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SYNTHESIS AND PROGESTERONE RECEPTOR BINDING AFFINITY OF SUBSTITUTED 1-PHENYL-7-BENZYL-4,5,6,7-TETRAHYDRO-1H-INDAZOLES

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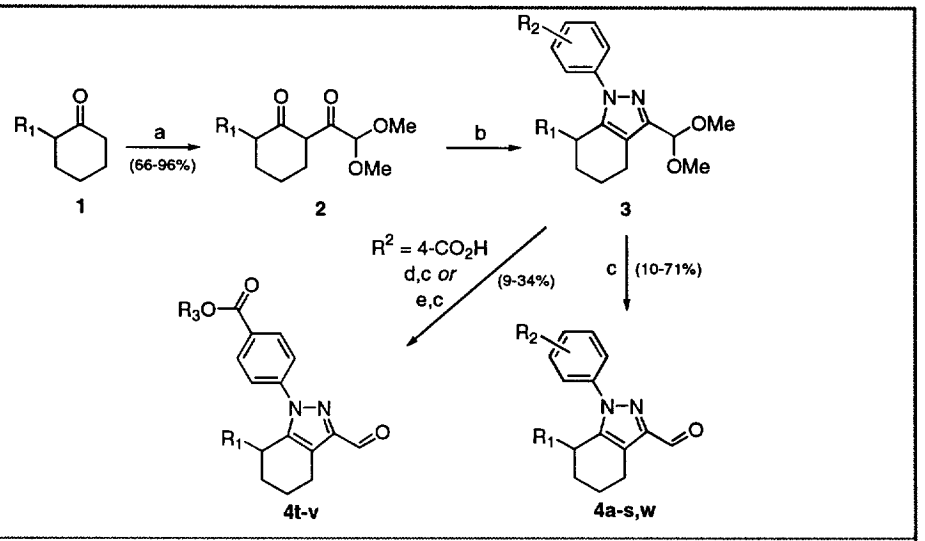
Abstract: Research directed toward the discovery of non-steroidal ligands for steroid receptors led to the preparation of a series of substituted 1-phenyl-7-benzyltetrahydroindazole-3-carboxaldehydes. Appropriately substituted 3-formyl analogs (**4**) were found to bind with high affinity to progesterone receptors and showed agonist activity in human T47D cells but were inactive in several in vivo models for progestational activity.

The interaction of naturally occurring steroid hormones and their receptors has been an important focus of medicinal chemistry for decades.[1] We have been interested in a receptor-based strategy directed toward novel agents that mimic (or block) the actions of the steroid sex hormone progesterone, which plays an essential role in regulating the menstrual cycle and in maintaining pregnancy.[2] Progesterone is secreted primarily by the corpus luteum and the placenta, and progesterone receptors are expressed in a variety of tissues throughout the body including the cervix, uterine endometrium, ovary, breast, and pituitary, illustrating the importance of the hormone in many aspects of female reproductive physiology. A number of steroids related to progesterone act as receptor agonists (i.e., ethynodiol diacetate, norgestimate) and are currently used in oral contraceptives, usually in conjunction with an estrogen. In addition, progesterone receptor antagonists (anti-progestins) would theoretically be attractive candidates for the treatment of progesterone-dependent pathologies such as endometriosis, uterine fibroids, and breast cancer. Non-steroidal anti-progestins would be of particular interest as contraceptive agents due to their potential for reducing progesterone-associated androgenic side effects (i.e., weight gain) and for their unique (and patentable) structures. A number of examples of non-steroidal progestin receptor ligands have been reported.[3-6] As part of a screening program directed toward the identification of novel non-steroidal progestins, we identified a series of 4,5,6,7-tetrahydro-1H-indazoles that bind to the progestin receptor. Examples having aromatic substitution at the 1- and 7-positions and formyl substitution at the 3-position (**4**) were found to bind selectively and with high affinity. The chemical synthesis of these compounds and our exploration of structure-activity relationships within the series are described below.

Chemistry

The preparation of 1-aryl-4,5,6,7-tetrahydro-1H-indazole-3-carboxaldehydes (**4**), shown in Scheme 1, has been described previously.[7-9] Starting from readily available 2-substituted cyclohexanones (**1**), treatment with lithium hexamethyldisilazide and reaction with methyl dimethoxyacetate afforded the intermediate diketo

Scheme 1

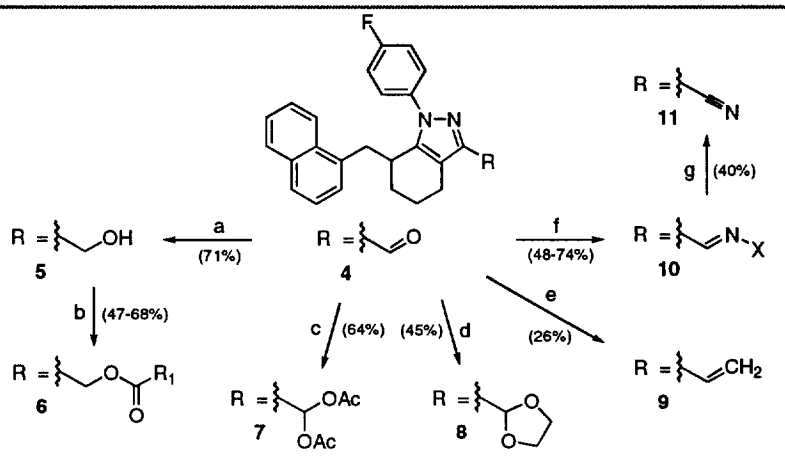


(a) $LiN(i\text{-}Pr)_2$ or $LiN(SiMe_3)_2$, THF, 0.5 equiv $(MeO)_2CHCO_2Me$, -78 to 25 °C; (b) R^2-$C_6H_4NHNH_2$•HCl, Et_3N or NaOAc, MeOH or EtOH; (c) aqueous HCl, THF; (d) R^3OH, cat. H_2SO_4, reflux; (e) CDI, R_3OH, CH_2Cl_2.

