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SWITCHING ANDROGEN RECEPTOR ANTAGONISTS TO AGONISTS BY MODIFYING C-RING SUBSTITUENTS ON PIPERIDINO[3,2-*g*]QUINOLINONE

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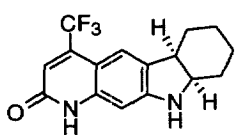
Abstract: New nonsteroidal human androgen receptor (hAR) agonists were developed from an hAR antagonist pharmacophore, 2(1*H*)-piperidino[3,2-*g*]quinolinone. (±)-*trans*-7,8-Diethyl-4-trifluoromethyl-2(1*H*)-piperidino-[3,2-*g*]quinolinone was synthesized and demonstrated potent hAR agonist activity (EC_{50} = 3 nM) in the cell-based cotransfection assay and high binding affinity (K_i = 16 nM) in the competitive receptor binding assay.

Introduction: In contrast to nonsteroidal antiandrogens, which have been successfully used in clinic,[2] nonsteroidal androgens had not been known in literature until recently. Both of the reported new nonsteroidal androgens (**1** and **2**)[3] were derived from analoguing antiandrogen pharmacophores. In our effort to develop novel human androgen receptor (hAR) modulators we reported the discovery of a linear tricyclic hAR antagonist pharmacophore, 2(1*H*)-piperidino[3,2-*g*]quinolinone (**3**).[4] To take advantage of this orally available pharmacophore, we systematically investigated the substitution pattern at the C-ring.



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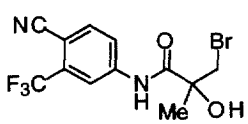

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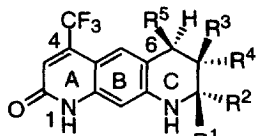

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Chemistry: The analogues of generic structure **3** (R^2 = H) were prepared by the synthetic sequence shown in Scheme 1. The preparation of **4** started from conjugate addition of aniline to an olefinic acid and a PPA mediated cyclization followed by nitrogen protection with a *t*-Boc group. The 2,4-disubstituted tetrahydroquinolines (**5**, $R^2 = R^3 = R^4$ = H) were synthesized by Grignard addition to **4** and deprotection of *t*-Boc followed by palladium catalyzed hydrogenation in the presence of an acid. The 2,3-di- or 2,3,3-trisubstituted tetrahydroquinolines (**5**, $R^2 = R^5$ = H) were prepared by alkylation of **4** with iodoalkane and deprotection of *t*-Boc followed by BF_3 catalyzed Et_3SiH reduction. Nitration of **5** followed by palladium catalyzed hydrogenation provided aminoquinolines **6**, which were subsequently converted to **3** via Knorr cyclization.[5] Stereoisomers were separated by HPLC and chiral compounds were tested as racemates. The preparation of compounds **24** and **25** are illustrative. [6]

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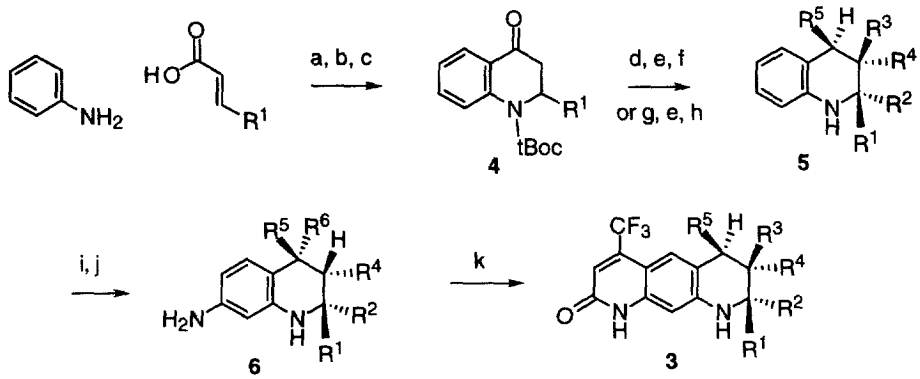
Scheme 1[a]



