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NEW NONSTEROIDAL ANDROGEN RECEPTOR MODULATORS BASED ON 4-(TRIFLUOROMETHYL)-2(1*H*)-PYRROLIDINO[3,2-*g*]QUINOLINONE

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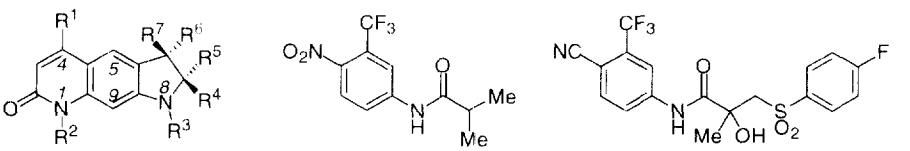
Abstract: A series of 2(1*H*)-pyrrolidino[3,2-*g*]quinolinones was prepared and tested for the ability to modulate the transcriptional activity of the human androgen receptor (hAR). The parent compound, 4-(trifluoromethyl)-2(1*H*)-pyrrolidino[3,2-*g*]quinolinone, displayed moderate interaction with hAR, but more substituted analogues, particularly 6,7-disubstituted compounds, were potent hAR agonists in vitro.

Introduction. Recent efforts to optimize a novel series of human androgen receptor (hAR) antagonists[2] based on a linear tricyclic pharmacophore, 4-(trifluoromethyl)-2(1*H*)-piperidino[3,2-*g*]quinolinone, led us to explore the ring-contracted pyrrolidino compounds, **1**. A number of nonsteroidal hAR antagonists have been described in the literature and two, flutamide (**2**)[3] and bicalutamide (**3**),[4] are used clinically in conjunction with LHRH agonists for the treatment of prostate cancer. Although steroidal hAR agonists are also useful clinically,[5] few nonsteroidal agonists have been disclosed. In this paper we describe initial efforts to determine the effects of alkyl-group substitution at various positions on the hAR modulatory activity of the 2(1*H*)-pyrrolidino[3,2-*g*]quinolinone pharmacophore **1**.



1 **2** **3**

Figure 1. 2(1*H*)-Pyrrolidino[3,2-*g*]quinolinone General Structure (**1**), Flutamide (**2**), and Bicalutamide (**3**).

Chemistry. The preparation of linear tricyclics of general structure **1** followed one of three routes. A number of compounds could be prepared from the corresponding indoles, several of which were commercially available (Scheme 1). The preparation of the non-commerical indoles followed a standard Fischer protocol[6] and reduction to the indoline proceeded uneventfully with $Na(CN)BH_3$,[7,8] to afford exclusively the *cis*-diastereomers for disubstituted indoles. Regiospecific *meta*-nitration (conc. H_2SO_4/70% HNO_3) followed by hydrogenation afforded the anilines necessary for Knorr cyclization[9] to the *N,N*-unsubstituted, 4-(trifluoromethyl)-2(1*H*)-pyrrolidino[3,2-*g*]quinolinones **1**. At this stage of the project, we concentrated on the 4-trifluoromethyl substituted analogues based on previous work.[2] The quinolinone nitrogen could be selectively alkylated by deprotonation with NaH followed by treatment with an alkyl halide, whereas reductive amination occurred only at the indoline nitrogen. All chiral compounds at this stage of the project were tested as racemates.[10]

