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NONSTEROIDAL ANDROGEN RECEPTOR AGONISTS BASED ON 4-(TRIFLUOROMETHYL)-2*H*-PYRANO[3,2-*g*]QUINOLIN-2-ONE

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Abstract: A series of 2*H*-pyrano[3,2-*g*]quinolin-2-ones was prepared and tested for the ability to modulate the transcriptional activity of the human androgen receptor (hAR). The parent compound, 4-(trifluoromethyl)-2*H*-pyrano[3,2-*g*]quinolin-2-one, displayed moderate interaction with hAR, but substituted analogues were potent hAR modulators in vitro as mesaured by an hAR cotransfection assay in CV-1 cells and bound to hAR with high affinity in a whole cell assay. Several analogues were able to activate hAR-mediated gene transcription more potently and efficaciously than dihydrotestosterone.

Introduction. A recent report from these laboratories described structure–activity relationship studies of a novel series of human androgen receptor (hAR) antagonists[5] based on a linear tricyclic pharmacophore, 4-(trifluoromethyl)-2(1*H*)-piperidino[3,2-*g*]quinolinone, typified by the lead hAR antagonist LG120907 (Figure 1, **1**).[6] We have also explored the lactone analogues, **2**, and discovered that these compounds interact with hAR to elicit very different biological responses. A number of nonsteroidal hAR antagonists has been described in the literature and two of these, flutamide[7] and bicalutamide (**3**),[8] are used clinically in conjunction with LHRH agonists for the treatment of prostate cancer. Although steroidal hAR agonists are also useful clinically,[9] few nonsteroidal androgen agonists have been disclosed.[10] Using an hAR cotransfection assay in CV-1 cells to guide SAR studies,[11] we set out to determine the effects of alkyl-group substitution at various positions on the hAR modulatory activity of the 2*H*-pyrano[3,2-*g*]quinolin-2-one pharmacophore **2**, leading to the discovery of several analogues with potent androgen agonist activity in vitro.

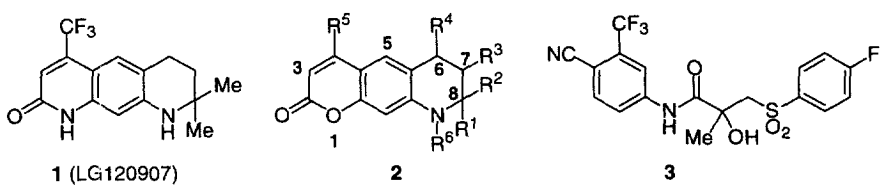

Figure 1. LG120907 (**1**), 2*H*-Pyrano[3,2-*g*]quinolin-2-one General Structure (**2**), and Bicalutamide (**3**).

Background. During preliminary investigations into the SAR of 6-aryl-1,2-dihydro-2,2,4-trimethylquinoline, a novel pharmacophore for the progesterone receptor,[12] we discovered that Skraup cyclization[13] of the 7-aminocoumarin **4a** afforded as a minor product the linear tricyclic compound **5a** in addition to the major angular isomer **6a** (Scheme 1). Although **6a** interacted only weakly with hPR and hAR, compound **5a** (LG120293) was discovered to be quite active as an *anti-androgen* (IC_{50} = 81 nM, 95% efficacy; K_i = 48 nM) and prompted us to investigate the SAR of this new hAR modulator lead. For example, reaction of **4b** with acetone in the presence of

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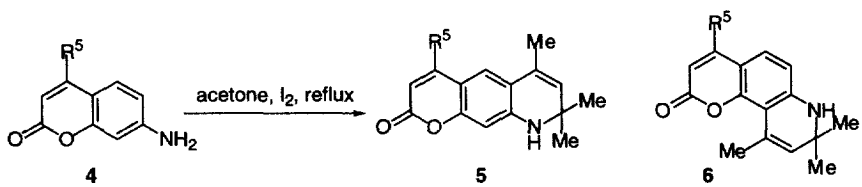
iodine afforded **5b** and **6b** in a ration of 1:19; **5b** not only bound to hAR with high affinity (K_i = 4 nM), but also behaved as an androgen receptor *agonist*, with potency and efficacy similar to that of dihydrotestosterone (EC_{50} = 7 nM, 89% efficacy). In contrast, **4c** afforded **5c** and **6c** in a ratio of 1:2; although **5c** bound to hAR (K_i = 3 nM), it had functional activity intermediate between **5a** and **5b** and acted as a weak partial agonist (EC_{50} = 0.7 μM, 60% efficacy). This hAR modulatory behavior was opposite to that observed for the C(8)-disubstituted nitrogen analogues such as **1**, which are hAR antagonists in this assay.[5] In line with earlier studies,[5,10a] further SAR investigations focused on modifications at C(6)-C(8) in order to improve hAR modulatory activity, leaving constant the coumarin system (e.g., $R^5 = CF_3$).

