AD_____

Award Number: W81XWH-05-1-0017

TITLE: Anticar Inhibitors of AR-Mediated Gene Expression

PRINCIPAL INVESTIGATOR: Blake R. Peterson, Ph.D.

CONTRACTING ORGANIZATION: Pennsylvania State University
University Park, PA 16802-5807

REPORT DATE: November 2005

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-11-2005	Annual	1 Nov 2004 – 31 Oct 2005

4. TITLE AND SUBTITLE
Anticar Inhibitors of AR-Mediated Gene Expression

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-05-1-0017

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Blake R. Peterson, Ph.D.

E-mail: brpeters@chem.psu.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Pennsylvania State University
University Park, PA 16802-5807

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
New drugs that halt the progression of prostate cancers are urgently needed. Because many prostate cancers require the androgen dihydrotestosterone to proliferate, antiandrogens such as casodex (bicalutamide) are often the first line therapy for treatment of this disease. However, this drug and other clinically employed antiandrogens generally suffer from low affinity for the androgen receptor (AR), low selectivity across the nuclear hormone receptor superfamily, and do not achieve complete androgen blockade. As an alternative, mifepristone (RU486) is under investigation as a potential anticancer agent effective against prostate cancers. This drug is a highly potent antiprogestin (IC50 = 25 pM) but also exhibits potent antiglucocorticoid (IC50 = 2.2 nM) and antiandrogen (IC50 = 10 nM) activities. Although mifepristone is effective against prostate cancer cells in vivo, the use of this drug as a chronically administered anticancer agent is severely limited by its potent antiglucocorticoid activity. We are investigating novel anticancer agents structurally related to mifepristone but that are designed to lack the antiglucocorticoid activity associated with this drug.

15. SUBJECT TERMS
anticancer agents, androgen receptor, prostate cancer

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
					USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
U	U	U	UU	19	

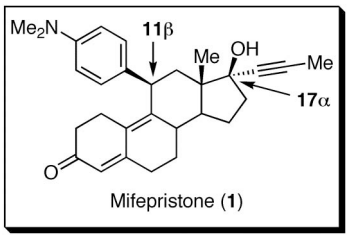
Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

Introduction

Mifepristone (RU486) is under investigation as an anticancer agent effective against prostate cancers.[1-3] This drug is a highly potent antiprogestin (IC_{50} = 25 pM)[4] with potent antiglucocorticoid (IC_{50} = 2.2 nM)[4] and antiandrogen (IC_{50} = 10 nM)[4] activities. Despite the efficacy of mifepristone against prostate cancer cells in vivo, this drug is severely limited as an anticancer agent by its potent antiglucocorticoid activity.[5] We are investigating analogues of mifepristone designed to dissociate the antiandrogen effects of this drug from the undesirable antiglucocorticoid effects. These compounds have the potential to provide improved drugs for the treatment of prostate cancer.

Body



Mifepristone (**1**)

Over the past year, we have synthesized and evaluated the biological activity of numerous novel nortestosterone derivatives structurally related to mifepristone (**1**). We have focused on modifications to the 11beta and 17alpha substituents, primarily investigating effects of length, flexibility and electronics. All new analogues were initially intitially evaluated using full-length androgen receptor (AR) in a ARE-reporter gene assay in CV-1 cells. These compounds were tested as agonists and as antagonists capable of blocking AR-mediated reporter gene expression activated by dihydrotestosterone (DHT). In addition, we employed a mammalian two-hybrid assay as a tool for studying effects on AR dimerization. Compounds were prepared in small quantities using parallel synthesis from common intermediates and evaluated at >90% purity.

Based on the structure of mifepristone (**1**), we varied both the 11beta-dimethylaniline substituent as well as the 17alpha substituent. As shown in Figure 1, we prepared mifepristone (**1**) analogues that modified the 17-propynyl group with ethynyl and methyl groups to investigate effects on activity. In addition, the dimethylaniline group was replaced with a phenyl group or an *o*-dichlorophenyl group. We investigated bi- and tri-heterocyclic ring systems at that position. Biological activity data compiled for these compounds is listed in Table 1.

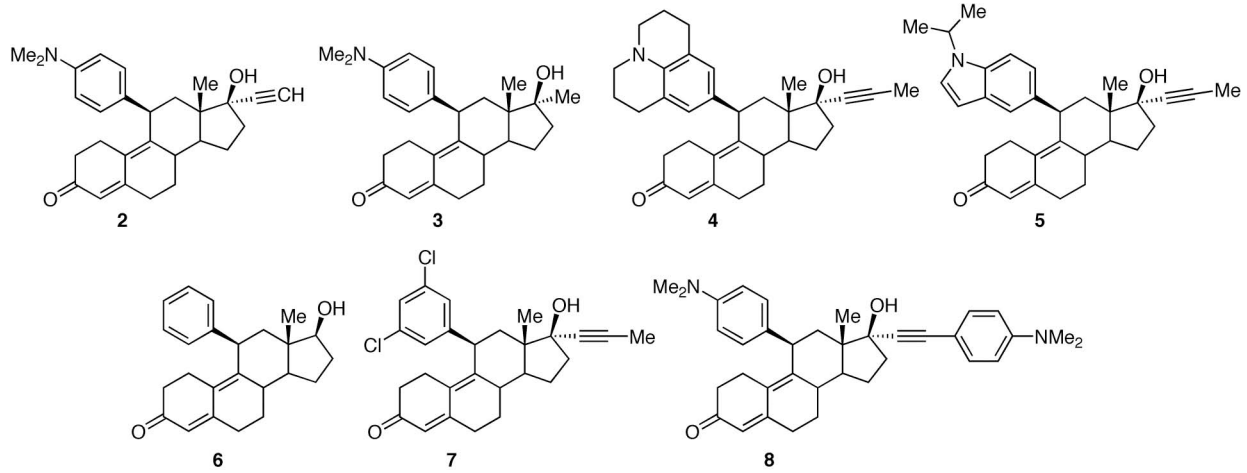
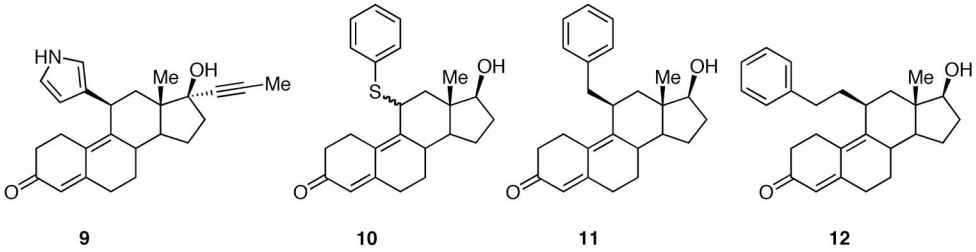