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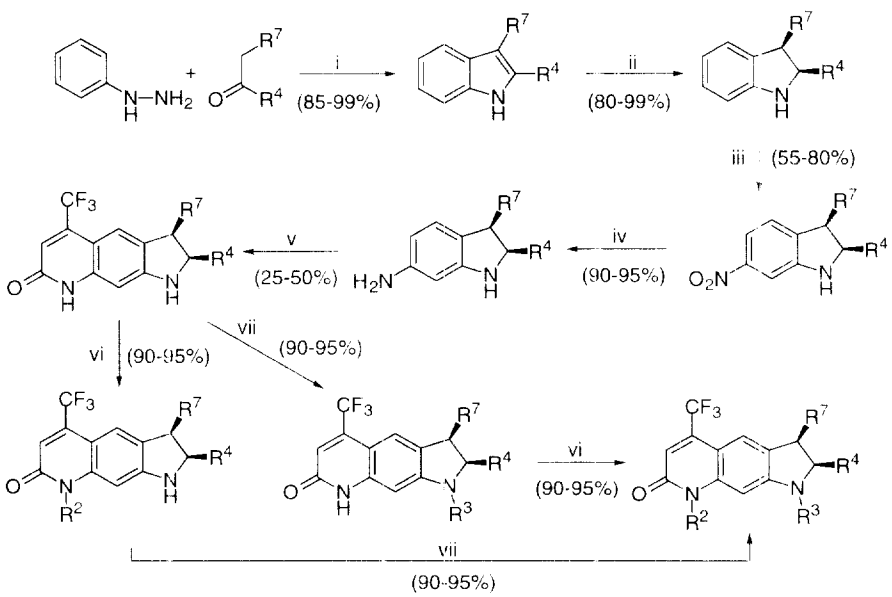
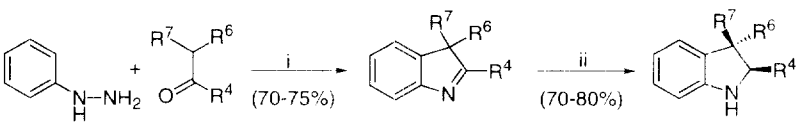
Scheme 1[a]



[a](i) AcOH, 60 °C; (ii) $Na(CN)BH_3$, AcOH, rt; (iii) 70% HNO_3, H_2SO_4, 0 °C; (iv) H_2, 10% Pd/C, EtOAc/EtOH; (v) ethyl 4,4,4-trifluoroacetoacetate, $ZnCl_2$, EtOH, Δ; (vi) R^2I, NaH, DMF, 0 °C; (vii) RCHO, $Na(CN)BH_3$, AcOH, rt

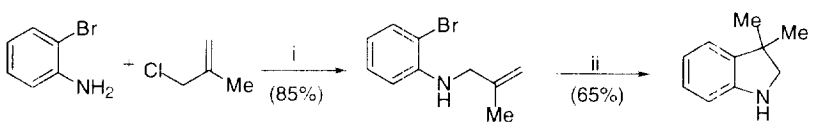
C(6)-Disubstituted compounds were prepared from either the corresponding indolenine (Scheme 2) or by utilizing an intramolecular Heck cyclization of an appropriately-substituted allyl aniline (Scheme 3).[11] The indolines thus obtained were converted to the linear tricyclics in a manner identical to that depicted in Scheme 1

Scheme 2[a]



[a](i) AcOH, 60 °C; (ii) $Na(CN)BH_3$, AcOH, rt

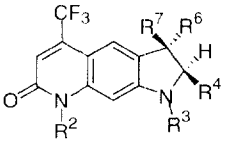
Scheme 3[a]



[a](i) K_2CO_3, DMF, rt; (ii) $Pd(OAc)_2$, DMF, $NaHCO_3$, 90 °C

In Vitro Biological Activity. The ability of the 2(1*H*)-pyrrolidino[3,2-*g*]quinolinones **1** to modulate the transcriptional activity of hAR in a cellular context was measured using a cotransfection assay in mammalian (CV-1) cells,[12,13] whereas binding to hAR was measured using a whole cell assay system in mammalian (COS) cells.[14] The data for 12 compounds designed to determine the effects of methyl substituents at various positions of the pharmacophore are depicted in Table 1, along with data for dihydrotestosterone (DHT) as a standard steroidal hAR agonist and bicalutamide as a standard nonsteroidal hAR antagonist.

Table 1. hAR Agonist and Antagonist Activity in Cotransfected CV-1 Cells and Binding Affinities to hAR Transfected into COS Cells.