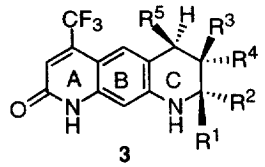
[a]Reagents: (a) toluene, reflux, 20 h; (b) PPA, 110 °C, 6 h; (c) *t*-Boc_2O, DMAP, THF, rt, 6 h; (d) R^5MgX, THF, rt; (e) TFA, CH_2Cl_2, rt, 30 min; (f) Pd/C, H_2, EtOAc, H_2SO_4, rt, 15 h; (g) R^3I or R^4I, NaH, DMF, rt, 15 h; (h) Et_3SiH, BF_3-OEt_2, CH_2Cl_2, sealed tube, 100 °C, 24 h; (i) H_2SO_4, HNO_3, -10 °C, 10 min; (j) Pd/C, H_2, EtOAc, rt, 15 h; (k) $EtO_2CCH_2COCF_3$, $ZnCl_2$, EtOH, reflux, 8 h.

Results and Discussion: A number of 8,8-dialkyl analogues have shown potent hAR antagonist activity in the cell-based cotransfection assay and high affinity in the receptor competitive binding assay, in addition, compound **7** demonstrated potent in vivo antiandrogenic activity following oral administration.[4] To reveal additional structure–activity relationship (SAR) of the pharmacophore, we continued to use the cotransfection and the binding assays as guides. Our initial attempt of removing one of the 8-alkyl substituents resulted in four analogues. Compounds **8** and **9** showed antagonist efficacy weaker than parent compound **7** but with stronger binding affinity to hAR (see Table 1). Additionally, compound **8** demonstrated potent partial agonist activity. To introduce a small alkyl group at C6, four *cis*-isomers (**12-15**) of 6,8-dialkyl analogues were prepared by the current synthetic route and all of them gave potent partial agonist activity similar to **8**. Interestingly, among the 7,8-dialkyl analogues compounds **22** (LG121091) and **24** (LG121104) showed potent full agonist activity similar to that of dihydrotestosterone in the hAR cotransfection assay. It was noticed that the proper size of 7-alkyl substituent was essential for the full agonist activity (i.e., 7-ethyl or propyl gave agonists and 7-methyl gave partial or full antagonists) and the size of 8-substitution is less crucial. The *trans*-isomers (**22** and **24**) afforded better agonist activity than their *cis*-isomers (**23** and **25**) although both have similar binding affinity. The 7,7,8-trialkyl analogues (**18**, **21** and **26**) were also examined and all of them are hAR antagonists.

The cross-reactivity of the new AR agonists **22** and **24** was evaluated using human progesterone, estrogen, glucocorticoid, and mineralocorticoid receptor (hPR, hER, hGR, and hMR) cotransfection assays and only hPR antagonist activity was observed. The IC_{50} values of **22** and **24** in hPR cotransfection assay are 280 and 260 nM with 78% and 73% efficacy.[7]

Conclusion: Several selective and potent hAR agonists were obtained from a known antagonist pharmacophore **3** (R^1, R^2 = alkyl) by modifying the substituents on the C-ring.[8] The size and relative stereochemistry at C7/C8 are essential for the agonist activity. This new discovery combined with other results[3a,9] from our laboratory provided a solid foundation of developing new nonsteroidal hAR agonists for clinical uses.