Figure 1. Structures of 11beta aryl-19-nortestosterone analogues.

Table 1. Compilation of data from full length AR reporter gene assays for compounds **1**-**8.** EC_{50} values were from dose response assays. IC_{50} values were from competition assays. % inhibition = maximal % decrease from DHT-mediated (1 nM for **1**, **2** and 0.1 nM for **3**) transcription.

Ligand	Character	EC50	IC50	% Inhibition
DHT	agonist	0.03 nM	-	-
Mifepristone (**1**)	partial agonist	1 nM	2.1 nM	81%
2	partial agonist	13.8 μM	7.6 nM	18%
3	partial agonist	67 nM	0.4 nM	65%
4	agonist	4.2 nM	-	-
5	agonist	66 nM	-	-
6	weak agonist	102 nM	-	-
7	agonist	43 nM	-	-
8	agonist	3.0 nM	-	-

Compounds shown in Figure 2 with aromatic rings and aromatic heterocycles linked to the 11beta position were strong agonists. Biological activity data is listed in Table 2.



9 **10** **11** **12**

Figure 2. Structures of nortestosterones linked to aromatic substitutents.

Table 2. Data from full length AR transactivational assays for compounds **9**-**12.** EC_{50} values were from dose response assays. IC_{50} values were from competition assays. % inhibition = maximal % decrease from activation by 1 nM DHT.

Ligand	Character	EC50	IC50	% Inhibition
DHT	agonist	0.03 nM	-	-
Mifepristone (**1**)	partial agonist	1 nM	2.1 nM	81%
9	agonist	3.9 μM	-	-
10	agonist	467 nM	-	-
11	agonist	130 nM	-	-
12	agonist	20 nM	-	-

As shown in Figure 3, we tested the steric requirements at the 11 position by projecting the substituted phenyl group further from the steroid backbone by inserting an ethynyl linker. We also substituted pyridine for the phenyl group. As listed in Table 3, none of these compounds exhibited any antagonistic activity.

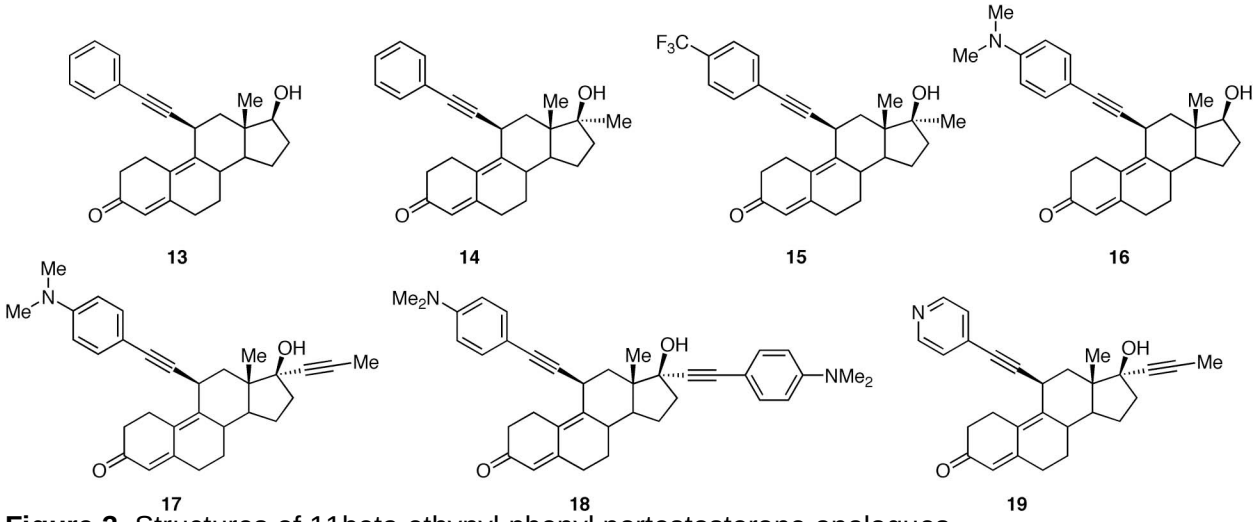


Figure 3. Structures of 11beta ethynyl phenyl nortestosterone analogues.

Table 3. Compilation of data from full length AR reporter gene assays for compounds **13**-**19.** EC_{50} values were derived from dose response assays, and IC_{50} values were from the competition assay. The % inhibition value represents the maximal % decrease from 1 nM DHT-mediated transcription in competition assays.

Ligand	Character	EC50	IC50	% Inhibition
DHT	agonist	0.03 nM	-	-
Mifepristone	partial agonist	1 nM	2.1 nM	81%
13	agonist	6.7 nM	-	-
14	agonist	4.9 μM	-	-
15	agonist	360 nM	-	-
16	agonist	2.9 nM	-	-
17	weak agonist	78 μM	-	-
18	agonist	1.2 μM	-	-
19	agonist	1.0 μM	-	-

As shown in Figure 4, we used alkyl linkers of varying lengths to append piperidine and substituted piperidine rings from the steroid backbone. We also varied the 17alpha substituent. Compound **26** was identified as a partial agonist. The biological activity data is listed in Table 4.