1

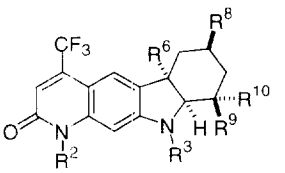
#	R^2	R^3	R^4	R^6	R^7	hAR Cotransfection Assay in CV-1 Cells: Agonist Efficacy (%)	Agonist EC_{50} (nM)	Agonist N	Antagonist Efficacy (%)	Antagonist IC_{50} (nM)	Antagonist N	hAR Whole Cell Binding K_i (nM)	N
	Dihydrotestosterone					100 ± 0	6 ± 1	(9)	--	--		2 ± 1	(9)
3	Bicalutamide					--	--		78 ± 3	157 ± 35	(9)	82 ± 21	(7)
4	H	H	H	H	H	27 ± 3	1897 ± 133	(2)	71 ± 4	23 ± 5	(7)	41 ± 3	(2)
5	Me	H	H	H	H	--	--	(2)	67 ± 6	56 ± 16	(3)	61 ± 18	(3)
6	Me	Me	H	H	H	--	--	(1)	94 ± 5	197 ± 52	(2)	167 ± 31	(2)
7	H	H	Me	H	H	40 ± 5	168 ± 89	(5)	55 ± 7	7 ± 3	(3)	10 ± 1	(3)
8	Me	H	Me	H	H	66 ± 10	20 ± 2	(3)	--	--	(3)	9 ± 3	(4)
9	H	H	Me	H	Me	81 ± 6	5 ± 1	(9)	--	--	(2)	6 ± 1	(9)
10	Me	H	Me	H	Me	37 ± 6	15 ± 3	(6)	35	9182	(1)	10 ± 2	(4)
11	H	H	H	Me	H	43 ± 11	62 ± 20	(3)	--	--	(3)	17 ± 3	(4)
12	H	H	H	Me	Me	38 ± 9	42 ± 8	(4)	39 ± 10	10 ± 6	(4)	28 ± 5	(2)
13	H	H	Me	Me	Me	35 ± 8	99 ± 33	(5)	23 ± 12	48 ± 43	(3)	21 ± 4	(5)
14	Me	H	Me	Me	Me	34 ± 3	380 ± 224	(6)	33 ± 9	22 ± 4	(8)	9 ± 1	(5)
15	Me	Me	Me	Me	Me	--	--	(1)	82 ± 2	56 ± 8	(4)	33 ± 7	(2)

[a]Efficacy for agonist assays is defined in % vs. DHT = 100. Efficacy for antagonist assays is % inhibition of transcriptional activity observed at an EC_{50} concentration of DHT.
[b]Values are in nM, mean ± SEM, N > 2. If no SEM is noted, value is from a single determination. N = number of independent determinations.
"--" = not active (<20% efficacy or > 10 μM potency)

While the parent compound **4** showed encouraging levels of activity, variability of this activity from week to week suggested some sort of metabolic degradation during the cellular assay. The simple addition of a methyl group to N1 (**5**) afforded a compound with comparable, and now more reproducible, activity. Methylation of

both nitrogens (**6**) resulted in a slight loss of activity. The addition of a methyl group to C7 was well-tolerated (**7**) and subsequent N1-methylation of this compound afforded **8**, a potent hAR partial agonist in this assay. The most interesting compound of this group was **9**, (R^4 = R^7 = Me), which mimicked the effects of DHT in the cotransfection assay with a comparable potency but with slightly attenuated efficacy. Other modifications led to decreased agonist activity, to the point that the pentamethyl analogue **15** proved to be a potent hAR antagonist. Notably, all of the methylated compounds **7**–**15** interacted strongly with hAR as indicated by their ability to displace DHT in the whole binding assay with K_is of 50 nM or less and three compounds (**8**, **9**, and **14**) had binding affinities (K_i) of less than 10 nM.

Table 2. hAR Agonist and Antagonist Activity in Cotransfected CV-1 Cells and Binding Affinities to hAR Transfected into COS Cells [a]



1'

#	R^2	R^3	R^6	R^8	R^9	R^{10}	hAR Cotransfection Assay in CV-1 Cells: Agonist Efficacy (%)	Agonist EC_{50} (nM)		Antagonist Efficacy (%)	Antagonist IC_{50} (nM)		hAR Whole Cell Binding K_i (nM)
	Dihydrotestosterone						100 ± 0	6 ± 1	(9)	--	--		2 ± 1
3	Bicalutamide						--	--		78 ± 3	157 ± 35	(9)	82 ± 21
16	H	H	H	H	H	H	91 ± 6	5 ± 1	(9)	--	--	(3)	5 ± 1
17	Me	H	H	H	H	H	61 ± 5	10 ± 2	(9)	--	--	(7)	4 ± 1
18	Me	Me	H	H	H	H	--	--	(3)	73 ± 6	36 ± 12	(9)	10 ± 1
19	Et	H	H	H	H	H	--	--	(1)	94 ± 2	230 ± 16	(3)	194 ± 51
20	Me	Pr	H	H	H	H	33	324	(1)	48 ± 2	27 ± 1	(4)	63 ± 21
21	H	H	H	Me	H	H	53 ± 6	64 ± 25	(5)	--	--	(1)	23 ± 10
22	Me	H	H	Me	H	H	--	--	(1)	77 ± 7	48 ± 15	(3)	94 ± 92
23	Me	Me	H	Me	H	H	--	--	(1)	89 ± 1	54 ± 20	(4)	78 ± 26
24	H	H	Me	H	H	H	60 ± 9	25 ± 2	(7)	--	--	(3)	21 ± 7
25	Me	H	Me	H	H	H	36 ± 5	33 ± 6	(6)	--	--	(4)	9 ± 2
26	Me	Me	Me	H	H	H	--	--	(2)	72 ± 5	53 ± 20	(4)	97
27	H	H	Me	H	Me	Me	--	--	(1)	76	51	(1)	77 ± 7
28	H	H	H	H	Me	Me	--	--	(5)	67 ± 5	26 ± 9	(4)	79
29	Me	Me	H	H	Me	Me	--	--	(1)	90 ± 1	27 ± 9	(2)	59

