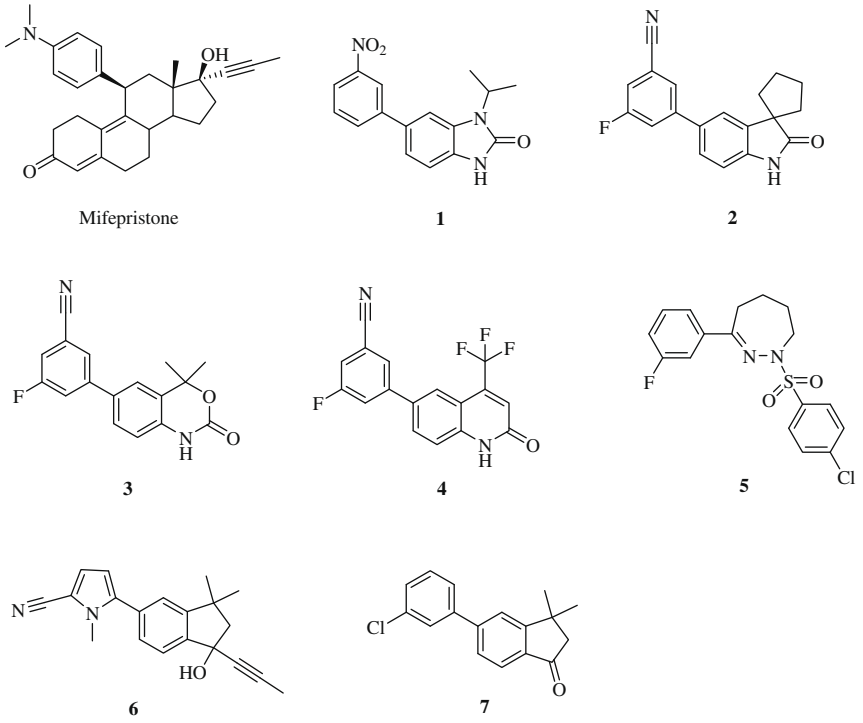


Figure 1. The PR antagonist Mifepristone and representative examples of non-steroidal PR antagonists (**1**–**7**) previously reported.

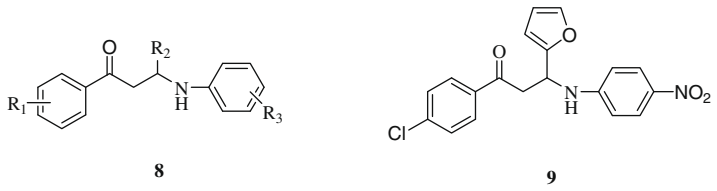


Figure 2. General structure of aromatic β-amino-ketone **8** and selective AR modulator **9**.

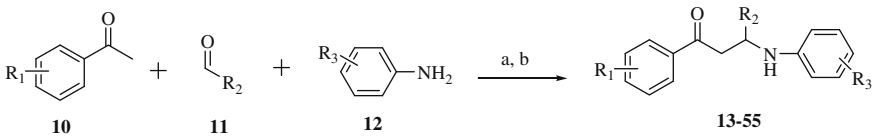
of aromatic ketones **10**, aldehydes **11** and aromatic amines **12** in an ethanolic hydrogen chloride solution (Scheme 1)[16] were conducted over periods of 12–24 h at room temperature. The crude products **13**–**55** collected by filtration and washed with absolute EtOH and 10% $NaHCO_3$ were purified by recrystallization from absolute EtOH (Table 1).

Compounds **13**–**55** were obtained as racemic products which were employed to detect biological activities unless further resolution was performed. To investigate chiral isomer-induced effect on biological activities HPLC separation of the enantiomers were explored for racemates **22** (having best PR antagonist activity in vivo) and **32** (for determining the absolute configuration of enantiomers of **22**). After a survey of a number of chiral stationary phases, it was found that the 'Chiralpak IA' column cleanly separated the enantiomers of **22** and **32**. Using a 20 mm id × 250 mm length 'Chiralpak IA' column, optimization of the mobile phase afforded conditions (hexane/THF 70/30 at a flow rate of 10 ml/min) suitable for separation of up to 10 mg racemate per 20-min injection, and each enantiomer could be separated to a purity of greater than 98% ee.

As shown in Figure 3 the absolute configuration of (+)-**32** was determined to be (+)-(*S*)-**32** isomer by X-ray crystallography. Comparing circular dichroism curves of the enantiomers of compound **22** with that of (+)-(*S*)-**32**, we found that isomer (+)-**22** was similar to (+)-**32** in CD curves and the absolute configuration of compound (+)-**22** was determined to be S. Isomer (−)-**22** that has the opposite CD curves with compound (+)-(*S*)-**32** was determined to be *R* (shown in Fig. 4).

3. Results and discussion

To investigate the effects of different β-aromatic groups (R_2) on the binding affinities to AR and PR, compounds **13**–**22** (Table 2) were designed and prepared, respectively. Compounds **13** and **14** with a 3-thiophenyl group were found to be more potent for AR, exhibiting a 10- to 33-fold more selectivity over PR. While the



Scheme 1. Reagents and conditions: (a) concd HCl/EtOH, rt, 12–24 h; (b) 10% $NaHCO_3$.

Table 1
Preparation of aromatic β-amino-ketone analogues

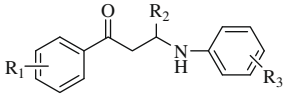
No.	R_1	R_2	R_3	Yield (%)
13	4-Me	Thiophen-3-yl	4-NO_2	83
14	4-CN	Thiophen-3-yl	4-NO_2	53
15	4-Me	Ph	4-NO_2	61
16	4-Me	Ph	4-MeO	62
17	4-Me	3,4,5-$(MeO)_3$Ph	4-NO_2	70
18	3-Me	3,4,5-$(MeO)_3$Ph	4-NO_2	67
19	3-F	3,4,5-$(MeO)_3$Ph	4-NO_2	72
20	3-Me	3-FPh	4-NO_2	96
21	3-F	3-FPh	4-NO_2	89
22	4-Me	3-FPh	4-NO_2	80
23	4-Me	3-FPh	3-NO_2	51
24	4-Me	3-FPh	4-CF_3	83
25	4-Me	3-FPh	4-F	59
26	4-Me	3-FPh	3,4-di-F	48
27	3-Cl	3-FPh	4-NO_2	86
28	3-Br	3-FPh	4-NO_2	83
29	H	3-FPh	4-NO_2	67
30	4-F	3-FPh	4-NO_2	51
31	4-Cl	3-FPh	4-NO_2	90
32	4-Br	3-FPh	4-NO_2	93
33	4-Ph	3-FPh	4-NO_2	68
34	4-Cy	3-FPh	4-NO_2	82
35	4-Morpholino	3-FPh	4-NO_2	89
36	3-Cl	4-FPh	4-NO_2	59
37	3-Cl	4-CF_3Ph	4-NO_2	81
38	3-F	4-CF_3Ph	4-NO_2	48
39	3-Me	4-CF_3Ph	4-NO_2	74
40	3-CN	4-CF_3 Ph	4-NO_2	76
41	3-MeO	4-CF_3 Ph	4-NO_2	80
42	4-MeO	4-CF_3 Ph	4-NO_2	59
43	4-Cl	4-CF_3 Ph	4-NO_2	89
44	4-Br	4-CF_3 Ph	4-NO_2	49
45	4-Me	Cy	4-NO_2	62
46	4-MeO	Cy	4-NO_2	48
47	4-Br	Cy	4-NO_2	28
48	4-Cl	Cy	4-NO_2	36
49	4-F	Cy	4-NO_2	43
50	3-F	Cy	4-NO_2	40
51	3-MeO	Cy	4-NO_2	48
52	3,4-di-Cl	Cy	4-NO_2	58
53	H	Cy	4-NO_2	49
54	4-Ph	Cy	4-NO_2	36
55	4-Cy	Cy	4-NO_2	51

two β-phenyl substituted products, compounds **15** and **16**, were less selective, compounds **17**, **18** and **19** displayed a inverse trend when the β-phenyl ring was substituted by a bulky 3,4,5-trimethoxyl group. Similarly, β-3-fluorophenyl substituted analogues **20**–**22** produced a more potent PR binding profile over that of AR. These results suggesting that steric variance of group R_2 can modulate the selectivity for PR and AR.

Next, 3-(phenylamino)-3-(3-fluorophenyl)-1-*p*-tolylpropan-1-one was chosen as general structure and a number of substituted aniline analogues (e.g., **23**–**26**, Table 3) were synthesized to study the structure–activity relationships. Both 4-nitroaniline and 3-nitroaniline derivatives (**22** and **23**) displayed higher PR binding affinity than AR, but replacement of the 4-nitro group (**22**) with a 4-trifluoromethyl group in **24** resulted in a dramatic decrease in PR binding (K_i >10,000 nM), substitution by a 4-fluoro atom in **25** produced a similar outcome (K_i = 444 nM), and the introduction of an additional fluoro atom in compound **26** did not alter the situation (K_i = 574 nM). So the preliminary optimization experiments (**24**, **25**, **26**) above suggest that nitro aniline analogues (e.g., **22**, **23**) are better for PR binding, indicating the importance of this structural moiety.

To further improve the binding affinity and selectivity for PR versus AR, several substituted groups were introduced into the ring of aromatic ketone to produce analogues **27**–**55** (Table 4), and the structure–activity relationships (SAR) of β-(*p*-nitrophenylamino)-ketones were studied. All the compounds (**27**–**55**) showed high PR binding affinity (K_i <100 nM). The trend observed for the β-(3-fluorophenyl) analogues (**21**, **22**, **27**–**35**) was that the substituted group (R_1) at the 3′-position was preferred to 4′-position (e.g., 21 vs **30**, 27 vs **31**) for PR binding potency and selectivity against AR. The data of β-(3-fluorophenyl) analogues **31**–**33** clearly demonstrated that a large steric group (R_1 = Ph, Cy, morpholino) at the 4′-position was tolerated for PR binding activity and good for PR selectivity. 3-chlorine analogue **27** (K_i = 23 nM) was the most potent compound in the β-(3-fluorophenyl) analogues in PR binding assay. Removing the fluoro atom in β-phenyl group (R_2) from 3′ (**27**) to 4′-position (**36**) resulted in threefold increase in the selectivity for PR against AR. The replacement of 4-fluoro atom (**36**) with 4-trifluoromethyl moiety (**37**) in β-phenyl group (R_2) further improved the PR binding selectivity (hAR/hPR >100-fold). The excellent selectivity could be accounted for the increase of steric hindrance from 4-trifluoromethyl moiety in **37** compared to 4-fluoro group in **36**. Most of the β-*p*-trifluoromethylphenyl analogues (e.g., **37**, **39**, **41** and **44**) exhibited significant selectivity for PR when optimizing the R_1 group. β-Cyclohexyl (R_2) analogues **45**–**53** displayed high PR binding affinity ranging from 6.7 to

Table 2
Effects of β-aromatic substituted groups on hPR-B and hAR[a] binding characteristics



Compound	R_1	R_2	R_3	hPR-B Binding K_i (nM) mean ± SEM	hAR Binding K_i (nM) mean ± SEM	Selectivity hAR/hPR
Progesterone				5.1 ± 0.6	35 ± 7	
DHT					4.7 ± 0.6	
13	4-Me	Thiophen-3-yl	4-NO_2	103 ± 8	10.4 ± 1.3	0.1
14	4-CN	Thiophen-3-yl	4-NO_2	75 ± 8	2.3 ± 0.2	0.03
15	4-Me	Ph	4-NO_2	66 ± 12	18 ± 3	0.3
16	4-Me	Ph	4-MeO	1119[b]	4523 ± 1021	4
17	4-Me	3,4,5-$(MeO)_3$Ph	4-NO_2	236 ± 23	490 ± 79	2.1
18	3-Me	3,4,5-$(MeO)_3$Ph	4-NO_2	173 ± 21	1531 ± 312	8.8
19	3-F	3,4,5-$(MeO)_3$Ph	4-NO_2	121 ± 12	3178 ± 881	26.1
20	3-Me	3-FPh	4-NO_2	35 ± 2	65 ± 1	1.8
21	3-F	3-FPh	4-NO_2	46 ± 4	51 ± 13	1.1
22	4-Me	3-FPh	4-NO_2	54 ± 6	96 ± 9	1.8

[a] Values represent triplicate determinations.
[b] Assayed once.

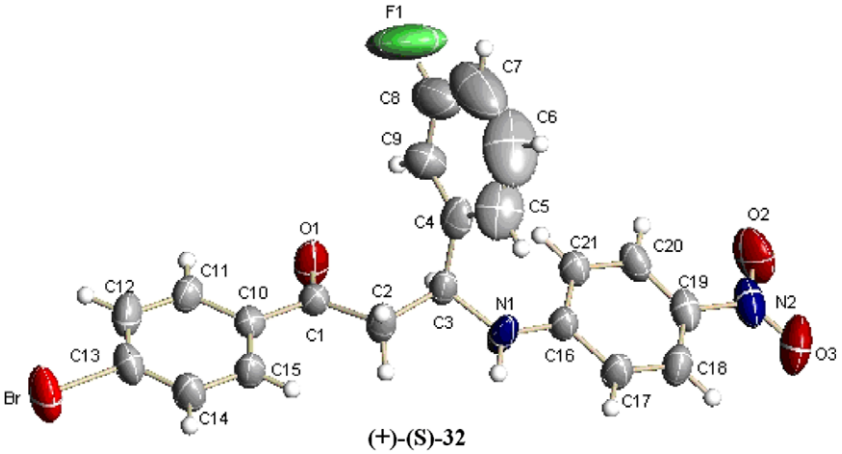


Figure 3. Single crystal X-ray structures of compound (+)-(*S*)-**32**.