Scheme 1

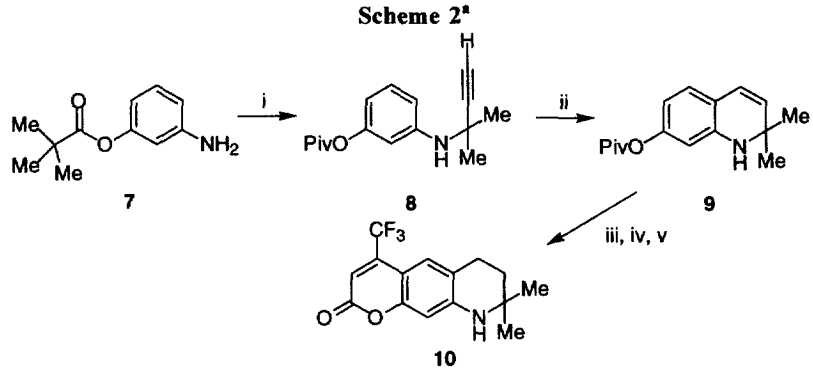


4 5 6

a: $R^5 = CH_2OMe$; b: $R^5 = CF_3$; c: R^5 = Me

Chemistry. In order to develop a general route to the linear tricyclic analogues required for potent hAR activity, several modifications of the previously disclosed routes to 4-(trifluoromethyl)-2(1*H*)-piperidino[3,2-*g*]quinolinone analogues were studied. For example, reaction of 3-aminophenylpivalate **7** with 3-acetoxy-3-methyl-1-butyne in the presence of CuCl afforded the propargyl aniline **8**,[14] which could be cyclized to **9** by treatment with catalytic CuCl in refluxing THF (Scheme 2).[15] Reduction of the olefin by catalytic hydrogenation, deprotection of the phenol, and von Pechman cyclization[16] using 4,4,4-trifluoroacetoacetate mediated by zinc chloride in ethanol, afforded **10**, the 1-oxa version of **1**.

Scheme 2[a]

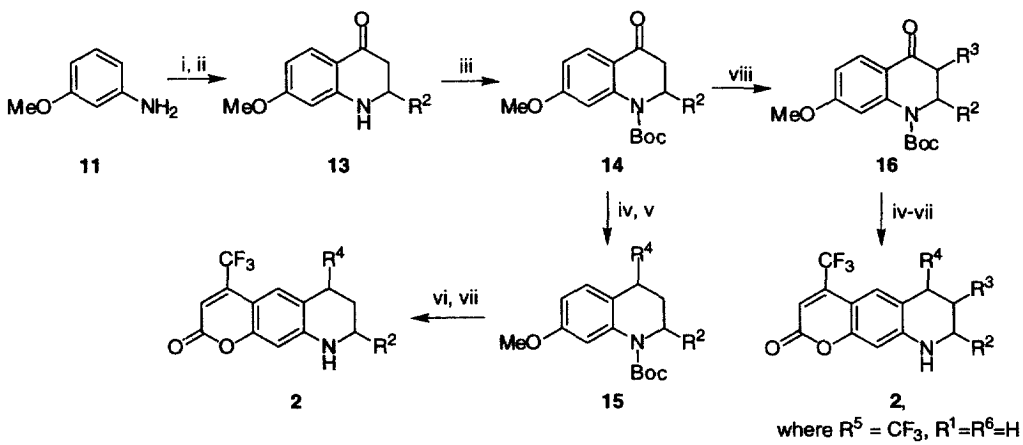


7 8 9 10

[a](i) 3-acetoxy-3-methyl-1-butyne (1.1 equiv), CuCl (5–10%), THF, 60 °C; (ii) CuCl (10–15%), THF, reflux; (iii) H_2, 10% Pd/C, EtOAc/ EtOH; (iv) KOH, MeOH; (v) 4,4,4-trifluoroacetoacetate (1.5 equiv), $ZnCl_2$ (1.5 equiv), EtOH (110–120 °C, sealed tube)