[a]See Table 1 for legend.

The potent in vitro activity of **9** combined with the fact that many of the compounds in Table 1 were sparingly soluble in most solvents prompted us to investigate the tetracyclic analogues **1'** (Table 2). Indeed, the parent

compound **16** (LG121100) was equipotent to **9**. N1-Methylation maintained agonist activity (**17**), but methylation of both nitrogens (**18**) afforded a potent hAR antagonist. Alkyl groups larger than methyl on either nitrogen resulted in attenuated activity (**19–20**). Additional substitution of the cyclohexyl moiety resulted in either attenuated agonist activity (**21** and **24**) or a switch to antagonist activity (**27** and **28**). An interesting effect of N-methylation was noted. Whereas N1-methylation of **16** had a small effect on agonist activity, N1-methylation of **21** resulted in a complete switch to antagonist activity (**22**). In addition, N1-methylation of **24** resulted in an almost complete loss of functional activity, but interaction with hAR was still high, as measured by whole-cell binding (**25**). These data can be contrasted with the tricyclic analogue **7**: the parent was a potent mixed agonist/antagonist which upon N1-methylation (**8**) became a potent agonist in the cotransfection assay.

The cross-reactivity of compounds **4–29** with related intracellular receptors was assessed using human progesterone (hPR), glucocortocoid (hGR), estrogen (hER), and mineralocortocoid (hMR) cotransfection assays (Table 3). No agonist activity was detected for any of these compounds using these assays, but moderate antagonist activity was observed (Table 3), particularly on the progesterone receptor. Compounds which showed the most significant (<500 nM) cross-reactivity are tabulated. The linear tetracyclic compounds **16** and **17** were fully efficacious hPR antagonists with significant potencies, comparable to several of the homologues prepared earlier.[2] No interaction with hER was observed, and with a single exception (**27**, 570 nM hMR antagonist) only weak μM activity on hGR and hMR was found.

Table 3. hPR, hGR, hER, and hMR Antagonist Activity in Cotransfected CV-1 Cells for Selected Quinolones.[a]

#	hPR Eff. (%)	hPR IC_{50} (nM)	hGR Eff. (%)	hGR IC_{50} (nM)	hER Eff. (%)	hER IC_{50} (nM)	hMR Eff. (%)	hMR IC_{50} (nM)
7	89	750	<20	>10000	<20	>10000	<20	>10000
9	88	1900	35	2500	<20	>10000	29	2000
16	91	170	33	5800	<20	>10000	54	2000
17	99	150	39	3000	<20	>10000	58	2900
18	98	1000	<20	>10000	<20	>10000	<20	>10000
22	97	380	<20	>10000	<20	>10000	<20	>10000
24	96	730	43	2100	<20	>10000	43	1900
27	96	430	31	1400	<20	>10000	77	570

[a]See Table 1 for legend.

Conclusion. The data presented for these series of linear tri- and tetracyclic pyrrolidino[3,2-*g*]quinolones form a basis for further development of nonsteroidal hAR modulators to address unmet clinical needs. The in vitro activity of the agonists presented above demonstrate for the first time that nonsteroidal pharmacophores can activate the human androgen receptor with potencies comparable to the natural hormone, dihydrotestosterone.[15] Simple substitutions of the parent structure afforded both hAR agonists and hAR

antagonists, demonstrating that this template will prove to be a valuable addition to the sex steroid receptor modulator field.