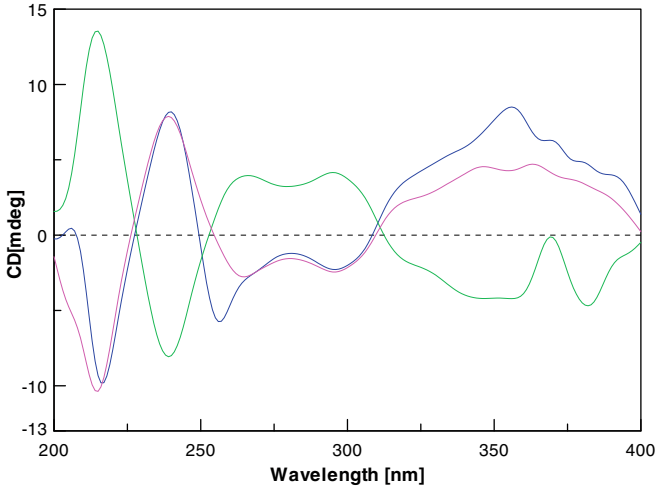
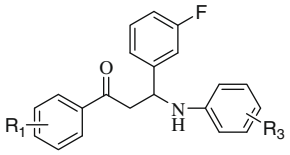


Figure 4. CD curves of compounds (−)-**22** (green), (+)-**22** (purple) and (+)-(*S*)-**32** (blue).

Table 3
hPR-B and hAR[a] binding characteristics of non-steroidal compounds **22**–**26** and SAR of substituted anilines



Compound	R_1	R_3	hPR-B Binding K_i (nM) mean ± SEM	hAR Binding K_i (nM) mean ± SEM	Selectivity hAR/hPR
Progesterone			5.1 ± 0.6	35 ± 7	
DHT				4.7 ± 0.6	
22	4-Me	4-NO_2	54 ± 6	96 ± 9	1.8
23	4-Me	3-NO_2	99 ± 4	203 ± 46	2.0
24	4-Me	4 -CF_3	NA[b]	NA[b]	
25	4-Me	4-F	444 ± 191	322 ± 48	0.7
26	4-Me	3,4-di-F	574 ± 109	1214 ± 397	2.1

[a] Values represent triplicate determinations.
[b] Not active, defined as K_i >10,000 nM.

13 nM. The significant improvement in PR binding affinity of β-cyclohexyl analogue **45**, compared with phenyl analogue **15**, may be explained by the steric effect caused by the change from β-phenyl group to β-cyclohexyl moiety. The β-cyclohexyl analogues with a large group (R_1 = Ph, Cy) substituted at the 4′-position, such as **54** and **55**, also displayed excellent PR selectivity. The SAR from compounds **27**–**55** above suggests that steric effect of group R_1 coordinating with group R_2 can also modulate the potency and selectivity for PR and AR.

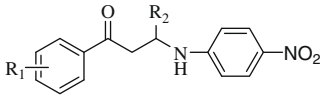
According to the binding activity results, compounds **22**, **26**, **27**, **30**, **41**, **42**, **44**, **46**, **47**, **48** and **50** were chosen for functional characterization (Table 5). In the hPR-B cotransfection assay with CV-1 cells, none of these compounds showed any PR agonist activity. Compound **22** appears to be the most efficacious PR antagonist in the assayed compound with an IC_{50} of 79 nM. The cross-reactivity profile of compound **22** was studied with a panel of steroid receptors. Apart from some moderate AR binding or activation properties, it did not react with hER, hGR and hMR (data shown in Table 6).

An in vivo experiment was conducted to verify the PR antagonist activity of compound **22** and **26** using a murine uterine decidulization assay.[17,18] As shown in Figure 5, control mice showed a dramatic increase in net uterine wet weight gain in response to the decidual stimulus. RU486 blocked this response at a dose of 0.1 mg/day intraluminally, resulting in net increases of only 3.1% of the control value. Compound **22** inhibited deciduomata formation by 83% at a dose of 5.0 mg/day, 65% at a dose of 1.0 mg/day, 45% at a dose of 0.5 mg/day, thereby establishing a dose-dependent effect that nearly reached the maximum at 5.0 mg/day. Compound **26** is structurally similar molecule but is less efficacious/potent than compound **22** in cotransfection/binding assay. It blocked decidualization by 52% at a dose of 5.0 mg/day as well, though it was ineffective at 1.0 mg/day (data not shown), which indicate the effectiveness of this type of scaffold.

There was no significant difference between the *R* and corresponding *S* enantiomers for PR binding affinities (Table 7). Compound (−)-(*R*)-**22** was slightly more active than (+)-(*S*)-**22** enantiomer for binding to both PR and AR.

The docking simulation on PR/mifepristone, PR/(−)-(*R*)-**22** binding was carried out using human PR crystal structure (PDB entry 2OVH) as the model. According the docking prediction (Fig. 6A), the docking model of mifepristone strongly resembled the binding model of Asoprisnil on the PR ligand-binding domain of 2OVH. The key features of the binding mode of mifepristone are summarized as follows: first, hydrogen bonds existed between the A-ring carbonyl group of mifepristone and the side-chains of Gln725 and ARG766 of the receptor. Second, *N*,*N*-dimethyl-aminophenyl group attached to C12 in mifepristone would probably force a displacement of helix 12 and the C-terminal extension, accounting for

Table 4
hPR-B and hAR[a] binding characteristics of non-steroidal compounds **27**–**55**



Compound	R_1	R_2	hPR-B Binding K_i (nM) mean ± SEM	hAR Binding K_i (nM) mean ± SEM	Selectivity hAR/hPR
27	3-Cl	3-FPh	23 ± 4	110 ± 3	4.8
28	3-Br	3-FPh	36 ± 1	124 ± 6	3.4
29	H	3-FPh	49 ± 7	63 ± 21	1.3
30	4-F	3-FPh	68 ± 14	30 ± 9	0.4
31	4-Cl	3-FPh	43 ± 5	18 ± 1	0.4
32	4-Br	3-FPh	39 ± 5	39 ± 1	1.0
33	4-Ph	3-FPh	50 ± 4	417 ± 60	8.3
34	4-Cy	3-FPh	76 ± 23	1010 ± 260	13.3
35	4-Morpholino	3-FPh	78 ± 14	283 ± 23	3.6
36	3-Cl	4-FPh	58 ± 16	493 ± 66	8.5
37	3-Cl	4-CF_3Ph	87 ± 15	>10,000	>100
38	3-F	4-CF_3Ph	47 ± 11	1314 ± 224	28.0
39	3-Me	4-CF_3Ph	85 ± 13	>10,000	>100
40	3-CN	4-CF_3 Ph	56 ± 8	883 ± 259	15.8
41	3-MeO	4-CF_3 Ph	31 ± 10	>10,000	>300
42	4-MeO	4-CF_3 Ph	18 ± 4	320 ± 43	17.8
43	4-Cl	4-CF_3 Ph	80 ± 19	636 ± 142	7.9
44	4-Br	4-CF_3 Ph	66 ± 7	>10,000	>150
45	4-Me	Cy	13 ± 1	115 ± 15	8.8
46	4-MeO	Cy	10.8 ± 1.4	37 ± 1	3.4
47	4-Br	Cy	10.2 ± 0.6	35 ± 10	3.4
48	4-Cl	Cy	6.7 ± 0.8	45 ± 1	6.7
49	4-F	Cy	8.5 ± 0.3	64 ± 10	7.5
50	3-F	Cy	8.5 ± 0.9	64 ± 6	7.5
51	3-MeO	Cy	11.2 ± 0.3	406 ± 78	36.2
52	3,4-di-Cl	Cy	9.1 ± 1.5	140 ± 34	15.3
53	H	Cy	13.0 ± 0.9	74 ± 10	5.7
54	4-Ph	Cy	28 ± 2	>10,000	>350
55	4-Cy	Cy	51 ± 1	>10,000	>196

[a] Values represent triplicate determinations.

the protease sensitivity[19] and increased accessibility of the PR to a presumed repressor.[20–22] The docking of (−)-(*R*)-**22** into the ligand-binding domain of PR together with mifepristone is shown in Figure 6B. It is interesting to note that the *p*-nitro group of (−)-(*R*)-**22** is networked to Gln725 and Arg766, and the *m*-fluorophenyl group and the *p*-methylphenyl group in (−)-(*R*)-**22** are superimposed with the D ring and *N*,*N*-dimethyl-aminophenyl group of mifepristone, respectively. All these factors demonstrate that the isomer (−)-(*R*)-**22** can mimic the interaction mode of mifepristone in the PR ligand-binding pocket.

Table 5
Activities of non-steroidal compounds on hPR-B in CV-1 cell cotransfection assay[a,d]

Compound	Agonist EC_{50} (nM) mean ± SEM	Antagonist IC_{50} (nM) mean ± SEM
Progesterone	15.2 ± 4.8	NA[b]
RU486	NA[b]	0.6 ± 0.1
22	NA[b]	79 ± 58
26	NA[b]	6064 ± 2368
27	NA[b]	3538 ± 1048
30	NA[b]	805[c]
41	NA[b]	1831 ± 811
42	NA[b]	2696 ± 873
44	NA[b]	1213 ± 688
46	NA[b]	139 ± 13
47	NA[b]	1555 ± 197
48	NA[b]	216 ± 85
50	NA[b]	193 ± 43

[a] Values represent triplicate determinations.
[b] Not active; defined as efficacy < 20%, potency >10,000 nM.
[c] Assayed once.

4. Conclusions

A novel structural class of non-steroidal PR antagonists, aromatic β-amino-ketone analogues, were discovered using PR binding and cotransfection assays. After optimizing the substitution groups at aromatic ketone and β-aromatic ring the resulted aromatic β-amino-ketone template significantly improved selectivity

Table 6
Binding affinities, agonist and antagonist cross-reactivities on hAR, hER, hGR, and hMR[a]

		Compound	
		RU486	**22**
Binding affinities K_i (nM) mean ± SEM	hAR	8.4 ± 1.4	96 ± 9
	hERα	NA[b]	NA[b]
	hERβ	2876[c]	NA[b]
	hGR	0.84 ± 0.11	NA[b]
	hMR	NA[b]	NA[b]
Agonist EC_{50} (nM) mean ± SEM	hAR	10 ± 2	NA[b]
	hERα	NA[b]	NA[b]
	hERβ	NA[b]	NA[b]
	hGR	872 ± 106	NA[b]
	hMR	NA[b]	NA[b]
Antagonist IC_{50} (nM) mean ± SEM	hAR	1.0 ± 0.2	140 ± 19
	hERα	>1000	NA[b]
	hERβ	812[b]	NA[b]
	hGR	0.95 ± 0.26	NA[b]
	hMR	>1000	NA[b]

[a] Values represent triplicate determinations.
[b] Not active; defined as efficacy <20%, potency >10,000 nM.
[c] Assayed once.

for PR. Compound **22** manifests itself as a potent PR antagonist both in vitro and in vivo, without observable cross-reactivities with ER, GR and MR. Preliminary structure–activity relationship analysis suggests that this scaffold of aromatic β-amino-ketone may serve as a starting point for development of novel PR antagonists.

5. Experimental section

5.1. Chemistry

Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. All final compounds were purified to >95% purity, as determined by high-performance liquid chromatography (HPLC: A, YMC ODS column, 4.6 × 50 mm, 8 min; flow rate = 2.0 mL/min; gradient = 10–95% 0.1% TFA in CH_3CN/90–5% 0.1% TFA in water; B, YMC ODS column, 4.6 × 50 mm, 8 min; flow rate = 2.0 mL/min; gradient = 10–90% 0.1% TFA in CH_3OH/90–10% 0.1% TFA in water). Chiralpak IA, AD-H columns were produced by Daicel Chemical Industries, LTD. Column chromatography was carried out on silica gel (200–300 mesh). All reactions were monitored using thin-layer chromatography (TLC) on silica gel plates. NMR spectra were determined using a Varian 300 or 400 MHz spectrometer. High-resolution mass spectra (HRMS) were recorded at an ionizing voltage of 70 eV on a Finnigan/MAT95 spectrometer. Elemental analyses were performed on a CE 1106 elemental analyzer. Optical rotations were determined on a Perkin–Elmer 341 polarimeter. Yields were of purified compounds and were not optimized.

5.1.1. 3-(4-Nitrophenylamino)-3-(thiophen-3-yl)-1-*p*-tolylpropan-1-one (13)

4-Nitroaniline (138 mg, 1 mmol) was dissolved or suspended in a absolute EtOH (3 mL), then 4-methylacetophenone (133 μL, 1 mmol) and thiophen-3-carboxaldehyde (88 μL, 1 mmol) was added, followed by saturation ethanol solution of HCl (30 μL) under stirring at room temperature for about 8–24 h. The product was collected by filtration and washed with absolute EtOH and 10% $NaHCO_3$, respectively. The pure product compound **13** (303 mg, 83%) was obtained by recrystallization from absolute EtOH. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.99–8.04 (m, 2H), 7.80 (d, 2H, J = 8.5 Hz), 7.29 (m, 1H), 7.23 (s, 1H), 7.18 (m, 1H), 7.06 (m, 1H), 6.53–6.60 (m, 2H), 5.25 (t, 1H, J = 5.8 Hz), 3.45–3.61 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 197.4, 152.2, 144.8, 142.3, 138.3, 133.9, 129.5 (2C), 128.2 (2C), 127.0, 126.2 (2C), 125.7, 121.4, 111.9 (2C), 50.2, 44.2, 21.7; Anal. ($C_{20}H_{18}N_2O_3S$) C, H, N.