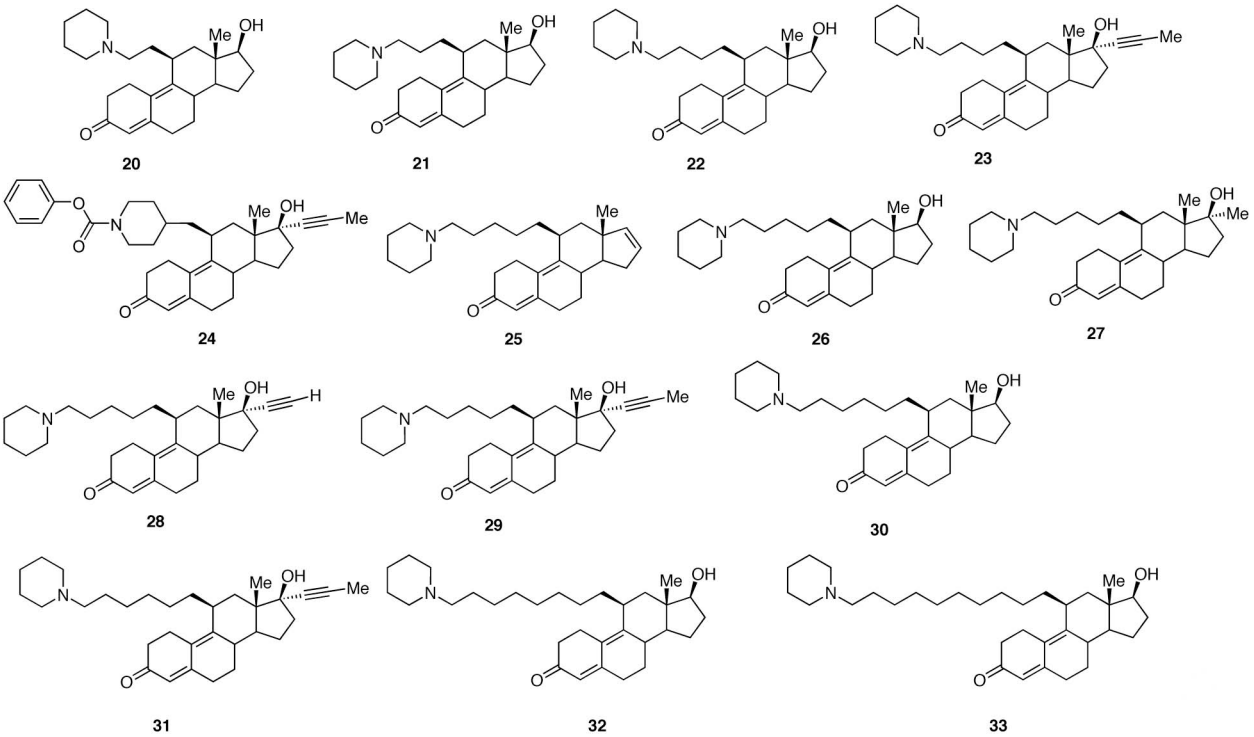


Figure 4. Structures of 11beta alkyl-linked piperidines.

Table 4. Compilation of data from full length AR transactivational assays for compounds **20**-**33.** EC_{50} values were derived from dose response assays, and IC_{50} values were from the competition assay. The % inhibition value represents the maximal % decrease from 1 nM DHT-mediated transcription in the competition assay format.

Ligand	Character	EC50	IC50	% Inhibition
DHT	agonist	0.03 nM	-	-
Mifepristone	partial agonist	1 nM	2.1 nM	81%
20	agonist	6.3 nM	-	-
21	agonist	0.5 nM	-	-
22	agonist	83 nM	-	-
23	agonist	4.4 μM	-	-
24	agonist	1.4 μM	-	-
25	agonist	9.3 nM	-	-
26	partial agonist	1.6 nM	2.2 μM	47%
27	agonist	361 μM	-	-
28	agonist	12 μM	-	-
29	agonist	44 μM	-	-
30	agonist	357 nM	-	-
31	agonist	1.2 mM	-	-
32	agonist	1.3 nM	-	-
33	agonist	52 nM	-	-

As shown in Figure 5, we replaced the mifepristone (**1**) dimethylaniline moiety with more flexible substituted piperidines. Isopropyl substituents stood in for the dimethyl amino group. Methyl groups were added to piperidine ring to test the effect of increasing steric bulk. We appended ring systems and *t*-butyl carbamate onto the piperidine, and also studied a bridged ring system. The 17alpha substituents were also varied. Biological activity data is listed in Table 5.