Table 1. Cotransfection and Competitive Binding Data for the Quinolinone Analogues.[a]



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#	R^1	R^2	R^3	R^4	R^5	hAR Agonist[b] mean ± SEM Eff(%)	hAR Agonist[b] mean ± SEM EC_{50} (nM)[c]	hAR Antagonist[b] mean ± SEM Eff(%)	hAR Antagonist[b] mean ± SEM IC_{50} (nM)[c]	hAR Binding[e] mean ± SEM K_i (nM)
		Dihydrotestosterone				100 ± 0	6 ± 1	-[d]	-	2 ± 0.4
		2-Hydroxyflutamide				-	-	83 ± 1	15 ± 2	27 ± 8
7	Me	Me	H	H	H	-	-	74 ± 2	27 ± 5	26 ± 5
8	Me	H	H	H	H	33 ± 5	21 ± 9	37 ± 8	19 ± 13	7 ± 1
9	Et	H	H	H	H	-	-	55 ± 5	11 ± 2	8 ± 2
10	Pr	H	H	H	H	-	-	84 ± 4	32 ± 6	24 ± 5*
11	i-Pr	H	H	H	H	-	-	82 ± 4	25 ± 9	109 ± 44
12	Me	H	H	H	Me	24 ± 2*	59 ± 9*	52 ± 6	15 ± 6	6 ± 3
13	Me	H	H	H	Et	30 ± 8*	66 ± 35*	-	-	23
14	Et	H	H	H	Et	47 ± 9	32 ± 8	-	-	9 ± 1*
15	Et	H	H	H	Me	37 ± 10	119 ± 72	40 ± 2	137 ± 98	21 ± 8
16	Me	H	H	Me	H	-	-	66 ± 4	33 ± 15	22 ± 6
17	Me	H	Me	H	H	26 ± 4	34 ± 8	41 ± 9	74 ± 48	15 ± 3
18	Me	H	Me	Me	H	-	-	78 ± 5	24 ± 7	82 ± 18
19	Et	H	H	Me	H	31	1668	40 ± 8	11 ± 3	12 ± 0
20	Et	H	Me	H	H	45	2468	66 ± 9	15 ± 6	11 ± 2
21	Et	H	Me	Me	H	-	-	90 ± 2*	42 ± 5*	50 ±6
22	Me	H	H	Et	H	89 ± 9	9 ± 1	-	-	11 ± 3
23	Me	H	Et	H	H	39 ± 7	10 ± 2	-	-	15 ± 4
24	Et	H	H	Et	H	114 ± 18	3 ± 0	-	-	16 ± 2
25	Et	H	Et	H	H	64 ± 7	20 ± 5	-	-	29 ± 4
26	Et	H	Et	Et	H	-	-	69 ± 8	48 ± 15	42 ± 20
27	Et	H	H	Pr	H	69 ± 9	16 ± 5	-	-	32 ± 10

[a]Values with standard errors (SEM) represent the mean value of at least three separate experiments with triplicate determinations and values without standard deviation represent a single experiment and values with * represent the mean value of two experiments with standard deviation. [b]Agonist efficacies were compared to that of dihydrotestosterone (100%) and antagonist efficacies were determined as a function (%) of maximal inhibition of dihydrotestosterone (EC_{50}). [c]All EC_{50} and IC_{50} values were determined from full dose-response curves ranging from 10^{-12} to 10^{-5} M in CV-1 cell. [d]A hyphen indicates an efficacy < 20% or a potency >10000 nM. [e]The radioligand used in the competitive binding assay was dihydrotestosterone.