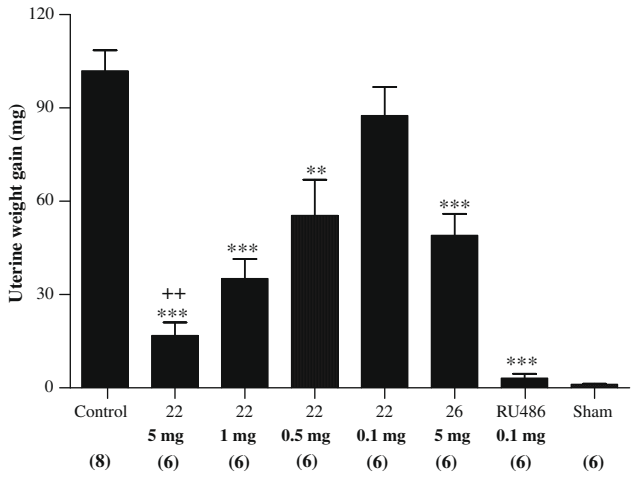


Figure 5. Effect of RU486 (0.1 mg, intrauterine), **22** (0.1 mg, 0.5 mg, 1 mg and 5 mg intrauterine), and **26** (5 mg, intrauterine) on sesame oil-induced uterine decidualization (single dose, day 4 of pseudopregnancy) in BALB/c mice; $^{**}P$ <0.01; $^{***}P$ <0.001 versus control, ^{++}P <0.01 versus **26** (5 mg, intrauterine). Numbers of mice in each group are given in parentheses.

5.1.2. 3-(4-Nitrophenylamino)-1-(4-cyanophenyl)-3-(thiophen-3-yl)propan-1-one (14)

4-Cyanoacetophenone (145 mg, 1 mmol), thiophen-3-carboxaldehyde (88 μL, 1 mmol), 4-nitroaniline (138 mg, 1 mmol), EtOH (3 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **14** (199 mg, 53%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.14 (d, 2H, J = 8.2 Hz), 7.94–8.03 (m, 4H), 7.45–7.52 (m, 2H), 7.19 (m, 1H), 6.64–6.70 (m, 2H), 5.30 (dd, 1H, J = 8.9, 4.1 Hz), 3.80 (dd, 1H, J = 17.9, 9.1 Hz), 3.56 (dd, 1H, J = 17.9, 4.1 Hz); ^{13}C NMR (CD_3COCD_3): δ 196.5, 153.9, 143.9, 140.4, 137.9, 133.1 (2C), 129.2 (2C), 126.9, 126.8, 126.3 (2C), 122.1, 118.3, 116.6, 112.2 (2C), 49.5, 45.8; HRMS (M+, EI) calcd for $C_{20}H_{15}N_3O_3S$: 377.0834; found, 377.0817. HPLC purity = 98.4% (system A), 97.3% (system B).

5.1.3. 3-(4-Nitrophenylamino)-3-phenyl-1-*p*-tolylpropan-1-one (15)

4-Methylacetophenone (133 μL, 1 mmol), benzaldehyde (101 μL, 1 mmol), 4-nitroaniline (138 mg, 1 mmol), EtOH (3 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **15** (219 mg, 61%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.93 (d, 2H, J = 8.7 Hz), 7.88 (d, 2H, J = 8.2 Hz), 7.45 (d, 2H, J = 7.3 Hz), 7.31–7.36 (m, 4H), 7.24 (t, 1H, J = 7.3 Hz), 6.61 (d, 2H, J = 9.1 Hz), 5.16 (dd, 1H, J = 9.0, 4.0 Hz), 3.71 (dd, 1H, J = 16.6, 9.1 Hz), 3.37 (dd, 1H, J = 17.6, 4.2 Hz), 2.37 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 197.5, 152.2, 144.8, 141.2, 138.3, 133.8, 129.5 (2C), 129.1 (2C), 128.3 (2C), 127.8, 126.1 (2C), 126.1 (2C), 112.1 (2C), 54.3, 45.4, 21.7; Anal. ($C_{22}H_{20}N_2O_3$) C, H, N.

5.1.4. 3-(4-Methoxyphenylamino)-3-phenyl-1-*p*-tolylpropan-1-one (16)

p-Anisidine (3 mmol, 369 mg), 4-methylacetophenone (3 mmol, 399 μL), benzaldehyde (3 mmol, 310 μL) EtOH (8 mL), EtOH/HCl (60 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **16** (641 mg, 62%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.70–7.72 (m, 2H), 7.43–7.46 (m, 2H), 7.31–7.37 (m, 4H), 7.23 (m, 1H), 6.66–6.69 (m, 2H), 6.51–6.54 (m, 2H), 4.92 (dd, 1H, J = 7.9, 5.2 Hz), 3.68 (s, 3H), 3.47 (dd, 1H, J = 16.2, 5.0 Hz), 3.38 (dd, 1H, J = 16.2, 7.8 Hz), 2.39 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 198.6, 152.2, 143.3, 141.2, 138.5, 136.7, 134.1, 128.8 (2C), 128.7, 128.5, 127.2, 126.4 (2C), 125.4, 115.3 (2C), 114.6 (2C), 55.6 (2C), 46.5, 21.3; HRMS (M+, EI) calcd for $C_{23}H_{23}NO_2$: 345.1729; found: 345.1729; HPLC purity = 99.2% (system A), 99.1% (system B).

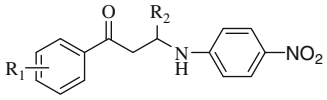
5.1.5. 3-(4-Nitrophenylamino)-3-(3,4,5-trimethoxyphenyl)-1-*p*-tolylpropan-1-one (17)

4-Methylacetophenone (133 μL, 1 mmol), 3,4,5-trimethoxybenzaldehyde (196 mg, 1 mmol), 4-nitro-benzenamine (138 mg, 1 mmol), EtOH (2 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **17** (315 mg, 70%). ^{1}H NMR (DMSO-d_6, 300 MHz): 7.95–7.98 (d, 2H, J = 9.1 Hz), 7.88–7.91 (d, 2H, J = 7.8 Hz), 7.33–7.36 (d, 2H, J = 8.2 Hz), 6.80 (s, 2H), 6.65–6.68 (d, 2H, J = 9.2 Hz), 5.10 (dd, 1H, J = 9.0, 3.3 Hz), 3.76 (s, 6H), 3.70 (dd, 1H, J = 17.7, 9.4 Hz), 3.62 (s, 3H), 3.35 (dd, 1H, J = 17.5, 3.8 Hz), 2.38 (s, 3H); Anal. ($C_{25}H_{26}N_2O_6$) C, H, N.

5.1.6. 3-(4-Nitrophenylamino)-3-(3,4,5-trimethoxyphenyl)-1-*m*-tolylpropan-1-one (18)

3-Methylacetophenone (133 μL, 1 mmol), 3,4,5-trimethoxybenzaldehyde (196 mg, 1 mmol), 4-nitro-benzenamine (138 mg,

Table 7
Binding affinities of *R* and *S* enantiomers for hPR-B and hAR[a]



Compound	R_1	R_2	hPR-B Binding K_i (nM) mean ± SEM	hAR Binding K_i (nM) mean ± SEM	Selectivity hAR/hPR
(−)-(*R*)-**22**	4-Me	3-FPh	35 ± 4	24.2 ± 0.8	0.69
(+)-(*S*)-**22**	4-Me	3-FPh	84 ± 15	342 ± 36	4.1

[a] Values represent triplicate determinations.

1 mmol), EtOH (2 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **18** (301 mg, 67%). ^{1}H NMR (DMSO-d_6, 300 MHz): 7.96–7.99 (d, 2H, J = 9.3 Hz), 7.78–7.80 (d, 2H, J = 7.1 Hz), 7.39–7.49 (m, 2H), 6.79 (s, 2H), 6.65–6.68 (d, 2H, J = 9.5 Hz), 5.10 (dd, 1H, J = 9.3, 3.8 Hz), 3.80 (s, 6H), 3.79 (s, 3H), 3.69 (d, 1H, J = 9.4 Hz), 3.38 (dd, 1H, J = 17.6, 3.8 Hz), 2.38 (s, 3H); Anal. ($C_{25}H_{26}N_2O_6$) C, H, N.

5.1.7. 3-(4-Nitrophenylamino)-1-(3-fluorophenyl)-3-(3,4,5-trimethoxyphenyl)propan-1-one (19)

3-Fluoroacetophenone (123 μL, 1 mmol), 3,4,5-trimethoxybenzaldehyde (196 mg, 1 mmol), 4-nitro-benzenamine (138 mg, 1 mmol), EtOH (2 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **19** (327 mg, 72%). ^{1}H NMR (DMSO-d_6, 300 MHz): 7.96–7.99 (m, 2H), 7.86 (d, 1H, J = 6.9 Hz), 7.77 (m, 1H), 7.49–7.64 (m, 2H), 6.81 (s, 2H), 6.66–6.69 (d, 2H, J = 9.1 Hz), 5.11 (dd, 1H, J = 9.2, 3.6 Hz), 3.77 (s, 6H), 3.75 (dd, 1H, J = 17.7, 9.6 Hz), 3.63 (s, 3H), 3.45 (d, 1H, J = 3.5 Hz); Anal. ($C_{24}H_{23}FN_2O_6$) C, H, N.

5.1.8. 3-(4-Nitrophenylamino)-3-(3-fluorophenyl)-1-*m*-tolylpropan-1-one (20)

3-Methylacetophenone (340 μL, 2.5 mmol), 3-fluorobenzaldehyde (265 μL, 2.5 mmol), 4-nitroaniline (345 mg, 2.5 mmol), EtOH (8 mL), EtOH/HCl (60 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **20** (907 mg, 96%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.93–7.97 (d, 2H, J = 9.2 Hz), 7.74–7.80 (m, 2H), 7.36–7.48 (m, 3H), 7.29–7.33 (t, 2H, J = 9.3 Hz), 7.05–7.11 (q, 1H, J = 7.8, 1.9 Hz), 6.62–6.65 (d, 2H, J = 9.4 Hz), 5.20 (dd, 1H, J = 8.9, 3.9 Hz), 3.75 (dd, 1H, J = 18.0, 9.1 Hz), 3.42 (dd, 1H, J = 17.6, 4.0 Hz), 2.37 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 196.6, 162.4 (d, J_{c-f} = 241.8 Hz), 153.5, 145.8 (d, J_{c-f} = 6.4 Hz), 138.3, 136.6, 136.4, 134.1, 130.7 (d, J_{c-f} = 8.1 Hz), 128.7, 128.6, 126.1 (2C), 125.3, 122.8, 114.1 (d, J_{c-f} = 20.9 Hz), 113.5 (d, J_{c-f} = 21.8 Hz), 111.7 (2C), 51.9, 45.7, 20.9; Anal. ($C_{22}H_{19}FN_2O_3$) C, H, N.

5.1.9. 3-(4-Nitrophenylamino)-1,3-bis(3-fluorophenyl)propan-1-one (21)

3-Fluoroacetophenone (308 μL, 2.5 mmol), 3-fluorobenzaldehyde (266 μL, 2.5 mmol), 4-nitroaniline (345 mg, 2.5 mmol), EtOH (8 mL), EtOH/HCl (60 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **21** (845 mg, 89%) as a yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.95 (d, 2H, J = 9.0 Hz), 7.84 (d, 1H, J = 7.6 Hz), 7.76 (d, 1H, J = 9.2 Hz), 7.48–7.63 (m, 2H), 7.29–7.44 (m, 3H), 7.05–7.11 (m, 1H), 6.64 (d, 2H, J = 8.9 Hz), 5.20 (dd, 1H, J = 9.6, 4.9 Hz), 3.77 (dd, 1H, J = 18.4, 9.4 Hz), 3.42 (m, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 195.7, 162.5 (d, J_{c-f} = 242.3 Hz), 162.3 (d, J_{c-f} = 244.1 Hz), 153.6, 145.6 (d, J_{c-f} = 5.9 Hz), 138.8 (d, J_{c-f} = 6.1 Hz), 136.5, 131.1 (d, J_{c-f} = 7.3 Hz), 130.8 (d, J_{c-f} = 7.7 Hz), 126.2 (2C), 124.5, 122.9, 120.4 (d, J_{c-f} = 21.4 Hz), 114.7 (d, J_{c-f} = 24.1 Hz), 114.2, (d, J_{c-f} = 20.5 Hz), 113.6 (d, J_{c-f} = 21.8 Hz), 111.8 (2C), 52.0, 45.8; Anal. ($C_{21}H_{16}F_2N_2O_3$) C, H, N.

5.1.10. 3-(4-Nitrophenylamino)-3-(3-fluorophenyl)-1-*p*-tolylpropan-1-one (22)

4-Methylacetophenone (2.67 mL, 20 mmol), 3-fluorobenzaldehyde (2.12 mL, 20 mmol), 4-nitroaniline (2.76 g, 20 mmol), EtOH (60 mL), EtOH/HCl (300 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **22** (6.08 g, 80%) as a yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.96 (d, 2H, J = 9.1 Hz), 7.89 (d, 2H, J = 8.2 Hz), 7.29–7.43 (m, 5H), 7.04–7.11 (m, 1H), 6.63–6.68 (m, 2H), 5.21 (dd, 1H, J = 9.1, 4.2 Hz), 3.73 (dd, 1H, J = 17.5, 9.1 Hz), 3.41 (dd, 1H, J = 17.8,4.4 Hz), 2.38 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 196.0, 162.4 (d, J_{c-f} = 242.7 Hz), 153.5, 145.8 (d, J_{c-f} = 6.9 Hz), 143.9, 136.3, 134.1, 130.7 (d, J_{c-f} = 7.8 Hz), 129.4 (2C), 128.3 (2C), 126.2 (2C), 122.8, 114.1 (d, J_{c-f} = 21 Hz), 113.5 (d, J_{c-f} = 21.9 Hz), 111.7 (2C), 51.9, 45.5, 21.3; Anal. ($C_{22}H_{19}FN_2O_3$) C, H, N.