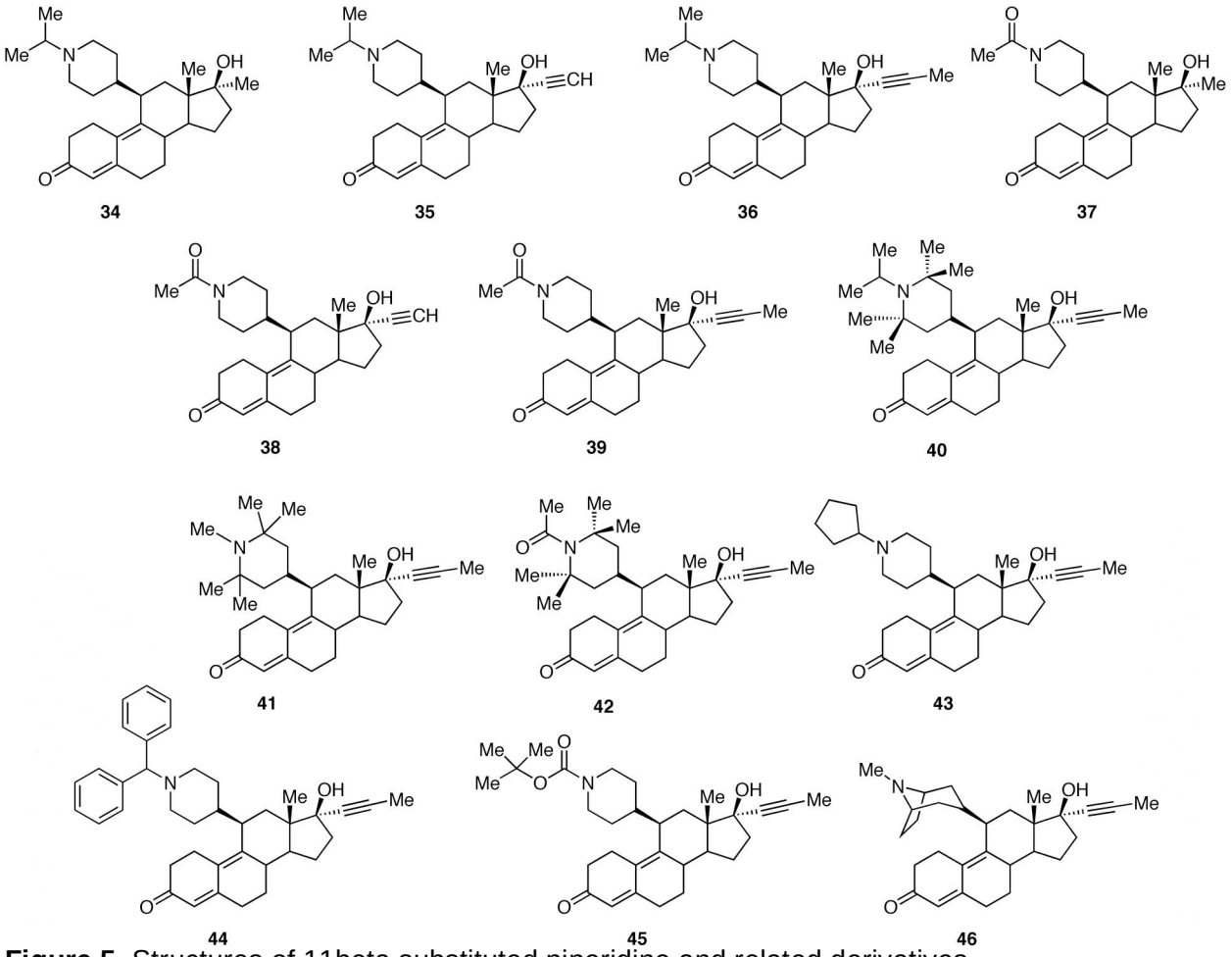


Figure 5. Structures of 11beta substituted piperidine and related derivatives.

Table 5. Compilation of data from full length AR transactivational assays for compounds **34**-**46.** EC_{50} values were derived from dose response assays, and IC_{50} values were from the competition assay. The % inhibition value represents the maximal % decrease from 1 nM DHT-mediated transcription in the competition assay format.

Ligand	Character	EC50	IC50	% Inhibition
DHT	agonist	0.03 nM	-	-
Mifepristone (**1**)	partial agonist	1 nM	2.1 nM	81%
34	agonist	35 nM	-	-
35	partial agonist	25 nM	150 nM	52%
36	partial agonist	617 nM	0.4 nM	30%
37	agonist	75 nM	-	-
38	partial agonist	19 nM	1.5 nM	45%
39	agonist	79 nM	-	-
40	agonist	11 μM	-	-
41	agonist	2 nM	-	-
42	agonist	14 μM	-	-
43	agonist	35 nM	-	-
44	partial agonist	75 μM	0.3 nM	37%
45	partial agonist	5 μM	5.5 nM	32%
46	agonist	2.1 nM	-	-

As shown in Figure 6, we varied the piperidines of the previous group to 1,2,3,6-tetrahydro-pyridines. Some of these molecules are very similar to ones in the previous section, differing only by a double bond that provides a geometry of the substituent more similar to mifepristone (**1**). As listed in Table 6, all of these analogues are strong agonists, varying only in potency. Addition of unsaturation to the ring increases the potency significantly.