The 4-ketotetrahydroquinoline intermediates **13** were utilized (Scheme 3) to investigate the effects of removing substitution at C(8). Condensation of *m*-anisidine (**11**) with acrylic acid (**12a**), 2-butenoic acid (**12b**), or 2-pentenoic acid (**12c**) followed by PPA-mediated cyclization afforded **13a–c**, respectively, in moderate yields. Protection of the amine as the *t*-butyl carbamate afforded **14a–c**. Addition of an organometallic reagent (or reduction with $LiAlH_4$) followed by reduction of the benzylic alcohol by hydrogenation over palladium on carbon in the presence of a protic acid afforded the tetrahydroquinolines **15** in modest yields. Deprotection of both the aniline and the phenol could be accomplished in a single operation by treatment with BBr_3; the resulting phenols were labile and subjected without purification to von Pechman cyclization conditions (4,4,4-trifluoroacetoacetate, $ZnCl_2$, EtOH, sealed tube, 100–120 °C) to afford the desired linear tricyclics **2**. Alternatively, substitution α to the ketone could be accomplished via enolate chemistry (KHMDS or NaH, iodoalkane, THF or DMF) to afford 4-ketotetrahydroquinolines **16**, which were then converted to linear tricyclics **2** in a manner identical to that of the less substituted analogues **14**.[17] In analogy to earlier studies, the nitrogen atom of **2** could be methylated using standard reductive amination conditions {paraformaldehdye, $Na(CN)BH_3$, AcOH}.[5,10a]

Scheme 3[a]

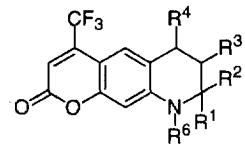


[a](i) **12a**, **b**, or **c** (1.5 equiv), toluene, reflux; (ii) PPA, 110 °C; (iii) *t*-Boc_2O, DMAP, THF, rt; (iv) R^4MgX (or $LiAlH_4$), 0 °C–rt; (v) Pd/C, H_2, AcOH, EtOAc/EtOH; (vi) BBr_3, CH_2Cl_2, -78 – 0 °C; (vii) 4,4,4-trifluoroacetoacetate (1.5 equiv), $ZnCl_2$ (1.5 equiv), EtOH (110–120 °C, sealed tube); (viii) NaH, R^3I, DMF, rt; or KHMDS, R^3I, THF, 0 °C.

In Vitro Biological Activity. The ability of the 2*H*-pyrano[3,2-*g*]quinolin-2-ones **2** to modulate the transcriptional activity of hAR in a cellular context was measured using a cotransfection assay in mammalian (CV-1) cells,[6,11] and binding to hAR was measured using a whole cell assay system in mammalian (COS) cells.[18] The data for C(4)-trifluoromethyl lactones designed to determine the effects of alkyl substitution on the tetrahydroquinoline ring of the pharmacophore are depicted in Table 1. Dihydrotestosterone (DHT) was used as the standard steroidal hAR agonist and bicalutamide as a standard nonsteroidal hAR antagonist.

The C(1)-oxa version of **1**, compound **10**, bound avidly to the receptor (K_i = 1 nM) and stimulated reporter gene expression with a potency comparable to DHT, but with partial efficacy (EC_{50} = 9 nM, 64% efficacy), confirming the widely differential hAR modulatory active of the lactone analogues relative to the lactams.[5]

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



#	R¹	R²	R³	R⁴	R⁶	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 IC_{50} (nM)
	Dihydrotestosterone					100 ± 0	6 ± 1	(9)	--	--		4 ± 1
3	Bicalutamide					--	--		78 ± 3	157 ± 35	(9)	117 ± 35
10	Me	Me	H	H	H	64 ± 13	9 ± 2	(5)	--	--		1 ± 0.3
17	H	H	H	H	H	68 ± 8	19 ± 6	(3)	--	--	(1)	1 ± 0.2
18	H	H	H	Me	H	100 ± 9	2 ± 0.2	(9)	--	--	(1)	1 ± 0.3
19	H	H	H	Et	H	119 ± 6	2 ± 0.2	(9)	--	--	(5)	1 ± 0.2
20	H	H	H	Pr	H	65 ± 4	5 ± 1	(9)	--	--	(1)	5 ± 2
21	H	H	H	CF_3	H	104 ± 12	2 ± 0.3	(6)	--	--	(1)	2 ± 0.2
22	H	H	H	C_2F_5	H	103 ± 16	13 ± 3	(7)	--	--	(1)	5 ± 2
23	H	H	Me	Et^{cis}	H	101 ± 22	7 ± 3	(3)	--	--	(2)	2
24	H	H	Me	Et^{trans}	H	113 ± 24	1 ± 0.2	(5)	--	--	(4)	0.3
25	H	H	Et	Et^{cis}	H	53 ± 17	5 ± 2	(2)	--	--	(2)	nd
26	H	H	Et	Et^{trans}	H	90 ± 7	5 ± 1	(3)	35 ± 16	1400	(2)	nd
27	H	Me	H	Et^{cis}	H	36 ± 3	27 ± 13	(4)	20 ± 10	16 ± 15	(3)	12 ± 2
28	H	Et	H	Et^{cis}	H	86 ± 21	26 ± 4	(4)	--	--	(2)	7
29	H	Et	H	Me^{cis}	H	86 ± 24	8 ± 3	(2)	--	--	(2)	11
30	H	Et	H	H	H	79 ± 13	7 ± 3	(3)	--	--	(3)	1
31	H	Et	Me trans	H	H	138 ± 7	1 ± 0.2	(3)	--	--	(3)	7 ± 3
32	H	H	H	Et	Me	70 ± 11	209 ± 45	(3)	--	--	(4)	276