5.1.11. 3-(3-Nitrophenylamino)-3-(3-fluorophenyl)-1-*p*-tolylpropan-1-one (23)

4-Methylacetophenone (133 μL, 1 mmol), 3-fluorobenzaldehyde (106 μL, 1 mmol), 3-nitroaniline (138 mg, 1 mmol), EtOH (3 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **23** (195 mg, 51%) as a yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.27 (d, 1H, J = 7.7 Hz), 8.21 (s, 1H), 8.02 (d, 1H, J = 8.1 Hz), 7.92–7.96 (m 2H), 7.79 (t, 1H, J = 8.0 Hz), 7.35 (d, 2H, J = 7.5 Hz), 7.15 (d, 2H, J = 7.5 Hz), 6.59–6.62 (t, 2H, J = 8.4 Hz), 5.13 (dd, 1H, J = 9.0, 4.0 Hz), 3.80 (dd, 1H, J = 18.0, 9.2 Hz), 3.51 (d, 1H, J = 3.6 Hz), 2.26 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 197.2, 163.2 (d, J_{c-f} = 245.4 Hz), 149.1, 147.6, 144.9, 144.6 (d, J_{c-f} = 6.3 Hz), 133.8, 130.6 (d, J_{c-f} = 8.2 Hz), 129.7, 129.5 (2C), 128.3 (2C), 121.9, 119.5, 114.6 (d, J_{c-f} = 21.4 Hz), 113.1 (d, J_{c-f} = 21.9 Hz), 112.6, 107.8, 54.3, 45.7, 21.7; Anal. ($C_{22}H_{19}FN_2O_3$) C, H, N.

5.1.12. 3-(4-(Trifluoromethyl)phenylamino)-3-(3-fluorophenyl)-1-*p*-tolylpropan-1-one (24)

4-Methylacetophenone (333 μL, 2.5 mmol), 3-fluorobenzaldehyde (265 μL, 2.5 mmol), 4-(trifluoro-methyl)-benzenamine (308 μL, 2.5 mmol), EtOH (6 mL), EtOH/HCl (60 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **24** (831 mg, 83%) as a white solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.88–7.91 (d, 2H, J = 8.1 Hz), 7.29–7.41 (m, 7H), 7.05 (m, 1H), 6.63–6.66 (d, 2H, J = 8.6 Hz), 5.10 (dd, 1H, J = 8.9, 4.1 Hz), 3.67 (dd, 1H, J = 17.4, 8.9 Hz), 3.34 (dd, 1H, J = 17.1, 4.3 Hz), 2.39 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 196.9, 162.8 (d, J_{c-f} = 245 Hz), 148.9, 144.7 (d, J_{c-f} = 6.4 Hz), 144.5, 133.5, 130.2 (d, J_{c-f} = 8.2 Hz), 129.2 (2C), 127.9 (2C), 126.1 (2C), 125.8, 121.5, 119.1 (q, J_{c-f} = 32.4 Hz), 114.2 (d, J_{c-f} = 21.4 Hz), 112.8 (d, J_{c-f} = 21.9 Hz), 112.6 (2C), 53.7, 45.3, 21.4; HRMS (M+, EI) calcd for $C_{23}H_{19}F_4NO$: 401.1403; found: 401.1405; HPLC purity = 100% (system A), 98.4% (system B).

5.1.13. 3-(4-Fluorophenylamino)-3-(3-fluorophenyl)-1-*p*-tolylpropan-1-one (25)

4-Methylacetophenone (133 μL, 1 mmol), 3-fluorobenzaldehyde (106 μL, 1 mmol), 4-fluoroaniline (96 μL, 1 mmol), EtOH (3 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **25** (173 mg, 59%) as a white solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.86–7.88 (d, 2H, J = 8.1 Hz), 7.26–7.38 (m, 5H), 7.00 (m, 1H), 6.84 (t, 2H, J = 8.7 Hz), 6.48–6.52 (m, 2H), 4.97 (dd, 1H, J = 8.6, 4.7 Hz), 3.58 (dd, 1H, J = 17.0, 8.8 Hz), 3.26 (dd, 1H, J = 17.2, 4.8 Hz), 2.38 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 196.8, 162.4 (d, J_{c-f} = 241.8 Hz), 154.5 (d, J_{c-f} = 230 Hz), 147.2 (d, J_{c-f} = 6.4 Hz), 144.3,

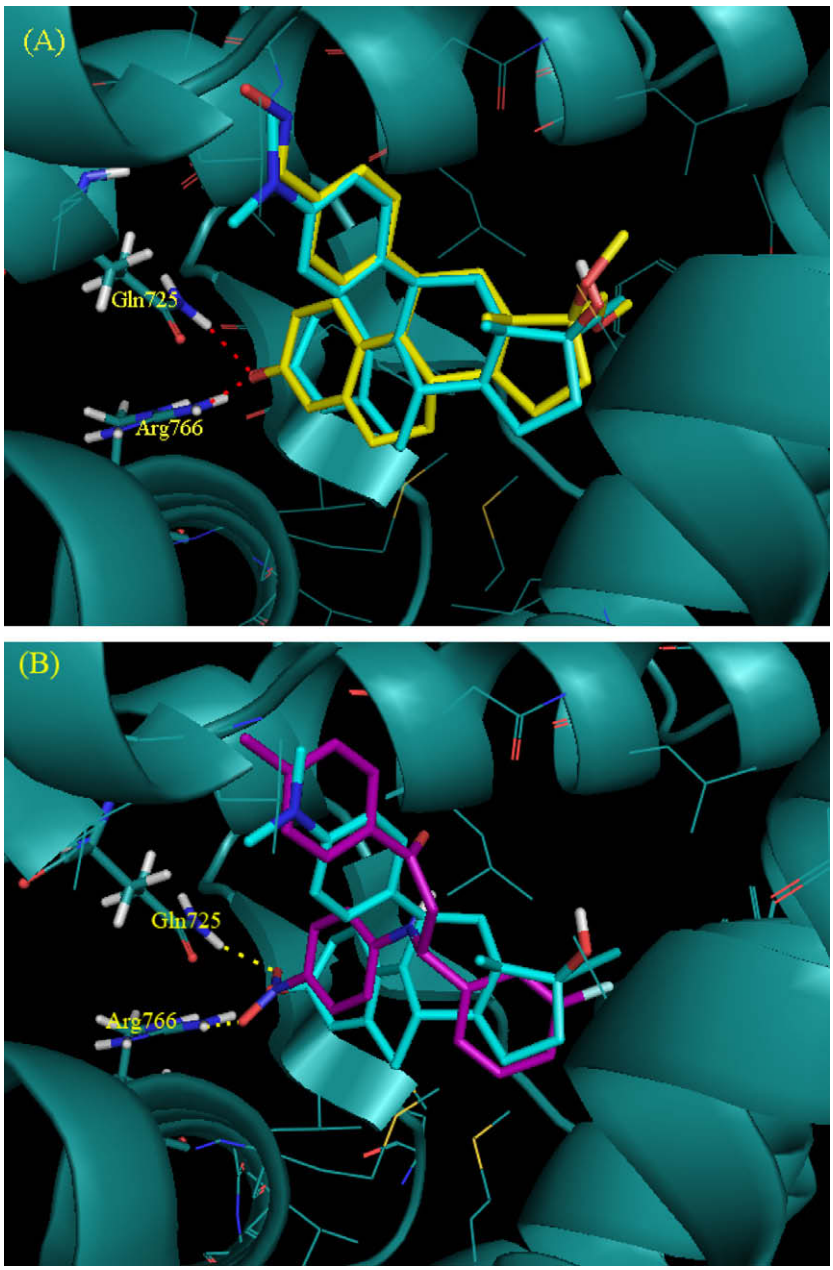


Figure 6. A The docking model of mifepristone (cyan) on the progesterone receptor ligand-binding domain of 2OVH bound with asoprisnil (yellow). Hydrogen bonding interactions are shown with dotted red lines. B Superimposition of the docking models of mifepristone (cyan) and (−)-(*R*)-**22** (pink) on the progesterone receptor ligand-binding domain of 2OVH. Hydrogen bonding interactions are shown with dotted yellow lines. These images were generated using the PyMol program (http://www.pymol.org/).

143.8, 134.3, 130.3 (d, J_{c-f} = 8.2 Hz), 129.4 (2C), 128.3 (2C), 123.0, 115.3 (2C, d, J_{c-f} = 21.9 Hz), 113.8 (2C, d, J_{c-f} = 6.9 Hz), 113.6, 113.5, 53.1, 46.1, 21.2; Anal. ($C_{22}H_{19}F_2NO$) C, H, N.

5.1.14. 3-(3,4-Difluorophenylamino)-3-(3-fluorophenyl)-1-*p*-tolylpropan-1-one (26)

4-Methylacetophenone (133 μL, 1 mmol), 3-fluorobenzaldehyde (106 μL, 1 mmol), 3,4-difluoroaniline (99 μL, 1 mmol), EtOH (3 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **26** (177.6 mg, 48%) as a pale white solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.85–7.88 (d, 2H, J = 8.2 Hz), 7.27–7.39 (m, 5H), 6.99–7.08 (m, 2H), 6.46 (m, 1H), 6.30 (t, 1H, J = 5.3 Hz), 4.97 (dd, 1H, J = 8.8, 4.3 Hz), 3.59 (dd, 1H, J = 16.9, 8.7 Hz), 3.28 (dd, 1H, J = 16.9, 4.4 Hz), 2.37 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 197.3, 162.2 (d, J_{c-f} = 245.4 Hz), 150.6 (dd, J_{c-f} = 230 Hz), 145.3 (d, J_{c-f} = 6.4 Hz), 144.7, 143.9 (d, J = Hz), 143.3 (dd, J_{c-f} = 223.1 Hz), 133.9, 130.5 (d, J_{c-f} = 7.8 Hz), 129.5 (2C), 128.3 (2C), 121.9, 117.3 (d, J_{c-f} = 16.9 Hz), 114.5 (d, J_{c-f} = 20.9 Hz), 113.2 (d, J_{c-f} = 21.9 Hz), 109.0, 102.6 (d, J_{c-f} = 20.9 Hz), 54.9, 45.8, 21.7; HRMS (M+, EI) calcd for $C_{22}H_{18}F_3NO$: 369.1341; found: 369.1345; HPLC purity = 100% (system A), 98.9% (system B).

5.1.15. 3-(4-Nitrophenylamino)-1-(3-chlorophenyl)-3-(3-fluorophenyl)propan-1-one (27)

3-Chloroacetophenone (325 μL, 2.5 mmol), 3-fluorobenzaldehyde (266 μL, 2.5 mmol), 4-nitroaniline (345 mg, 2.5 mmol), EtOH (8 mL), EtOH/HCl (60 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **27** (859 mg, 86%) as a yellow solid. ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 7.97–8.01 (m,

4H), 7.68 (m, 1H), 7.57 (m, 1H), 7.33–7.43 (m, 3H), 7.04 (m, 1H), 6.72–6.74 (m, 2H), 5.36 (dd, 1H, J = 8.7, 4.2 Hz), 3.88 (dd, 1H, J = 18.0, 9.0 Hz), 3.59 (dd, 1H, J = 17.9, 4.2 Hz); ^{13}C NMR (CD_3COCD_3, 100 MHz): δ 195.6, 163.5 (d, J_{c-f} = 243.2 Hz), 153.8, 146.2 (d, J_{c-f} = 6.4 Hz), 139.1, 138.0, 134.8, 133.5, 131.0 (3C), 128.3 (d, J_{c-f} = 13.6 Hz), 127.1, 126.3, 123.2, 114.5 (d, J_{c-f} = 20.9 Hz), 114.0 (d, J_{c-f} = 21.8 Hz), 112.3 (2C), 53.0, 46.4; Anal. ($C_{21}H_{16}ClFN_2O_3$) C, H, N.

5.1.16. 3-(4-Nitrophenylamino)-1-(3-bromophenyl)-3-(3-fluorophenyl)propan-1-one (28)

3-Methylacetophenone (134 μL, 1 mmol), 3-fluorobenzaldehyde (106 μL, 1 mmol), 4-nitroaniline (138 mg, 1 mmol), EtOH (3 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **28** (363 mg, 83%) as a yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.13 (s, 1H), 7.95–8.00 (m, 3H), 7.86 (m, 1H), 7.51 (t, 1H, J = 8.0 Hz), 7.39 (q, 1H, J = 8.1, 6.1 Hz), 7.30–7.33 (d, 2H, J = 7.9 Hz), 7.08 (m, 1H), 6.62–6.65 (d, 2H, J = 9.3 Hz), 5.19 (dd, 1H, J = 9.4, 4.0 Hz), 3.78 (dd, 1H, J = 18.3, 9.8 Hz), 3.46 (dd, 1H, J = 18.0, 3.9 Hz); ^{13}C NMR (DMSO-d$_6$, 100 MHz) δ 195.5, 162.4 (d, J_{c-f} = 242.3 Hz), 153.4, 145.6 (d, J_{c-f} = 6.3 Hz), 138.5, 136.3, 136.0, 131.0 (2C), 130.6 (d, J_{c-f} = 8.6 Hz), 127.1, 126.1 (2C), 122.7, 122.2, 114.1 (d, J_{c-f} = 21.4 Hz), 113.5 (d, J_{c-f} = 21.4 Hz), 111.6 (2C), 51.7, 45.7; Anal. ($C_{21}H_{16}BrFN_2O_3$) C, H, N.