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8. The relative stereochemistry of all diastereomeric compounds in this paper was unambiguously assigned by NOE experiments.
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10. The preparation of **16** is illustrative. *1,2,3,4,4a,9b-Hexahydro-7-nitrocarbazole.* In a 100-mL r.b. flask, a solution of 1,2,3,4,4a,9b-hexahydrocarbazole (1.34 g, 7.73 mmol) in conc. H_2SO_4 (10 mL) was cooled to -5 °C and 90% fuming HNO_3 (0.35 mL) was added dropwise (approx. 8 sec between drops). After the addition was complete, the reaction mixture was stirred an additional 5 min and poured onto ice (100 g). To this slurry, K_2CO_3 (approx. 6 g) was CAREFULLY added portionwise to neutralize the acid. The product was extracted with CH_2Cl_2 (3 x 100 mL). The extracts were washed with sat'd $NaHCO_3$ (1 x 60 mL), combined, dried ($MgSO_4$), filtered through a pad of Celite, and concentrated to an orange oil which solidfies upon cooling (1.63 g, 97%): R_f 0.45 (hexane:EtOAc, 4:1); ^1H NMR (400 MHz, $CDCl_3$) 7.63 (dd, J = 7.9, 2.0, 1H), 7.41 (d, J = 2.0, 1H), 7.13 (d, J = 7.9, 1H), 3.94 (br s, 1H), 3.84 (dd, J = 12.7, 6.6, 1H), 3.15 (dd, J = 13.2, 6.6, 1H), 1.80-1.67 (m, 3H), 1.55 (m, 2H), 1.42-1.25 (m, 3H). *7-Amino-1,2,3,4,4a,9b-hexahydrocarbazole.* In a 200-mL r.b. flask, a solution of 1,2,3,4,4a,9b-hexahydro-7-nitrocarbazole (1.61 g) in 1:1 EtOAc:EtOH (60 mL) was treated with 10% Pd/C (0.41 g) and purged with N_2. The reaction mixture was stirred under an atmosphere of H_2 for 5 h, purged, and filtered through a pad of Celite. The pad was rinsed with EtOAc (1 x 50 mL) and MeOH (1 x 50 mL) and the filtrate was concentrated to a tan solid (1.38 g, 99%) which was used without purification. *(R/S-7aI, 10aα)-6b,7,8,9,10,10a-Hexahydro-5-(trifluoromethyl)-3-pyridono[5,6-b]carbazole* (**16**). In a 50-mL r.b. flask, a solution of 7-amino-1,2,3,4,4a,9b-hexahydrocarbazole (0.50 g, 2.7 mmol) in EtOH (10 mL) was treated with ethyl 4,4,4-trifluoroacetoacetate (0.39 mL, 2.7 mmol, 1.0 equiv), and $ZnCl_2$ (0.54 g, 4.0 mmol, 1.5 equiv), and heated to reflux for 16 h. The reaction mixture was allowed to cool to rt, the bulk of the EtOH was removed in vacuo, and the residue was poured into water (30 mL). The product was extracted with EtOAc (3 x 30 mL). The extracts were washed with 0.5% HCl (1 x 30 mL) and brine (1 x 30 mL), combined, dried ($MgSO_4$), filtered, and concentrated. Two major products are observed (R_f 0.15 and 0.80 in CH_2Cl_2:MeOH, 15:1), corresponding to the quinolinone 16 and its *O*-ethyl quinoline analogue.[2] The residue was purified by silica gel chromatography (CH_2Cl_2:MeOH, 60:1 to 15:1 gradient) to afford 0.28 g of the ethoxyquinoline (R_f 0.85; 33%) and 0.28 g (34%) of **16**: R_f 0.15 (CH_2Cl_2:MeOH, 15:1), ^1H NMR (400 MHz, acetone-d_6) 10.85 (br s, 1H), 7.34 (s, 1H), 6.61 (s, 1H), 6.49 (s, 1H), 5.92 (s, 1H), 3.87 (m, 1H), 3.17 (dd, J = 13.6, 6.8, 1H), 1.85-1.70 (m, 3H), 1.56 (m, 2H), 1.44 (m, 3H).
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14. A complete description of these assays, including materials and methods, can be found in reference 2.
15. The in vitro agonist activity of **16** has been confirmed in vivo using a rodent model (castrated rat acute LH suppression assay): Wang, X.-N., unpublished results.