[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 and/or > 10 μM potency)

Removal of the C(8) methyls afforded a compound (**17**) with reduced activity in the functional assay, but which still bound to the receptor with high affinity (K_i = 1 nM). The addition of a methyl (**18**) or an ethyl (**19**) group to C(6) gave ligands which stimulated reporter gene expression with full efficacy compared to DHT (100% and

119%, respectively) and were more potent (EC_{50} = 2 nM) than the natural hormone. A trifluoromethyl group at C(6) gave similar results (**21**), but propyl and perfluoroethyl groups afforded less potent compounds (**20**, **22**).

Since **19** (LG121011) was consistently slightly more active than **18** in the functional assay, we next investigated the effects of additional substitution of the tetrahydroquinoline ring, initially keeping the ethyl group at C(6) constant. Addition of a methyl group at C(7) afforded two diastereomeric compounds: the *cis* diastereomer (**23**) was less active than the parent, but the *trans* diastereomer (**24**) was at least as active (K_i = 1 nM, 113% efficacy). Likewise, the C(6)-C(7) *cis*-diethyl derivative (**25**) was less active than the *trans* isomer (**26**), but both were less active than **19**. Substitution at C(8) was detrimental to hAR activity (**27-28**) when the C(6) substituent was an ethyl group, but interestingly the *cis*-C(6)-methyl, C(8)-ethyl compound **29** activated gene expression with good potency and efficacy (EC_{50} = 8 nM, 86%). Moving the methyl group of **29** from C(6) to C(7) afforded **31**, which was one of the most potent and efficacious compounds in this series (EC_{50} = 1 nM, 138%). The free NH was crucial for potent hAR activity, as exemplified by N-methyl **19** (compound **32**). This dramatic effect of N-methylation on activity was observed consistently for this series.[19]

The cross-reactivity of **5a**, **5b**, **10**, and **17–32** with other steroid receptors was assessed using human PR, glucocorticoid (hGR), estrogen (hER), and mineralocortocoid (hMR), cotransfection assays (Table 2). No agonist activity was observed for any of the test compounds, but antagonist activity was detected, most notably on the progesterone receptor.[20] Compounds which displayed the most significant (<400 nM) cross-reactivity are tabulated. The separation between hAR agonist and hPR antagonist activity was generally 100-fold or greater, particularly for the more active androgens (e.g., **19**, **24**, **31**).

Table 2. hPR, hGR, hER, and hMR Antagonist Activity in Cotransfected CV-1 Cells for Selected Pyrano[3,2-*g*]quinolin-2-ones.[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)
5b	79	100	<20	--	<20	--	<20	--
10	84	570	<20	--	<20	--	50	1500
18	83	280	<20	--	<20	--	<20	--
19	91	200	<20	--	<20	--	<20	--
20	89	106	<20	--	<20	--	<20	--
21	68	270	<20	--	<20	--	<20	--
23	87	130	57	340	33	3100	39	2800
24	98	130	70	2300	<20	--	60	3000
26	98	65	63	2800	<20	--	50	1900
31	88	440	<20	--	<20	--	<20	--

[a] See Table 1 for legend.

Conclusion. The in vitro data presented for this series of linear tricyclic 2*H*-pyrano[3,2-g]quinolin-2-ones further demonstrate the potential for the discovery of nonsteroidal androgen receptor agonists with activities comparable to the natural hormone, DHT. These results, along with structurally related nonsteroidal hAR agonists recently described,[10a,20,21,22] form a basis for the further development of nonsteroidal androgens to complement the currently available steroidal treatments in the field of androgen replacement therapy.[9]

References and Notes

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