5.1.17. 3-(4-Nitrophenylamino)-3-(3-fluorophenyl)-1-phenylpropan-1-one (29)

Acetophenone (117 μL, 1 mmol), 3-fluorobenzaldehyde (106 μL, 1 mmol), 4-nitroaniline (138 mg, 1 mmol), EtOH (3 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **29** (245 mg, 67%) as a yellow soild. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.95–8.01 (q, 4H, J = 9.3, 6.9 Hz), 7.67 (m, 1H), 7.53–7.57 (t, 2H, J = 8.0 Hz), 7.31–7.44 (m, 3H), 7.09 (m, 1H), 6.64–6.67 (d, 2H, J = 9.5 Hz), 5.23 (dd, 1H, J = 9.4, 4.1 Hz), 3.77 (dd, 1H, J = 18.0, 9.5 Hz), 3.48 (d, 1H, J = 4.1 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 196.6, 162.4 (d, J_{c-f} = 242.3 Hz)), 153.5, 145.8 (d, J_{c-f} = 6.3 Hz), 136.5, 136.4, 133.5, 130.6 (d, J_{c-f} = 7.8 Hz), 128.8 (2C), 128.2 (2C), 126.2 (2C), 122.8, 114.1 (d, J_{c-f} = 20.9 Hz), 113.5 (d, J_{c-f} = 21.4 Hz), 111.7 (2C), 51.9, 45.6; HRMS (M+, EI) calcd for $C_{21}H_{17}N_2FO_3$: 364.1223 (M+); found: 364.1208; HPLC purity = 100% (system A), 95.2% (system B).

5.1.18. 3-(4-Nitrophenylamino)-3-(3-fluorophenyl)-1-(4-fluorophenyl)propan-1-one (30)

4-Flluoroacetophenone (63 μL, 0.5 mmol), 3-fluorobenzaldehyde (55 μL, 0.5 mmol), 4-nitroaniline (69 mg, 0.5 mmol), EtOH (1.5 mL), EtOH/HCl (20 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **30** (97 mg, 51%) as a yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.05–8.10 (m, 2H), 7.96 (d, 2H, J = 9.5 Hz), 7.28–7.41 (m, 5H), 7.04–7.11 (m, 1H), 6.64 (d, 2H, J = 9.1 Hz), 5.20 (dd, 1H, J = 9.0, 3.9 Hz), 3.75 (dd, 1H, J = 17.8, 9.3 Hz), 3.41 (d, 1H, J = 4.1 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 195.8, 166.1 (d, J_{c-f} = 255.1 Hz), 163.2 (d, J_{c-f} = 245.6 Hz), 151.8, 143.7 (d, J_{c-f} = 6.4 Hz), 138.6, 132.6, 130.9, 130.8, 130.7, 126.2 (2C), 121.8, 116.1, 115.9, 114.9 (d, J_{c-f} = 20.9 Hz), 113.1 (d, J_{c-f} = 21.9 Hz), 112.1 (2C), 53.7, 45.1; Anal. ($C_{21}H_{16}F_2N_2O_3$) C, H, N.

5.1.19. 3-(4-Nitrophenylamino)-1-(4-chlorophenyl)-3-(3-fluorophenyl)propan-1-one (31)

4-Chloroacetophenone (325 μL, 2.5 mmol), 3-fluorobenzaldehyde (266 μL, 2.5 mmol), 4-nitroaniline (345 mg, 2.5 mmol), EtOH (8 mL), EtOH/HCl (60 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **31** (891 mg, 90%) as a yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.94–8.02 (m, 4H), 7.76 (d, NH), 7.59–7.62 (t, 2H, J = 8.5 Hz), 7.40 (m, 1H), 7.29–7.33 (m, 2H), 7.05–7.11 (m, 1H), 6.62–6.65 (d, 2H, J = 9.1 Hz), 5.20 (dd, 1H, J = 8.7, 4.0 Hz), 3.75 (dd, 1H, J = 17.8, 9.0 Hz), 3.45 (dd, 1H, J = 17.8, 3.9 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 195.6, 162.5 (d, J_{c-f} = 242.3 Hz), 153.5 (d, J_{c-f} = 9.1 Hz), 145.7 (d, J_{c-f} = 6.4 Hz), 138.5, 136.3, 135.2, 130.7 (d, J_{c-f} = 8.2 Hz), 130.2 (2C), 128.9 (2C), 126.2, 122.8, 114.2 (d, J_{c-f} = 20.5 Hz), 113.5 (d, J_{c-f} = 21.9 Hz), 111.6 (2C), 51.9, 45.7; Anal. ($C_{21}H_{16}ClFN_2O_3$) C, H, N.

5.1.20. 3-(4-Nitrophenylamino)-1-(4-bromophenyl)-3-(3-fluorophenyl)propan-1-one (32)

3-bromoacetophenone (498 mg, 2.5 mmol), 3-fluorobenzaldehyde (266 μL, 2.5 mmol), 4-nitroaniline (345 mg, 2.5 mmol), EtOH (8 mL), EtOH/HCl (60 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **32** (1.03 g, 93%) as a yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.92–7.98 (t, 4H, J = 9.1 Hz), 7.74–7.77 (d, 2H, J = 8.5 Hz), 7.39 (m, 1H), 7.29–7.33 (t, 2H, J = 9.4 Hz), 7.09 (t, 1H, J = 8.6 Hz), 6.61–6.66 (d, 2H, J = 9.2 Hz), 5.19 (dd, 1H, J = 9.1, 4.4 Hz), 3.75 (dd, 1H, J = 18.1, 9.3 Hz), 3.43 (dd, 1H, J = 17.2, 3.6 Hz); ^{13}C NMR (CD_3COCD_3, 100 MHz): δ 195.9, 163.5 (d, J_{c-f} = 242.7 Hz), 153.8, 146.2 (d, J_{c-f} = 6.3 Hz), 137.9, 136.2, 132.3 (2C), 131.1131.0, 130.5, 128.2126.3, 123.1 (2C), 114.5 (d, J_{c-f} = 21.4 Hz), 114.0 (d, J_{c-f} = 22.3 Hz), 112.3 (2C), 53.0, 46.3; Anal. ($C_{21}H_{16}BrFN_2O_3$) C, H, N.

5.1.21. 3-(4-Nitrophenylamino)-1-(4-phenyl-phenyl)-3-(3-fluorophenyl)propan-1-one (33)

4-acetylbiphenyl (134 μL, 1 mmol), 3-fluorobenzaldehyde (106 μL, 1 mmol), 4-nitroaniline (138 mg, 1 mmol), EtOH (3 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **33** (298 mg, 68%) as a yellow soild. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.08 (d, 2H, J = 8.4 Hz),7.97 (d, 2H, J = 9.2 Hz),7.85(d, 2H, J = 8.4 Hz), 7.76 (d, 2H, J = 7.7 Hz), 7.31–7.54 (m, 6H), 7.08 (t, 1H, J = 9.0 Hz), 6.66 (d, 2H, J = 9.0 Hz), 5.25 (dd, 1H, J = 9.0, 3.9 Hz), 3.80 (dd, 1H, J = 17.5, 9.0 Hz), 3.50 (d, 1H, J = 3.9 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 197.0, 163.1 (d, J_{c-f} = 245.9 Hz), 151.9, 146.5, 144.0 (d, J_{c-f} = 6.4 Hz), 139.4, 138.5, 134.8, 130.7 (d, J_{c-f} = 7.8 Hz), 129.0 (2C),128.8 (2C), 128.5, 127.4 (2C), 127.3 (2C), 126.2 (2C), 121.8, 114.8 (d, J_{c-f} = 21 Hz), 113.0 (d, J_{c-f} = 21.9 Hz), 112.2 (2C), 53.8, 45.3; Anal. ($C_{27}H_{21}FN_2O_3$) C, H, N.

5.1.22. 3-(4-Nitrophenylamino)-1-(4-cyclohexylphenyl)-3-(3-fluorophenyl)propan-1-one (34)

4-Cyclohexylacetophenone (202 mg, 1 mmol), 3-fluorobenzaldehyde (106 μL, 1 mmol), 4-nitroaniline (138 mg, 1 mmol), EtOH (3 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **34** (367 mg, 82%) as a yellow soild. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.94–7.97 (d, 2H, J = 9.3 Hz), 7.90–7.93 (d, 2H, J = 8.2 Hz), 7.29–7.41 (m, 5H), 7.07 (m, 1H), 6.62–6.65 (d, 2H, J = 9.6 Hz), 5.21 (dd, 1H, J = 8.8, 3.8 Hz), 3.73 (dd, 1H, J = 17.8, 9.2 Hz), 3.37 (d, 1H, J = 4.3 Hz), 1.69–1.80 (m, 5H), 1.25–1.44 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 197.1, 163.1 (d, J_{c-f} = 245.4 Hz), 154.8, 151.9, 144.0 (d, J_{c-f} = 6.4 Hz), 138.5, 134.0, 130.7 (d, J_{c-f} = 8.2 Hz), 128.4 (2C), 127.3 (2C), 126.1 (2C), 121.8, 114.8 (d, J_{c-f} = 20.9 Hz), 113.1 (d, J_{c-f} = 21.8 Hz), 112.2 (2C), 53.9, 45.1, 44.7, 33.9 (2C), 26.6 (2C), 25.9; Anal. ($C_{27}H_{27}FN_2O_3$) C, H, N.

5.1.23. 3-(4-Nitrophenylamino)-3-(3-fluorophenyl)-1-(4-morpholinophenyl)propan-1-one (35)

4-morpholinoacetophenone (205 mg, 1 mmol), 3-fluorobenzaldehyde (106 μL, 1 mmol), 4-nitroaniline (138 mg, 1 mmol), EtOH (3 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **35** (400 mg, 89%) as a yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.94–7.97 (d,

2H, J = 9.2 Hz), 7.85–7.88 (d, 2H, J = 8.9 Hz), 7.28–7.43 (m, 3H), 7.07 (m, 1H), 6.98–7.01 (d, 2H, J = 8.7 Hz), 6.63–6.66 (d, 2H, J = 9.0 Hz), 5.20 (dd, 1H, J = 8.5, 4.1 Hz), 3.72–3.75 (t, 4H, J = 4.4 Hz), 3.65 (dd, 1H, J = 17.2, 8.8 Hz), 3.34 (d, 1H, J = 4.4 Hz), 3.27–3.32 (t, 4H, J = 4.8 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 194.2, 162.4 (d, J_{c-f} = 242.3 Hz), 154.3, 153.5, 146.0 (d, J_{c-f} = 6.3 Hz), 136.3, 130.7 (d, J_{c-f} = 7.7 Hz), 130.1 (2C), 126.6, 126.2, 122.8, 114.1 (d, J_{c-f} = 21 Hz), 113.5 (d, J_{c-f} = 21.9 Hz), 113.1(2C), 111.7 (2C), 65.9 (2C), 52.2, 46.9 (2C), 44.9; Anal. ($C_{25}H_{24}FN_3O_4$) C, H, N.

5.1.24. 3-(4-Nitrophenylamino)-1-(3-chlorophenyl)-3-(4-fluorophenyl)propan-1-one (36)

3-chloroacetophenone (130 μL, 1 mmol), 4-fluorobenzaldehyde (108 μL, 1 mmol), 4-nitroaniline (138 mg, 1 mmol), EtOH (3 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **36** (235 mg, 59%) as a yellow solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.02 (t, 1H, J = 2.0 Hz), 7.96–7.98(m, 3H), 7.80 (br, NH), 7.74 (m, 1H), 7.59 (t, 1H, J = 7.9 Hz), 7.52–7.55 (m, 2H), 7.18–7.22 (m, 2H), 6.64 (d, 2H, J = 9.3 Hz), 5.19 (dd, 1H, J = 9.1, 4.0 Hz), 3.79 (dd, 1H, J = 18.0, 9.3 Hz), 3.45 (dd, 1H, J = 17.9, 3.8 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 195.7, 161.4 (d, J_{c-f} = 241.8 Hz), 153.4, 138.5, 138.4, 136.2, 133.8, 133.1, 130.8, 128.6 (2C, d, J_{c-f} = 8.2 Hz), 127.9, 126.7, 126.1 (2C), 115.3 (2C, d, J_{c-f} = 21.4 Hz), 111.6 (2C), 51.6, 45.9; Anal. ($C_{21}H_{16}ClFN_2O_3$) C, H, N.