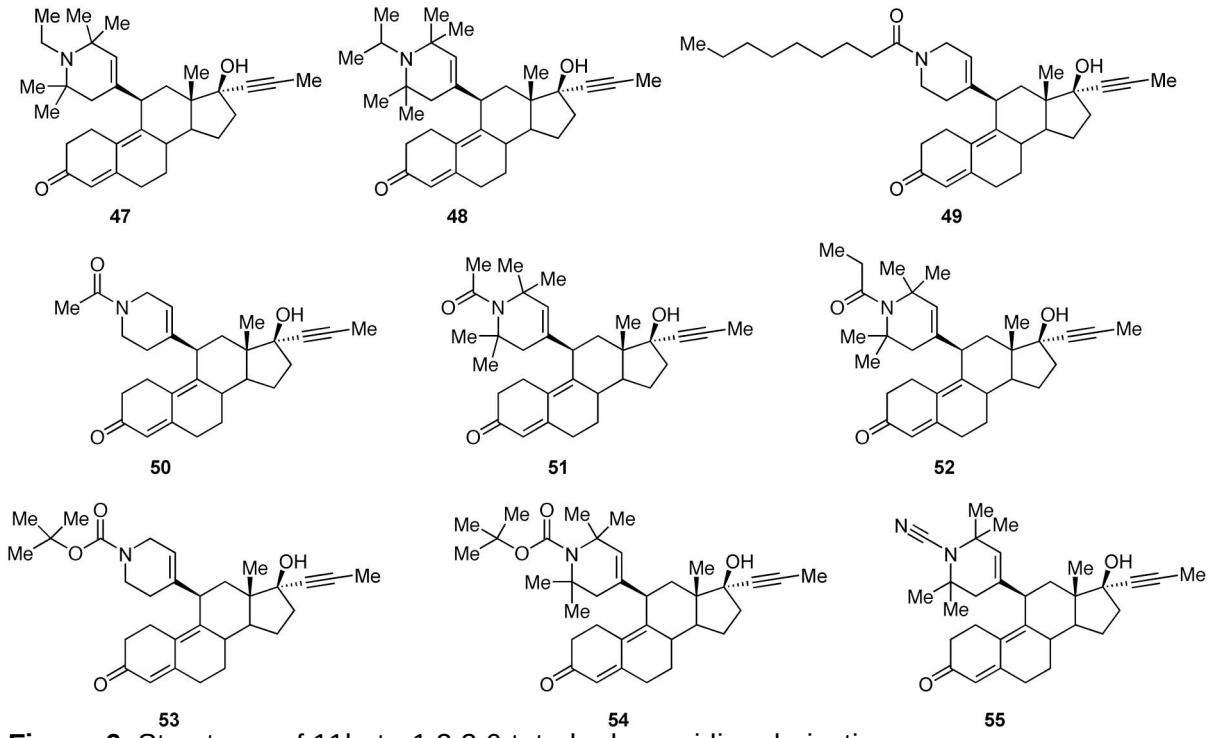


Figure 6. Structures of 11beta-1,2,3,6-tetrahydro-pyridine derivatives.

Table 6. Compilation of data from full length AR transactivational assays for compounds **47-55.** EC_{50} values were derived from dose response assays, and IC_{50} values were from the competition assay. The % inhibition value represents the maximal % decrease from 1 nM DHT-mediated transcription in the competition assay format.

Ligand	Character	EC50	IC50	% Inhibition
DHT	agonist	0.03 nM	-	-
Mifepristone (**1**)	partial agonist	1 nM	2.1 nM	81%
47	agonist	2.0 μM	-	-
48	agonist	2.8 nM	-	-
49	agonist	59 nM	-	-
50	agonist	27 nM	-	-
51	agonist	436 nM	-	-
52	agonist	68 nM	-	-
53	agonist	3.5 nM	-	-
54	agonist	1.5 μM	-	-
55	agonist	90 nM	-	-

As shown in Figure 7, expanding on the 11beta-alkyl piperidines, we made a series of 11beta-alkyl *N*-substituted piperazines. Substituted with either *t*-butyl carbamate or isopropyl, we varied the length of the alkyl linker. Biological activity data is listed in Table 7.

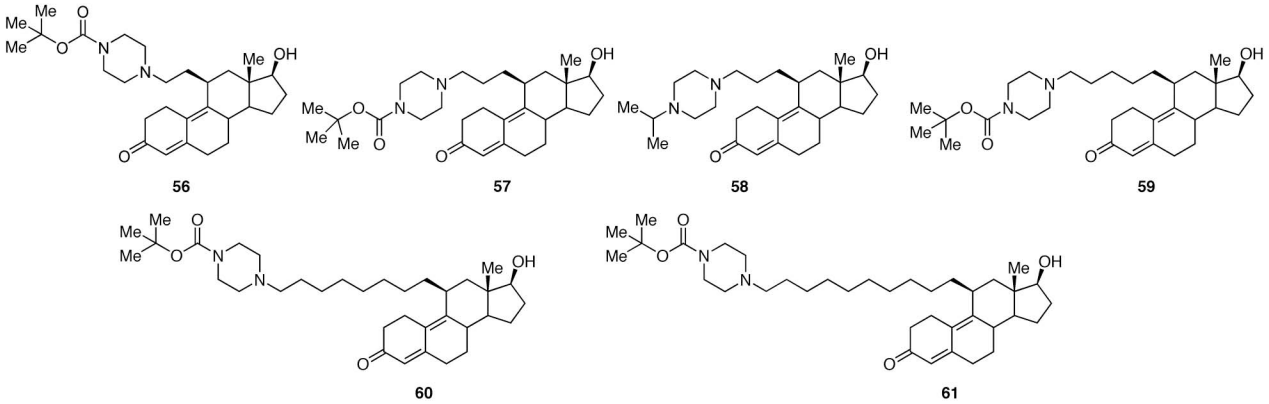


Figure 7. Structures of the 11beta-alkyl piperazines.

Table 7. Compilation of data from full length AR transactivational assays for compounds **56**-**61.** EC_{50} values were derived from dose response assays, and IC_{50} values were from the competition assay. The % inhibition value represents the maximal % decrease from 1 nM DHT-mediated transcription in the competition assay format. *GraphPad Prism was unable to calculate IC_{50} for compound **59**.

Ligand	Character	EC50	IC50	% Inhibition
DHT	agonist	0.03 nM	-	-
Mifepristone (**1**)	partial agonist	1 nM	2.1 nM	81%
56	agonist	3.7 nM	-	-
57	partial agonist	1.0 μM	5.4 nM	30%
58	agonist	5.9 nM	-	-
59	partial agonist	2.9 nM	*	25%
60	partial agonist	670 nM	1.0 μM	33%
61	agonist	560 nM	-	-

As shown in Figure 8, we made an analogue of our previously published 11beta-octyl derivative by installing a 17alpha propyne group (**62)**. This compound was a potent partial agonist (EC_{50}(**62**) = 0.14 nM), although it could only decrease DHT-mediated transcription by 34%. This is comparable both in magnitude of the effect and potency to the original 11beta-octyl compound; it appears that addition of the propyne group in this case does not have a big effect on activity. An analogue with a longer 16 atom linker, including an N-methyl amide (**63**), also displayed partial agonist activity, though with very low potency. The other ligands of this group, including a compound with a terpenoid side chain (**66**) and two compounds with dimethyl butyne side chains (**64** and **65**) were all agonists (Table 8).