References and Notes

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6. Preparation of **24** and **25**: A mixture of aniline (3.0 g, 32 mmol) and 2-pentenoic acid (2.5 g, 25 mmol) in toluene (20 mL) was heated at reflux for 20 h and removal of solvent followed by chromatography (EtOAc/hexane, 9/1) afforded 3.3 g (72%) of 3-phenylaminopentanoic acid. The acid was treated with PPA (20 mL) at 110 °C for 6 h and the reaction mixture was poured into ice water (50 mL) and then neutralized with Na_2CO_3 to pH 7. Extraction of the mixture with EtOAc (3 × 60 mL) followed by chromatography (EtOAc/hexane, 4/6) afforded 1,2,3,4-tetrahydro-2-ethyl-4-quinolinone (1.7 g, 54%) as a yellow solid. The quinolinone (1.0 g, 6.2 mmol) in THF (25 mL) was treated with di-*t*-butyl dicarbonate (1.6 g, 7.4 mmol) and DMAP (0.94 g, 7.4 mmol) at rt. for 6 h. Removal of solvent and chromatography afforded **4** (R^1 = Et, 1.6 g, 80%). To a solution of **4** (R^1 = Et, 0.20 g, 0.72 mmol) and iodoethane (0.50 mL, 6.3 mmol) in DMF (10 mL) was added NaH (60 % in mineral oil, 40 mg, 1.0 mmol) and the resulting mixture was stirred at rt. for 15 h. The reaction was quenched with water (10 mL) and extracted with EtOAc (2 × 25 mL). Removal of solvent and chromatography (EtOAc/hexane, 1/9) afforded a mixture of products, which was treated with TFA (1.0 ml) in methylene chloride (2.0 mL) for 3 h. The reaction was adjusted to pH 10 by 5% NaOH and extracted with EtOAc (2 × 20 mL). Chromatography (5–20% EtOAc/hexane, gradient) afforded a cis/trans mixture of 2,3-diethyl-1,2-dihydro-4-quinolones (85 mg, 58%) as a colorless oil. The quinolinone mixture (85 mg, 0.42 mmol) was treated with Et_3SiH (1.0 mL) and BF_3-OEt_2 (0.05 mL, 0.4 mmol) in CH_2Cl_2 (1.0 mL) at 100 °C for 15 h in a sealed tube followed by chromatography (EtOAc/hexane, 1/9) afforded a mixture of **5** (R^1 = R^3/R^4 = Et, 70 mg, 88%). The quinoline mixture (70 mg, 0.37 mmol) was converted to the final compounds according to a general nitration-hydrogenation-Knorr procedure[4] in 30% (81%, 83%, 44%) three-step yield as a yellow solid (36 mg, 0.11 mmol) and HPLC separation provided the pure isomers. Compound **24**: mp, 263–264 °C; ^{1}H NMR (400 MHz, acetone-d_6) 10.71 (brs, 1 H), 7.30 (s, 1 H), 6.52 (s, 1 H), 6.41 (s, 1 H), 6.25 (brs, 1 H), 3.15 (m, 1 H), 2.95 (dd, J = 15.8, 4.8, 1 H), 2.58 (dd, J = 15.8, 6.2, 1 H), 1.71 (m, 1 H), 1.63 (m, 2 H), 1.49 (m, 1 H), 1.23 (m, 1 H), 0.99 (t, J = 7.3, 3 H), 0.97 (t, J = 7.3, 3 H); ^{13}C NMR (100 MHz, acetone-d_6) 161.8, 149.0, 141.8, 125.5, 125.2, 122.8, 117.9, 114.3, 105.0, 96.8, 56.8, 56.6, 36.2, 28.7, 25.6, 11.5, 9.8. Compound **25**: ^{1}H NMR (400 MHz, acetone-d_6) 10.67 (bs, 1 H), 7.27 (s, 1 H), 6.52 (s, 1 H), 6.41 (s, 1 H), 6.30 (bs, 1 H), 3.35 (m, 1 H), 2.82 (m, 1 H), 2.71 (dd, J = 16.2, 8.1, 1 H), 1.89 (m, 1 H), 1.60–1.21 (m, 4 H), 1.01 (t, J = 7.5, 3 H) and 0.97 (t, J = 7.5, 3 H).
7. The cross-reactivity profile of **22** and **24** with other steroid receptors is consistent with related series; see ref. 3a and 9.
8. The substituent effects that switch agonist/antagonist activities have been seen in other steroid receptors, e.g. PR see (a) Cook, C. E.; Wani, M. C.; Lee, Y.-W.; Fail, P. A.; and Petrow, V. *Life Sciences* **1992**, *52*, 155, and ER see (b) Bowler J.; Lilley, T. J.; Pittam, J. D.; and Wakeling, A. E. *Steroids* **1989**, *54(1)*, 71.
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