5.1.25. 3-(4-Nitrophenylamino)-1-(3-chlorophenyl)-3-(4-(trifluoromethyl)phenyl)propan-1-one (37)

3-Choloroacetophenone (324 μL, 2.5 mmol), 4-trifluoromethylbenzaldehyde (343 μL, 2.5 mmol), 4-nitroaniline (345 mg, 2.5 mmol), EtOH (8 mL), EtOH/HCl (60 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **37** (326 mg, 81%) as a yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.94–8.01 (m, 4H), 7.69–7.75 (m, 5H), 7.58 (t, 1H, J = 7.8 Hz), 6.62–6.65 (d, 2H, J = 9.2 Hz), 5.27 (dd, 1H, J = 9.2, 3.6 Hz), 3.81 (dd, 1H, J = 17.7, 9.0 Hz), 3.50 (dd, 1H, J = 18.0, 3.8 Hz); ^{13}C NMR (CD_3COCD_3, 100 MHz): δ 195.5, 153.6, 147.8, 139.0, 138.1, 134.8, 133.5, 131.0, 129.4 (q, J_{c-f} = 32 Hz), 128.3, 128.1 (2C), 127.0, 126.3 (2C), 126.1, 126.0, 123.6, 112.3 (2C), 53.1, 46.3; HRMS (M+, EI) calcd for $C_{22}H_{16}ClF_3N_2O_3$: 448.0802; found: 448.0799. HPLC purity = 98.1% (system A), 99.0% (system B).

5.1.26. 3-(4-Nitrophenylamino)-3-(4-(trifluoromethyl)phenyl)-1-(3-fluorophenyl)propan-1-one (38)

3-Fluoroacetophenone (123 μL, 1 mmol), 4-trifluoromethylbenzaldehyde (137 μL, 1 mmol), 4-nitroaniline (138 mg, 1 mmol), EtOH (3 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **38** (205 mg, 48%) as a yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.95–7.98 (d, 2H, J = 9.6 Hz), 7.86 (d, 1H, J = 7.6 Hz), 7.69–7.79 (m 5H), 7.49–7.64 (m, 2H), 6.62–6.65 (d, 2H, J = 9.3 Hz), 5.27 (t, 1H, J = 8.7 Hz), 3.81 (dd, 1H, J = 17.8, 9.1 Hz), 3.51 (dd, 1H, J = 17.9, 3.5 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 195.5, 162.3 (d, J_{c-f} = 243.7 Hz), 153.4, 147.4, 138.6 (d, J_{c-f} = 5.9 Hz), 136.4, 131.0 (d, J_{c-f} = 8.2 Hz), 128.5(q, J_{c-f} = 31.5 Hz), 127.6 (2C), 126.2 (2C), 125.6 (2C), 124.4, 122.9, 120.5 (d, J_{c-f} = 21.4 Hz), 114.6 (d, J_{c-f} = 21.8 Hz), 111.6 (2C), 51.9, 45.7; HRMS (M+, EI) calcd for $C_{22}H_{16}N_2F_4O_3$: 432.1097 (M+); found: 432.1093; HPLC purity = 96.3% (system A), 97.8% (system B).

5.1.27. 3-(4-Nitrophenylamino)-3-(4-(trifluoromethyl)phenyl)-1-m-tolylpropan-1-one (39)

3-Methylacetophenone (136 μL, 1 mmol), 4-trifluoromethylbenzaldehyde (137 μL, 1 mmol), 4-nitroaniline (138 mg, 1 mmol), EtOH (3 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **39** (315 mg, 74%) as a yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.95 (d, 2H, J = 9.3 Hz), 7.65–7.80 (m, 6H), 7.39–7.50 (m, 2H), 6.59–6.65 (m, 2H), 5.28 (dd, 1H, J = 9.1, 3.8 Hz), 3.78 (dd, 1H, J = 9.0, 17.9 Hz), 3.39–3.48 (dd, 1H, J = 17.9, 3.9 Hz), 2.32 (s, 3H); ^{13}C NMR (CD_3COCD_3, 100 MHz): δ 196.6, 153.7, 148.1, 138.9, 138.0, 137.3, 134.5, 129.4 (q, J_{c-f} = 32 Hz), 129.1, 129.0, 128.1 (2C), 126.3 (2C), 126.1, 126.0, 125.8, 123.6, 112.3 (2C), 53.1, 46.3, 20.8; HRMS (M+, EI) calcd for $C_{23}H_{19}F_3N_2O_3$: 428.1348; found: 428.1345. HPLC purity = 99.4% (system A), 99.5% (system B).

5.1.28. 3-(4-Nitrophenylamino)-1-(3-cyanophenyl)-3-(4-(trifluoromethyl)phenyl)propan-1-one (40)

3-cyanoacetophenone (145 mg, 1 mmol), 4-trifluoromethylbenzaldehyde (137 μL, 1 mmol), 4-nitroaniline (138 mg, 1 mmol), EtOH (3 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **40** (331 mg, 76%) as a yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.49(s, 1H), 8.27(d, 1H, J = 8.0 Hz), 8.12 (1H, d, J = 8.0 Hz), 7.96(d, 2H, J = 8.7 Hz), 7.69–7.78 (m, 5H), 6.63 (d, 2H, J = 9.3 Hz), 5.27 (dd, 1H, J = 9.5, 3.4 Hz), 3.85 (dd, 1H, J = 18.3, 9.6 Hz), 3.52 (dd, 1H, J = 17.95, 3.4 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 195.2, 153.4, 147.3, 137.1, 136.6, 136.4, 132.4, 132.2, 130.2, 127.8 (q, J_{c-f} = 30.9 Hz), 127.6 (2C), 126.1 (2C), 125.6 (2C), 122.9, 118.2, 112.1, 111.7 (2C), 51.8, 45.7; Anal. ($C_{23}H_{16}F_3N_3O_3$) C, H, N.

5.1.29. 3-(4-Nitrophenylamino)-3-(4-(trifluoromethyl)phenyl)-1-(3-methoxyphenyl)propan-1-one (41)

3-Methoxyacetophenone (138 μL, 1 mmol), 4-trifluoromethylbenzaldehyde (137 μL, 1 mmol), 4-nitroaniline (138 mg, 1 mmol), EtOH (3 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **41** (356 mg, 80%) as a yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.95–7.98 (d, 2H, J = 9.3 Hz), 7.69–7.75 (m, 4H), 7.60 (d, 1H, J = 7.5 Hz), 7.43–7.49 (q, 2H, J = 7.9, 2.6 Hz), 7.23 (dd, 1H, J = 17.7, 2.7 Hz), 6.62–6.66 (d, 2H, J = 9.3 Hz), 5.29 (dd, 1H, J = 9.0, 3.6 Hz), 3.82 (s, 3H), 3.78 (dd, 1H, J = 18.1, 9.0 Hz), 3.49 (dd, 1H, J = 18.0, 4.0 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 196.2, 159.5, 153.4, 147.5, 137.9, 136.4, 130.0, 128.0 (q, J_{c-f} = 31.4 Hz), 127.6 (2C), 126.2 (2C), 125.6 (2C), 122.9, 120.7, 119.6, 112.5, 111.6 (2C), 55.5, 51.9, 45.7; Anal. ($C_{23}H_{19}F_3N_2O_4$) C, H, N.

5.1.30. 3-(4-Nitrophenylamino)-3-(4-(trifluoromethyl)phenyl)-1-(4-methoxyphenyl)propan-1-one (42)

4-methoxyacetophenone (150 mg, 1 mmol), 4-trifluoromethylbenzaldehyde (137 μL, 1 mmol), 4-nitroaniline (138 mg, 1 mmol), EtOH (3 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **42** (262 mg, 59%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.00–8.05 (m, 4H), 7.74–7.80(dd, 4H, J = 11.0, 8.7 Hz), 7.15 (d, 2H, J = 9.3 Hz), 6.69 (d, 2H, J = 9.1 Hz), 5.33 (dd, 1H, J = 8.3, 4.2 Hz), 3.90 (s, 3H), 3.79 (dd, 1H, J = 17.7, 9.3 Hz), 3.45 (dd, 1H, J = 17.6, 4.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 195.7, 164.2, 151.9, 145.4, 138.6, 130.5 (2C), 130.1 (d, J_{c-f} = 31.9 Hz), 129.1, 126.6 (2C), 126.2 (2C), 126.0 (2C), 125.2, 113.9 (2C), 112.2 (2C), 55.5, 54.0, 44.7; Anal. ($C_{23}H_{19}F_3N_2O_4$) C, H, N.

5.1.31. 3-(4-Nitrophenylamino)-1-(4-chlorophenyl)-3-(4-(trifluoromethyl)phenyl)propan-1-one (43)

3-Chloroacetophenone (130 μL, 1 mmol), 4-trifluoromethylbenzaldehyde (137 μL, 1 mmol), 4-nitroaniline (138 mg, 1 mmol), EtOH (3 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **43** (398 mg, 89%) as a yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.38 (d, 2H, J = 8.3 Hz), 8.25 (d, 2H, J = 8.7 Hz), 7.99 (d, 2H, J = 9.2 Hz), 7.72–7.78 (m, 4H), 6.65 (d, 2H, J = 9.3 Hz), 5.31 (dd, 1H, J = 8.9, 3.4 Hz), 3.88 (dd, 1H, J = 18.1, 9.1 Hz), 3.60 (1H, dd, J = 18.2, 3.7 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 195.8, 152.0, 145.3, 140.4, 138.3, 134.3, 130.0

(q, J_{c-f} = 32.3 Hz), 129.4 (2C),129.1 (2C), 126.6 (2C), 126.2 (2C), 126.1 (2C), 123.9 (d, J_{c-f} = 270.5 Hz), 112.2 (2C), 53.4, 45.2; Anal. ($C_{22}H_{16}ClF_3N_2O_3$) C, H, N.

5.1.32. 3-(4-Nitrophenylamino)-1-(4-bromophenyl)-3-(4-(trifluoromethyl)phenyl)propan-1-one (44)

4-bromoacetophenone (199 mg, 1 mmol), 4-trifluoromethylbenzaldehyde (137 μL, 1 mmol), 4-nitroaniline (138 mg, 1 mmol), EtOH (3 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **44** (240 mg, 49%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.92–7.98 (m, 4H), 7.68–7.77 (m, 6H), 6.63 (d, 2H, J = 9.2 Hz), 5.26 (dd, 1H, J = 9.1, 3.9 Hz), 3.77(dd, 1H, J = 17.6, 9.3 Hz), 3.48 (dd, 1H, J = 17.5, 4.3 Hz); ^{13}C NMR (CD_3COCD_3, 100 MHz): δ 195.7, 153.7, 147.9, 138.1, 136.2, 132.4 (2C), 130.5 (2C), 129.4 (q, J_{c-f} = 32 Hz), 128.2, 128.1 (2C), 126.3 (2C), 126.1, 126.0123.6, 111.7 (2C), 51.8, 45.6; HRMS (M+, EI) calcd for $C_{22}H_{16}BrN_2O_3F_3$: 492.0296; found: 492.0281. HPLC purity = 99.4% (system A), 98.2% (system B).

5.1.33. 3-(4-Nitrophenylamino)-3-cyclohexyl-1-*p*-tolylpropan-1-one (45)

4-Methylacetophenone (133 μL, 1 mmol), cyclohexanecarboxaldehyde (122 μL, 1 mmol), 4-nitroaniline (138 mg, 1 mmol), EtOH (3 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **45** (226 mg, 62%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ8.02–8.06 (m, 2H), 7.82 (d, 2H, J = 8.3 Hz), 7.25 (d, 2H, J = 7.9 Hz), 6.51–6.55 (m, 2H), 4.87(d, 1H, J = 9.8 Hz), 3.96 (m, 1H), 3.15–3.26 (m, 2H), 2.42 (s, 3H), 1.62–1.93 (m, 6H), 0.97–1.28 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 198.2, 153.1, 144.5, 137.5, 134.1, 129.4 (2C), 128.1 (2C), 126.5 (2C), 111.1 (2C), 54.5, 42.1, 39.8, 29.9, 29.6, 26.2, 26.0 (2C), 21.7; Anal. ($C_{22}H_{26}N_2O_3$) C, H, N.

5.1.34. 3-(4-Nitrophenylamino)-3-cyclohexyl-1-(4-methoxyphenyl)propan-1-one (46)

4-Methoxyacetophenone (150 mg, 1 mmol), cyclohexanecarboxaldehyde (122 μL, 1 mmol), 4-nitroaniline (138 mg, 1 mmol), EtOH (3 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **46** (182 mg, 48%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.93–7.96 (m, 4H), 7.04 (d, 2H, J = 8.8 Hz), 6.63 (d, 2H, J = 9.4 Hz), 4.04 (m, 1H), 3.84 (s, 3H), 3.20 (d, 2H, J = 6.2 Hz), 1.51–1.80 (m, 6H), 1.00–1.21 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 197.1, 163.8, 153.2, 137.4, 130.3 (2C), 129.7, 126.5 (2C), 113.9 (2C), 111.1 (2C), 55.5, 54.6, 42.1, 39.5, 29.9, 29.6, 26.2, 26.0 (2C); Anal. ($C_{22}H_{26}N_2O_4$) C, H, N.

5.1.35. 3-(4-Nitrophenylamino)-1-(4-bromophenyl)-3-cyclohexylpropan-1-one (47)

4-Bromoacetophenone (398 mg, 2 mmol), cyclohexanecarboxaldehyde (242 μL, 2 mmol), 4-nitroaniline (276 mg, 2 mmol), EtOH (7 mL), EtOH/HCl (50 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **47** (245 mg, 28%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.89–7.96 (q, 4H, J = 8.7, 8.7 Hz), 7.73 (d, 2H, J = 8.3 Hz,), 6.64 (d, 2H, J = 9.1 Hz), 4.03 (dd, 1H, J = 11.7, 5.8 Hz), 3.26 (d, 2H, J = 6.3 Hz), 1.56–1.79 (m, 6H), 1.03–1.21 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 197.5, 153.0, 137.6, 135.3, 132.0 (2C), 129.5 (2C), 128.8, 126.5 (2C), 111.2 (2C), 54.3, 42.1, 40.0, 29.9, 29.5, 26.1, 26.0 (2C); Anal. ($C_{21}H_{23}BrN_2O_3$) C, H, N.