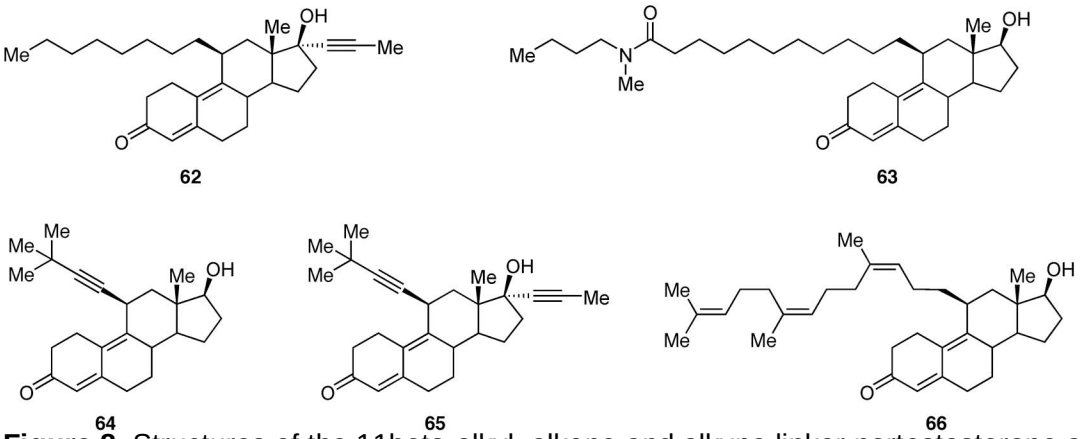


Figure 8. Structures of the 11beta-alkyl, alkene and alkyne linker nortestosterone analogues.

Table 8. Compilation of data from full length AR transactivational assays for compounds **62**-**66.** EC_{50} values were derived from dose response assays, and IC_{50} values were from the competition assay. The % inhibition value represents the % decrease from 1 nM DHT-mediated (**1**, **63**) or 0.1 nM DHT-mediated (**62**) transcription in competition assay format.

Ligand	Character	EC50	IC50	% Inhibition
DHT	agonist	0.03 nM	-	-
Mifepristone (**1**)	partial agonist	1 nM	2.1 nM	81%
62	partial agonist	101 nM	0.14 nM	34%
63	partial agonist	12 μM	70 μM	34%
64	agonist	390 nM	-	-
65	agonist	2.8 nM	-	-
66	agonist	820 nM	-	-

We retained the 11beta-phenyl group and replaced the dimethyl amino functionality with a series of alkyl ethers linked to piperidine. We increased the steric bulk, but maintained side chain flexibility through use of this linker. We varied the length of the linker and also the 17alpha substituent. We also removed the phenyl intermediary and directly linked the alkyl ether piperidine to the steroid. As listed in Table 9, this group of compounds displayed better characteristics. Compounds **67** and **68** are potent partial agonists.

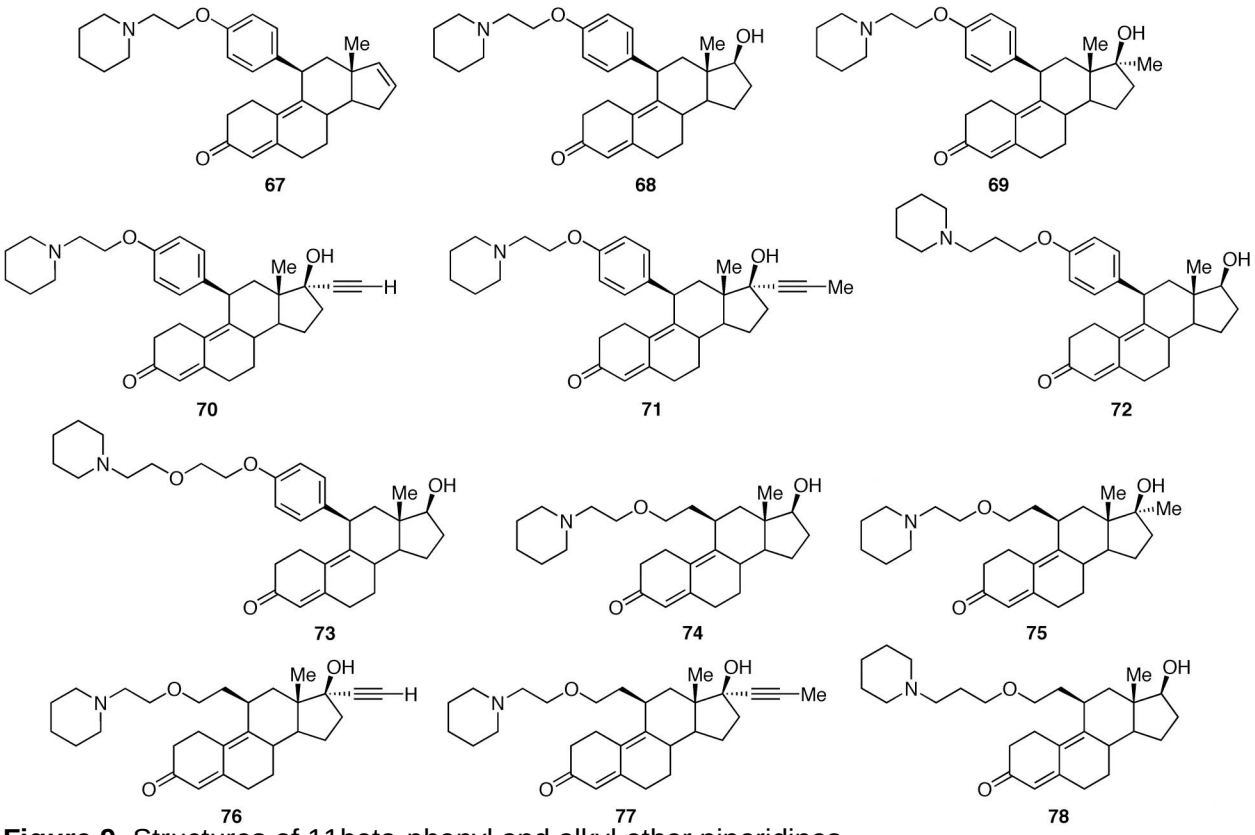


Figure 9. Structures of 11beta-phenyl and alkyl ether piperidines.

Table 9. Compilation of data from full-length AR reporter gene assays for compounds **67**-**78.** EC_{50} values were derived from dose response assays, and IC_{50} values were from the competition assay. The % inhibition value represents the maximal % decrease from 1 nM DHT-mediated transcription in competition assay format.

Ligand	Character	EC50	IC50	% Inhibition
DHT	agonist	0.03 nM	-	-
Mifepristone (**1**)	partial agonist	1 nM	2.1 nM	81%
67	partial agonist	15 μM	0.016 nM	45%
68	partial agonist	210 nM	8.4 nM	60%
69	agonist	1.6 nM	-	-
70	agonist	2.3 nM	-	-
71	agonist	2.8 nM	-	-
72	agonist	1.3 nM	-	-
73	agonist	17.6 nM	-	-
74	partial agonist	1.7 μM	74 nM	48%
75	-	-	-	-
76	weak agonist	183 μM	-	-
77	weak agonist	527 μM	-	-
78	weak agonist	917 μM	-	-

Because **67** and **68** represented inhibitors with high potency and significant antagonism, we tested them in a GR reporter gene assay and in a AR two hybrid assay. We compared the structure activity series from **67**-**71**. In the GR assay (Figure 10), none of these compounds exhibited agonist activity. In the competition assay, although certain compounds activated slightly, no GR antagonism was observed.

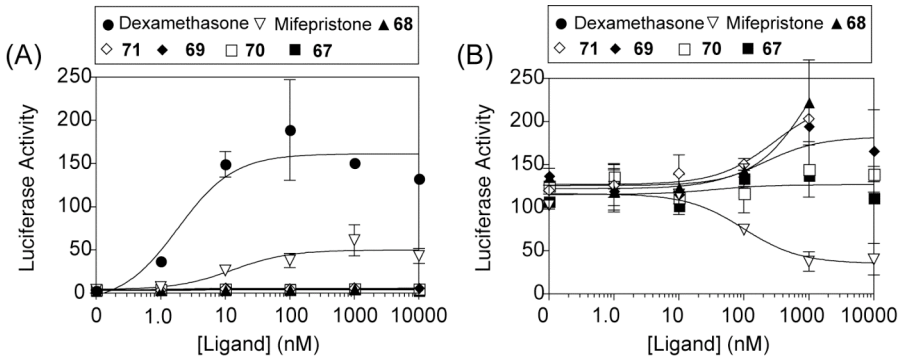


Figure 10. GR reporter gene assay data. Panel A: Dose response of controls dexamethasone and mifeprisone (**1**) as well as compounds **68**-**71**. Panel B: Competition assay for the same compounds in the presence of 100 nM dexamethasone.

In the AR two hybrid assay shown in Figure 11, **68** was a more potent antagonist than mifepristone (**1**), although it could not inhibit DHT-mediated dimerization to quite the same degree. Compound **67** was a less potent partial agonist. In contrast, **69** and **70** were agonists. The compound with the largest 17alpha substituent (**71**) displayed partial agonist characteristics, with weaker antagonism that **67**.

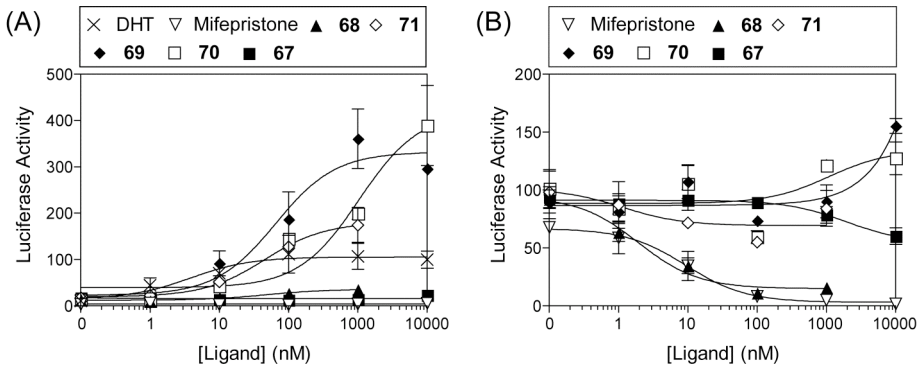


Figure 11. AR two hybrid assay. Panel A: Dose response. Panel B: Competition in the presence of 1 nM DHT.

Table 10. Compilation of data for AR two hybrid assay data.

Ligand	Character	EC50	IC50	% Inhibition
DHT	agonist	3.9 nM	-	-
Mifepristone (**1**)	antagonist	-	9.4 nM	97%
67	partial agonist	0.24 nM	1.9 μM	42%
68	antagonist	48 nM	1.9 nM	83%
69	agonist	65 nM	-	-
70	agonist	1 μM	-	-
71	partial agonist	46 nM	1.1 nM	20%

We also compared compounds **68**, **26** and **36**, which showed the greatest antagonism in the full length AR reporter gene assays (Table 11). In the two hybrid AR assay, as shown in Figure 12, **68** is nearly as good as mifepristone (**1**) at knocking out dimerization of the two AR fragments. Compound **26** and **35** are also able to decrease dimerization-mediated reporter gene expression but much less potently and to a lesser degree.