5.1.36. 3-(4-Nitrophenylamino)-1-(4-chlorophenyl)-3-cyclohexylpropan-1-one (48)

4-Chloroacetophenone (130 μL, 1 mmol), cyclohexanecarboxaldehyde (122 μL, 1 mmol), 4-nitroaniline (138 mg, 1 mmol), EtOH (3 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **48** (137 mg, 36%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.93–7.99 (m, 4H), 7.59 (m, 2H), 6.63 (d, 2H, J = 9.4 Hz), 4.03 (dd, 1H, J = 11.6, 5.9 Hz), 3.27 (d, 2H, J = 6.2 Hz), 1.57–1.79 (m, 6H), 1.04–1.79 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 197.3, 153.0, 140.0, 137.6, 134.9, 129.4 (2C), 129.1 (2C), 126.5 (2C),111.2 (2C), 54.3, 42.1, 40.1, 29.9, 29.5, 26.1, 26.0 (2C); Anal. ($C_{21}H_{23}ClN_2O_3$) C, H, N.

5.1.37. 3-(4-Nitrophenylamino)-3-cyclohexyl-1-(4-fluorophenyl)-propan-1-one (49)

4-Fluoroacetophenone (122 μL, 1 mmol), cyclohexanecarboxaldehyde (122 μL, 1 mmol), 4-nitroaniline (138 mg, 1 mmol), EtOH (3 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **49** (160 mg, 43%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.03–8.08 (m, 2H), 7.95 (d, 2H, J = 9.0 Hz), 7.35 (t, 2H, J = 8.9 Hz), 6.63 (d, 2H, J = 9.1 Hz), 4.04 (dd, 1H, J = 11.5, 6.0 Hz), 3.27 (d, 2H, J = 6.2 Hz), 1.57–1.80 (m, 6H), 1.05–1.21 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 196.9, 165.9 (d, J_{c-f} = 254 Hz), 153.0, 137.6, 133.0, 130.6 (d, J_{c-f} = 9.1 Hz), 126.6 (2C), 115.89 (d, J_{c-f} = 21.9 Hz), 111.1 (2C), 54.3, 42.1, 39.9, 29.9, 29.5, 26.1, 26.0, 25.9; HRMS (M+, EI) calcd for $C_{21}H_{23}FN_2O_3$: 370.1693; found: 370.1681. HPLC purity = 100% (system A), 98.7% (system B).

5.1.38. 3-(4-Nitrophenylamino)-3-cyclohexyl-1-(3-fluorophenyl)-propan-1-one (50)

3-Fluoroacetophenone (108 μL, 1 mmol), cyclohexanecarboxaldehyde (122 μL, 1 mmol), 4-nitroaniline (138 mg, 1 mmol), EtOH (3 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **50** (149 mg, 40%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.95 (d, 2H, J = 8.4 Hz), 7.83 (d, 1H, J = 7.3 Hz), 7.74 (d, 1H, J = 9.2 Hz), 7.59 (dd, 1H, J = 13.9, 7.9 Hz), 7.50 (t, 1H, J = 8.6 Hz), 6.65 (d, 2H, J = 8.6 Hz), 4.05 (m, 1H), 3.30 (d, 2H, J = 5.9 Hz), 1.60–1.81 (m, 6H), 1.06–1.21 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 197.3, 162.8 (d, J_{c-f} = 247.3 Hz), 153.0, 138.6 (d, J_{c-f} = 5.9 Hz), 137.6, 130.4 (d, J_{c-f} = 7.8 Hz), 126.5 (2C), 123.7, 120.5 (d, J_{c-f} = 21.4 Hz), 114.7 (d, J_{c-f} = 21.8 Hz), 111.2 (2C), 54.3, 42.1, 40.3, 29.9, 29.5, 26.1, 26.0, 25.9; HRMS (M+, EI) calcd for $C_{21}H_{23}FN_2O_3$: 370.1693; found: 370.1703. HPLC purity = 98.8% (system A), 97.3% (system B).

5.1.39. 3-(4-Nitrophenylamino)-3-cyclohexyl-1-(3-methoxyphenyl)propan-1-one (51)

4-Methoxyacetophenone (150 mg, 1 mmol), cyclohexanecarboxaldehyde (122 μL, 1 mmol), 4-nitroaniline (138 mg, 1 mmol), EtOH (3 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **51** (182 mg, 48%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.92 (d, 2H, J = 9.1 Hz), 7.57 (d, 1H, J = 7.7 Hz), 7.41–6.46 (m, 2H), 7.20 (m, 1H), 6.63 (d, 2H, J = 9.6 Hz), 4.04 (dd, 1H, J = 11.7, 5.8 Hz), 3.80 (s, 3H), 3.26 (d, 2H, J = 6.2 Hz), 1.55–1.79 (m, 6H), 0.98–1.14 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 198.4, 159.9, 153.1, 137.9, 137.6, 129.7 (2C), 126.5 (2C), 120.5, 119.9, 112.3, 111.2, 55.4, 54.4, 42.1, 40.2, 29.9, 29.5, 26.2, 26.1 (2C); Anal. ($C_{22}H_{26}N_2O_4$) C, H, N.

5.1.40. 3-(4-Nitrophenylamino)-1-(3,4-dichlorophenyl)-3-cyclohexylpropan-1-one (52)

3,4-Dichloroacetophenone (189 mg, 1 mmol), cyclohexanecarboxaldehyde (122 μL, 1 mmol), 4-nitroaniline (138 mg, 1 mmol), EtOH (3 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **52** (243 mg, 58%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.16 (d, 1H, J = 2.2 Hz), 7.88–7.95 (m, 3H), 7.79 (d, 1H, J = 8.6 Hz), 6.62 (d, 2H, J = 9.1 Hz), 4.01 (dd, 1H, J = 11.7, 5.6 Hz), 3.28 (d, 2H, J = 6.1 Hz), 1.52–1.79 (m, 6H), 1.00–1.17 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 196.2, 152.9, 138.2, 137.8, 136.1, 133.5, 130.9, 130.0, 126.9, 126.6 (2C), 111.2 (2C), 54.2, 42.1, 40.2, 29.9, 29.5, 26.1, 26.0, 25.9; Anal. ($C_{21}H_{22}Cl_2N_2O_3$) C, H, N.

5.1.41. 3-(4-Nitrophenylamino)-3-cyclohexyl-1-phenylpropan-1-one (53)

Acetophenone (117 μL, 1 mmol), cyclohexanecarboxaldehyde (122 μL, 1 mmol), 4-nitroaniline (138 mg, 1 mmol), EtOH (3 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **53** (173 mg, 49%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.92–7.97 (t, 4H, J = 8.3 Hz), 7.65 (m, 1H), 7.50–7.55 (m, 2H), 6.63 (d, 2H, J = 8.8 Hz), 4.05 (dd, 1H, J = 12.0, 5.7 Hz), 3.28 (d, J = 6.3 Hz), 1.55–1.80 (m, 6H), 1.05–1.22 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 198.6, 153.1, 137.5, 136.6, 133.6, 128.8 (2C), 128.0 (2C), 126.5 (2C), 111.2 (2C), 54.4, 42.1, 40.0, 29.9, 29.5, 26.2, 26.1 (2C); Anal. ($C_{21}H_{24}N_2O_3$) C, H, N.

5.1.42. 3-(4-Nitrophenylamino)-1-(4-phenyl-phenyl)-3-cyclohexylpropan-1-one (54)

4-acetylbiphenyl (196 mg, 1 mmol), cyclohexanecarboxaldehyde (122 μL, 1 mmol), 4-nitroaniline (138 mg, 1 mmol), EtOH (3 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **54** (154 mg, 36%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.05 (d, 2H, J = 8.4 Hz), 7.95 (d, 2H, J = 9.0 Hz), 7.83 (d, 2H, J = 8.4 Hz), 7.75 (m, 2H), 7.41–7.55 (m, 3H), 6.65 (d, 2H, J = 9.6 Hz), 4.07 (dd, 1H, J = 12.0, 6.2 Hz), 3.30 (d, 2H, J = 6.1 Hz), 1.54–1.82 (m, 6H), 1.06–1.25 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 198.1, 153.1, 146.2, 139.6, 137.6, 135.3, 128.9 (2C), 128.6 (2C), 128.4, 127.4 (2C), 127.2 (2C), 126.6 (2C), 111.2 (2C), 54.5, 42.1, 40.0, 29.9, 29.6, 26.2, 26.1 (2C); Anal. ($C_{27}H_{28}N_2O_3$) C, H, N.

5.1.43. 3-(4-Nitrophenylamino)-3-cyclohexyl-1-(4-cyclohexylphenyl)propan-1-one (55)

4-Cyclohexylacetophenone (202 mg, 1 mmol), cyclohexanecarboxaldehyde (122 μL, 1 mmol), 4-nitroaniline (138 mg, 1 mmol), EtOH (3 mL), EtOH/HCl (30 μL) were subjected to conditions similar to those employed in the procedure for **13** to give **55** (221 mg, 51%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.85–7.95 (q, 4H, J = 9.1, 8.2 Hz), 7.35 (d, 2H, J = 8.2 Hz), 6.62 (d, 2H, J = 8.9 Hz), 4.04 (dd, 1H, J = 11.6, 6.4 Hz, CH), 3.23 (d, 2H, J = 5.6 Hz), 1.03–1.79 (m, 22H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 198.2, 154.3, 153.1, 137.5, 134.4, 128.2 (2C), 127.2 (2C), 126.5 (2C), 111.1 (2C), 54.4, 44.6, 42.0, 39.8, 34.0 (2C), 29.9, 29.5, 26.6 (2C), 26.2, 26.1 (2C), 25.9; Anal. ($C_{27}H_{34}N_2O_3$) C, H, N.

HPLC separation of enantiomers of **22** and **32:** An 'Chiralpak IA' preparative column (20 mm id × 250 mm length) on a YMC K-Prep was equilibrated with an eluent of hexane/THF (80:20) at a flow rate of 10 ml/min. A 1 mg/ml solution of **22** in hexane/THF (70:30) was prepared, and 4.0 mL of this solution was injected per run. Eluent was monitored by absorbance detection at 280 nm. The retention time of the first peak was approximately 15 min. The first eluting enantiomer (levorotatory) was collected until the absorbance began to decline at which point the mixed fractions were collected separately. Once the absorbance had returned to a fixed height, the second (dextrorotatory) enantiomer was collected until all of the second enantiomer had been collected, then another injection was made. The mixed fractions were combined and resubjected to the HPLC conditions. The enantiomers of **32** were resolved in an identical manner to that described above for **22**.

5.1.44. (−)-(*R*)-3-(4-Nitrophenylamino)-3-(3-fluorophenyl)-1-*p*-tolylpropan-1-one ((−)-22)

Compound (−)-**22** was prepared on an 'Chiralpak IA' preparative column (20 mm id × 250 mm length) with 98% recovery and analyzed on an 'Chiralpak AD-H' column (4.6 mm id × 250 mm length), 0.6 ml/min, (hexane/ethanol, 90:10), t_R = 60.9 min, 99.9% ee; $[\alpha]_D^{20}$ = −95.5° (*c* 0.53 g/100 mL, CH_3CN). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.97–8.01 (m, 2H), 7.78 (d, 2H, J = 8.3 Hz), 7.31 (m, 1H), 7.24 (d, 2H, J = 8.1 Hz), 7.18 (d, J = 7.9 Hz), 7.10 (m, 1H), 6.96 (m, 1H), 6.50 (m, 2H), 5.65 (br, NH), 5.08 (dd, 1H, J = 12.1, 6.0 Hz), 3.51 (dd, J = 17.4, 4.4 Hz), 3.46 (dd, J = 16.6, 5.1 Hz), 2.40 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 197.1, 163.0 (d, J_{c-f} = 245.9 Hz), 151.9, 145.0, 144.0 (d, J_{c-f} = 6.4 Hz), 138.5, 133.7, 130.7 (d, J_{c-f} = 8.2 Hz), 129.5 (2C), 128.3 (2C), 126.1 (2C), 121.8, 114.8 (d, J_{c-f} = 21.4 Hz), 113.1 (d, J_{c-f} = 22.4 Hz), 112.2 (2C), 53.9, 45.1, 21.7; Anal. ($C_{22}H_{19}FN_2O_3$) C, H, N.

5.1.45. (+)-(*S*)-3-(4-Nitrophenylamino)-3-(3-fluorophenyl)-1-*p*-tolylpropan-1-one ((+)-22)

Compound (+)-**22** was prepared and analyzed in a manner identical to that described above for (−)-**22**, t_R = 68.1 min, 99.7% ee; $[\alpha]_D^{20}$ = +96.0° (*c* 0.57 g/100 mL, CH_3CN). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.97–8.01 (m, 2H), 7.78 (m, 2H), 7.31 (m, 1H), 7.24 (d, 2H, J = 7.9 Hz), 7.18 (d, 1H, J = 7.9 Hz), 7.10 (m, 1H), 6.95 (m, 1H), 6.49 (m, 2H), 5.64 (br, NH), 5.07 (dd, 1H, J = 11.9, 5.8 Hz), 3.50 (dd, 1H, J = 16.4, 6.8 Hz), 3.45 (dd, 1H, J = 16.4, 5.3 Hz), 2.38 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 197.1, 163.1 (d, J_{c-f} = 245.5 Hz), 151.9, 145.0, 144.0 (d, J_{c-f} = 6 Hz), 138.5, 133.7, 130.7 (d, J_{c-f} = 8.2 Hz), 129.5 (2C), 128.3 (2C), 126.1 (2C), 121.8, 114.8 (d, J_{c-f} = 20.9 Hz), 113.1 (d, J_{c-f} = 21.9 Hz), 122.2 (2C), 53.9, 45.1, 21.7; Anal. ($C_{22}H_{19}FN_2O_3$) C, H, N.

5.1.46. (+)-(*S*)-3-(4-Nitrophenylamino)-1-(4-bromophenyl)-3-(3-fluorophenyl)propan-1-one((+)-32)

Compound (+)-**32** was prepared on an 'Chiralpak IA' preparative column (20 mm id × 250 mm length) in a manner identical to that described above for (−)-**22** and analyzed on an 'Chiralpak AD-H' column (4.6 mm id × 150 mm length), 1.0 ml/min, (hexane/2-propanol = 82/18), t_R = 13.9 min, 98.1% ee; $[\alpha]_D^{20}$ = +107.5 (*c* 0.48 g/100 mL, CH_3CN). ^{1}H NMR (CD_3COCD_3, 400 MHz): 7.92–7.98 (m, 4H), 7.68–7.72 (m, 2H), 7.38–7.40 (m, 2H), 7.32 (m, 1H), 7.02 (m, 1H), 6.68–6.72 (m, 2H), 5.32 (dd, 1H, J = 8.8, 4.3 Hz), 3.82 (dd, 1H, J = 17.7, 8.7 Hz), 3.54 (dd, 1H, J = 17.4, 4.5 Hz). Anal. ($C_{21}H_{16}BrFN_2O_3$) C, H, N.

5.2. Receptor binding assay

Steroid receptor binding assays were performed as previously described.[23] An appropriate amount of baculovirus derived nuclear receptor protein extract (hPR-B, hAR, hERα, hERβ, hGR and hMR) was loaded into each well of Isoplate containing the assay buffer followed by addition of 5 nM [^{3}H] progesterone, 5 nM [^{3}H]DHT, 5 nM [^{3}H]E_2, 5 nM [^{3}H]E_2, 5 nM [^{3}H]dexamethasone, and 5 nM [^{3}H] aldosterone, respectively. Increasing concentrations of test compounds (1 pM to 10 μM) were added thereafter (2.5 μL) to give a final volume of 100 μL well^{-1}. The plates were sealed and incubated for 16 h at 4 °C. Hydroxyapatite [HA 25% (v/v), 25 μL] was added to each well the next day and the plates were gently agitated twice for 5 min each. Following centrifugation at 2500 rpm for 3 min at 4 °C, the supernatant was decanted and 100 μL ice-cold assay buffer added to each well. This washing procedure was repeated twice before adding 150 μL scintillation liquid, gently agitating the plates to resuspend HA and counting bound radioactivity with a MicroBeta counter. Non-linear regression analyses were performed to generate dose–response curves. K_i values were calculated from IC_{50} using the equation of Cheng and Prusoff [$K_i = IC_{50}/(1 + [\text{radioligand}]/K_d)$], where K_d is the dissociation constant of the radioligand.[24]

5.3. Cotransfection assay

Transient cotransfection assay was performed in CV-1 cells according to the method described previously.[23] CV-1 cells (African green monkey kidney fibroblasts) were cultured in the presence of

Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% CDT-FBS and seeded 24 h before transfection in 6 cm dish (6×10^5 cells per dish). Two micrograms of the reporter plasmid (MMTV-Luc) and 0.4 μg of pSG5-hPR-B were introduced simultaneously into cells with a ratio of 5 to 1. Cells were transfected for 8 h, and harvested with 0.05% trypsin and 0.02% EDTA prior to reseeding onto a 96-well microtiter plate (8000 cells well^{-1}). They were incubated for 24 h with or without various concentrations of control or test compounds. For antagonist assay, test samples were added 30 min ahead of progesterone at its EC_{50} concentration. Cell extracts were prepared and the expressed luciferase activity was determined in a Wallac 1420 multi label counter (VICTOR^2, Perkin-Elmer) using a Steady-Glo luciferase kit from Promega. To detect potential cytotoxicity of compounds, treated cells were reacted with AlamarBlue™ (United States Biological) for 4 h and the fluorescence is monitored at 540 nm excitation wavelength and 590 nm emission wavelength on a FlexStation 384^{II} (Molecular Devices, Sunnyvale, CA, USA) prior to luciferase activity measurement. The relative luciferase activity was normalized against cell viability (% growth) assessed with AlamarBlue in the same well. To determine cross-reactivity of test compounds, cotransfection assays with hAR, hMR, and hGR with the MMTV-Luc reporter and hERα, hERβ with the ERE-MMTV-Luc reporter were performed.

5.4. Murine uterine decidulization assay

Murine uterine decidulization assay was performed as described in the literature.[17,18] BALB/c mice were used in the experiment. The animals were housed at 22 ± 0.8 °C in a 12 h light:dark cycle and kept on a standard laboratory diet and drinking water ad libitum. 7-week old male BALB/c mice were vasectomized 3 weeks before decidulization assay. Mature female virgin BALB/c (21 g mean weight) were caged together with vasectomized male BALB/c mice with a ratio of 2 to 1 between 17.00 and 10.00 h. It was regarded as pseudopregnant when a vaginal plug was detected the next morning (day 1). Mating was presumed to have taken place at 02.00 (time 0). Pseudopregnant mice were treated intraluminally with either RU486 or test compounds (**22**, **26**). RU486 and test compounds were first dissolved in 100% ethanol and then diluted with sesame oil. The sesame oil mixtures were blown with nitrogen to make sure that no residual ethanol was left. On day 4 (16.00 h), 10 μL of sesame oil containing RU486 or test compounds was injected intraluminally into the right uterine horn (stimulated) and the left horn served as an internal control (non-stimulated). Control animals were injected 10 μL of sesame oil into the right uterine horns only. Seventy-two h after decidual stimulation, the mice were killed and the uterine horns were removed and weighed. For each mouse, uterine wet weight gain was calculated by subtracting the weight of the non-stimulated horn from that of the stimulated horn. Percent inhibition was calculated using the formula: % inhibition = (1 − weight gain in treated group/weight gain in control group) × 100.

5.5. Statistical analysis

Statistical analysis with Student's *t*-test was performed using GraphPad Prism software (GraphPad, San Diego, CA, USA) and data are presented as means ± SEM. The criterion for significance was a probability of less than 0.05 or 0.01.

5.6. Molecular docking

The crystal structure of the progesterone binding domain of PR (residues 678–933) in complex with asoprisnil was retrieved from the Brookhaven Protein Data Bank (PDB entry 2OVH). To prepare the receptor suitable for docking studies, the PDB structure 2OVH was processed in graphic software AutoDockTools. According the default setting in AutodockTools, the hydrogens were added and the atoms were typing as the rules in AutoDock. The Gasteiger charges were calculated and assigned for the atoms of the structure. The coordinates along with the charge, salvation information of the receptor were saved in pdbqt format for later docking studies. The initial structures of mifepristerone, distomers of (−)-(*R*)-**22** was optimized using the Cerius2 software with OPEN force field. The conjugated gradient method was used for energy minimization with an energy convergence gradient value of 0.001 kcal/(mol Å).

The advance docking program AUTODOCK 4.0 was used to dock ligands to the progesterone receptor ligand-binding domain. The Lamarckian genetic algorithm[25] was applied to analyze protein–ligand interactions. A Solis and Wets local search[25] was performed for energy minimization on a user-specified proportion of the population. The docked structures of the ligands were generated after a reasonable number of evaluations. The whole docking operation could be stated as follows:

1. Each ligand molecule was checked for polar hydrogens and assigned atom types, the partial atomic charges with Gasteiger method and the atomic solvation parameters.
2. The three-dimensional grid with $60 \times 60 \times 60$ points and a spacing of 0.375 Å was created by the AutoGrid4 algorithm to evaluate the binding energies between the ligands and the proteins. In this stage, the protein was embedded in the three-dimensional grid, and a probe atom was placed at each grid point. The affinity and electrostatic potential grid were calculated for each type of atom in the ligands. The energetics of a particular ligand configuration was found by trilinear interpolation of affinity values and electrostatic interaction of the eight grid points surrounding each of the atoms in a ligand.
3. A series of the docking parameters were set on. Not only the atom types but also the generations and the number of runs for the Lamarckian genetic algorithm were edited and properly assigned according to the requirement of the Amber force field. The number of generations, energy evaluations, and docking runs were set to 27×10^3, 2.5×10^6, and 10, respectively.

Acknowledgment

This work was supported in part by a grant from the Ministry of Science and Technology of China (2009ZX09302-001).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.04.092.

References and notes

1. Mangelsdorf, D. J.; Thummel, C.; Beato, M.; Herrlich, P.; Schutz, G.; Umesono, K.; Blumberg, B.; Kastner, P.; Mark, M.; Chambon, P.; Evans, R. M. *Cell* **1995**, *83*, 835.
2. Murphy, A. A.; Kettel, L. M.; Morales, A. J.; Roberts, V. J.; Yen, S. S. *J. Clin. Endocrinol. Metab.* **1993**, *76*, 513.
3. Bakker, G. H.; Setyono-Han, B.; Portengen, H.; De Jong, F. H.; Foekens, J. A.; Klijn, J. G. *J. Steroid Biochem. Mol. Biol.* **1990**, *37*, 789.
4. Kettel, L. M.; Murphy, A. A.; Mortola, J. F.; Liu, J. H.; Ulmann, A.; Yen, S. S. *Fertil. Steril.* **1991**, *56*, 402.
5. Lamberts, S. W.; Tanghe, H. L.; Averaat, C. J.; Braakman, R.; Wijngaarde, R.; Koper, J. W.; Jong, W. *J. Neurol. Neurosurg. Psychiatry* **1992**, *55*, 486.
6. Batista, M. C.; Bristow, T. L.; Mathews, J.; Stokes, W. S.; Loriaux, D. L.; Nieman, L. K. *Am. J. Obstet. Gynecol.* **1991**, *165*, 82.
7. Zhang, P.; Fensome, A.; Jay, W.; Richard, W.; Zhiming, Z. *Expert Opin. Ther. Patents* **2003**, *13*, 1839.
8. Kevin, P. M.; Euqene, L. S.; Shawn, P. W. *Med. Res. Rev.* **2007**, *27*, 374.

9. Zhang, P.; Terefenko, E. A.; Wrobel, J.; Zhang, Z.; Zhu, Y.; Cohen, J.; Marschke, B. K.; Mais, D. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2747.
10. Terefenko, A. E.; Kern, J.; Fensome, A.; Wrobel, J.; Zhu, Y.; Cohen, J.; Winneker, R.; Zhang, Z.; Zhang, P. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3600.
11. Fensome, A.; Bender, R.; Cohen, J.; Collins, A. M.; Mackner, A. V.; Miller, L. L.; Ullrich, W. J.; Winneker, R.; Wrobel, J.; Zhang, P.; Zhang, Z.; Zhu, Y. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3487.
12. Zhang, P.; Terefenko, E. A.; Fensome, A.; Wrobel, J.; Winneker, R.; Lundeen, S.; Marschke, K. B.; Zhang, Z. *J. Med. Chem.* **2002**, *45*, 4379.
13. Wiethe, W. R.; Stewart, L. S.; Drewry, H. D.; Gray, W. D.; Mehbob, A.; Hoekstra, J. W. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3777.
14. Kern, J. C.; Terefenko, E.; Trybulski, E.; Berrodin, T. J.; Cohen, J.; Winneker, R. C.; Yudt, M. R.; Zhang, Z.; Zhu, Y.; Zhang, P. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6666.
15. Zhou, C.; Wu, G.; Feng, Y.; Li, Q.; Su, H.; Mais, D. E.; Zhu, Y.; Li, N.; Deng, Y.; Yang, D.; Wang, M.-W. *Br. J. Pharmaco.* **2008**, *154*, 440.
16. Yin, L.; Lei, H.; Zou, J.; Xu, X. *Synthesis* **1991**, *9*, 717.
17. Crombie, D. L.; Mukherjee, R.; McDonnell, D. P.; Hayes, J. S.; Wang, M. W. *J. Steroid Biochem. Mol. Biol.* **1994**, *49*, 123.
18. Hamann, L. G.; Farmer, L. J.; Johnson, M. G.; Bender, S. L.; Mais, D. E.; Wang, M. W.; Crombie, D.; Goldman, M. E.; Jones, T. K. *J. Med. Chem.* **1996**, *39*, 1778.
19. Allan, G. F.; Leng, X.; Tsai, S. Y.; Weigel, N. L.; Edwards, D. P.; Tsai, M.; O'Malley, B. W. *J. Biol. Chem.* **1992**, *267*, 19513.
20. Xu, J.; Nawaz, Z.; Tsai, S. Y.; Tsai, M. J.; O'Malley, B. W. *Proc. Natl. Acad. Sci.* **1996**, *93*, 12195.
21. Klotzbucher, M.; Schwerk, C.; Holewa, B.; Klein-Hitpass, L. *Mol. Endocrinol.* **1997**, *11*, 768.
22. Willians, S. P.; Sigler, P. B. *Nature* **1998**, *393*, 392.
23. Ning, M.; Zhou, C.; Weng, J.; Zhang, S.; Chen, D.; Yang, C.; Wang, H.; Ren, J.; Zhou, L.; Jin, C.; Wang, M. W. *Br. J. Pharmacol.* **2007**, *150*, 19.
24. Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
25. Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. *J. Comput. Chem.* **1998**, *19*, 1639.