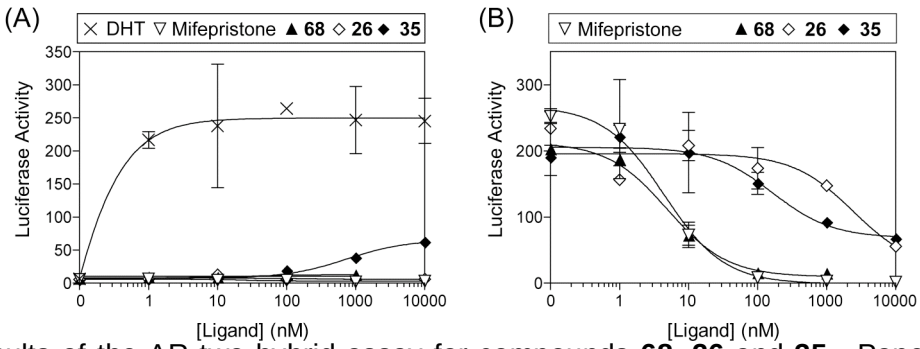


Figure 12. Results of the AR two hybrid assay for compounds **68**, **26** and **35**. Panel A: Dose response with controls DHT and mifepristone (**1**). Panel B: Competition in the presence of 1 nM DHT.

Table 11. Compilation of data for AR two hybrid assay for compounds **68**, **26**, **35** and the control mifepristone (**1**).

Ligand	Character	EC50	IC50	% Inhibition
DHT	agonist	50 pM	-	-
Mifepristone (**1**)	antagonist	29 nM	4.5 nM	99%
68	antagonist	30 nM	5.0 nM	93%
26	antagonist	123 nM	2.4 μM	77%
35	partial agonist	743 nM	163 nM	65%

As shown in Figure 13, in a GR reporter gene assay, **68** activates very slightly at 1 mM, but the three compounds studied do not significantly activate the receptor. In the competition assay, **26** demonstrates slight antagonism at the highest concentration, but the control mifepristone (**1**) shows a much greater decrease.

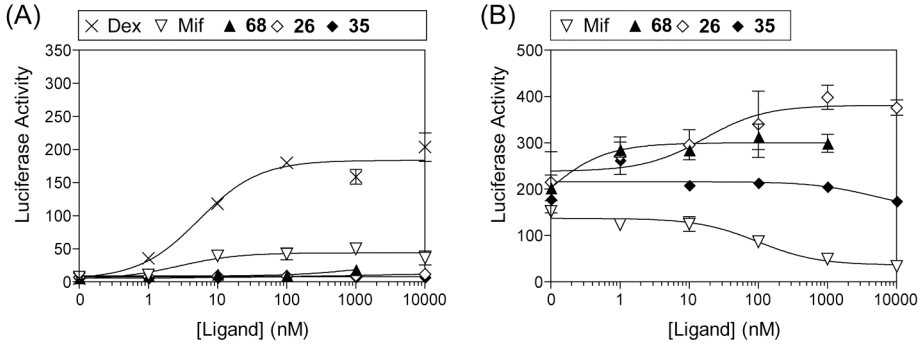


Figure 13. GR reporter gene assay data for compounds **99**, **57** and **66** with controls dexamethasone (**23**) and mifepristone (**1**). Panel A: Dose response assay. Panel B: Competition in the presence of 100 nM dexamethasone (**23**).

Key Research Accomplishments

We identified several novel and potent partial agonists of the AR. We are further optimizing these compounds to identify novel agents effective against prostate cancer.

Reportable Outcomes

None

Conclusions

We evaluated a large number of nortestosterone analogues as AR ligands. Mifepristone (**1**) was used as a model AR antagonist, and related compounds were synthesized by altering the 11beta and 17alpha positions of this steroid. The 17alpha propyne of mifepristone proved to be an important structural feature; replacement with smaller groups created partial agonists with less inhibitory capacity. Insertion of a rigid ethyne linker between the phenyl group and the steroid backbone created agonist compounds, no matter how the phenyl or 17alpha position was substituted. Replacement of the phenyl group with a piperidine proved to be a better strategy. A number of substituted piperidine compounds were potent partial agonists. Compound **35** decreased DHT-mediated AR transactivation by about 50%, and inhibited AR dimerization to a greater extent. This compound displayed slight GR antagonism, but much less than mifepristone (**1**). Interestingly, some closely related versions with unsaturated piperidine side chains displayed only agonist activity.

Substituted piperazines linked by alkyl chains to the steroid displayed moderate antagonism, but we were more successful in appending piperidines to the phenyl group through an alkyl or alkyl ether linkage. Compound **26** was a partial agonist in the AR transactivation assays able to reduce DHT-mediated activity by about 50%. This compound reduced AR dimerization significantly, but with low potency, and it demonstrated little GR activity. Another of this class of compounds, **68**, was the best compound identified, better than our previously published 11beta-alkyl analogues. Compound **68** is a potent and complete inhibitor of dimerization in the AR two hybrid assay, and more importantly it is also a potent partial agonist, with the ability to decrease DHT-mediated AR transactivation by 60%. Whereas mifepristone significantly cross-reacts with the glucocorticoid receptor, **68** demonstrated little GR activity in dose response and competition formats. Future work will involve experiments to determine whether **68** and related compounds inhibit dimerization of the full length receptor in living cells and function as agents effective against prostate cancers in vitro and in vivo.

References

1. Lin, M. F.; Kawachi, M. H.; Stallcup, M. R.; Grunberg, S. M.; Lin, F. F. *Prostate* **1995,** 26, (4), 194-204.
2. El Etreby, M. F.; Liang, Y.; Johnson, M. H.; Lewis, R. W. *Prostate* **2000,** 42, (2), 99-106.
3. Liang, Y.; Eid, M. A.; El Etreby, F.; Lewis, R. W.; Kumar, M. V. *Int. J. Oncol.* **2002,** 21, (6), 1259-1267.
4. Fuhrmann, U.; Hess-Stumpp, H.; Cleve, A.; Neef, G.; Schwede, W.; Hoffmann, J.; Fritzemeier, K.-H.; Chwalisz, K. *J. Med. Chem.* **2000,** 43, 5010-5016.
5. Honer, C.; Nam, K.; Fink, C.; Marshall, P.; Ksander, G.; Chatelain, R. E.; Cornell, W.; Steele, R.; Schweitzer, R.; Schumacher, C. *Mol. Pharmacol.* **2003,** 63, (5), 1012-1020.

Appendices - None