acetal **2**. Reaction of **2** with an appropriately substituted arylhydrazine was followed by acid hydrolysis, giving a 2:1 mixture of **4** and its 2-aryl isomer.[10] Several 1-[4-(alkoxycarbonyl)phenyl] analogs (**4t–v**) were synthesized by isolation of the intermediate dimethyl acetal **3** (R^2 = 4-CO_2H) and esterification of the free carboxyl group prior to deprotection.

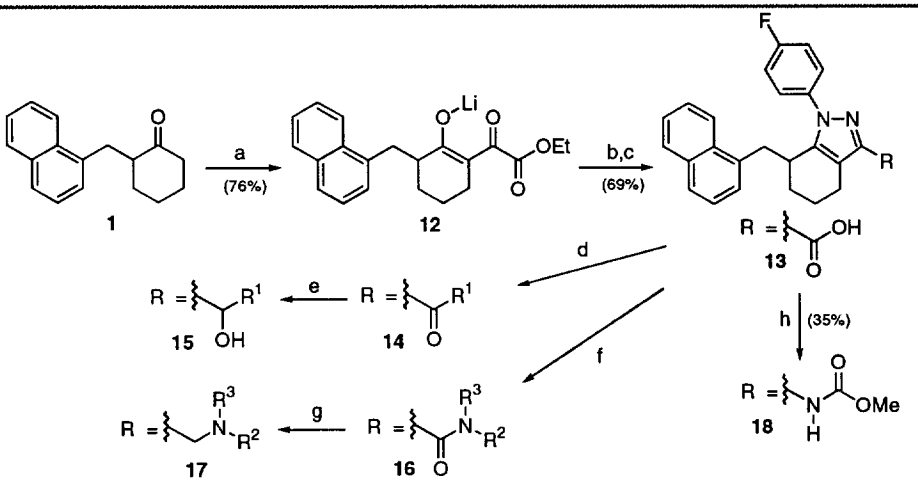
In order to explore the effect of the 3-substituent on binding affinity, **4c** was used as a starting material for the synthesis of a variety of derivatives (Scheme 2). Reduction with sodium borohydride provided alcohol **5**, which was subsequently acylated to give **6**; alternatively, acetal formation gave **7** and **8** and Wittig olefination provided **9**. Several imino analogs (**10**) were prepared by reaction of **4c** with the appropriate amine or hydrazine. The oxime (**10c**, X = OH) was converted further into the nitrile (**11**). Additional 3-substituted analogs of **4c** were prepared from carboxylic acid **14** according to the route outlined in Scheme 3. Following literature precedent, the lithium enolate of 2-(1-naphthylmethyl)cyclohexanone was reacted with diethyl oxalate to give lithium salt **12**, which was condensed with 4-fluorophenylhydrazine to provide, after saponification, carboxylic acid **13**.[11] Acid **13** served as an intermediate for the synthesis several of secondary alcohols (**15**) by reduction of the corresponding ketones (**14**), which were themselves prepared from **13** via the Weinreb amide (**16**, R^2 = OMe, R^3 = Me).[12] Compound **13** was also converted to other amides (**16a,b**) which were reduced to the corresponding amines (**17**) with lithium aluminum hydride. Finally, reaction of **13** with diphenylphosphoryl azide and methanol provided the Curtius rearrangement product **18**.

Scheme 2



(a) $NaBH_4$, $EtOH/CH_2Cl_2$; (b) $R^1(C=O)Cl$, pyridine, CH_2Cl_2; (c) Ac_2O, $SOCl_2$; (d) $HOCH_2CH_2OH$, TsOH, benzene, reflux; (e) Ph_3PCH_3Br, NaH, DMSO; (f) H_2NPh or $H_2NOH{\bullet}HCl$ or H_2NNR^2 or $H_2NNH(C=O)R$ or $H_2NNH(SO_2)R$, pyridine, EtOH or MeOH; (g) X = OH only: Ac_2O, NaOAc.

Scheme 3



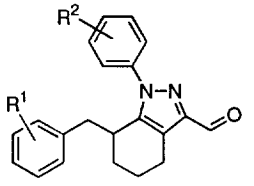
(a) $LiN(SiMe_3)_2$, THF, $(CO_2Et)_2$, -78 to 25 °C; (b) $4\text{-}FC_6H_4NHNH_2{\bullet}HCl$, NaOAc, EtOH; (c) aqueous NaOH; (d) $(COCl)_2$, $(MeO)MeNOH{\bullet}HCl$, pyridine; then R^1MgX, THF/ether; (e) $NaBH_4$, EtOH; (f) $(COCl)_2$, $R^2R^3NH{\bullet}HCl$, pyridine; (g) $LiAlH_4$, THF; (h) DPPA, Et_3N, CH_2Cl_2, then MeOH, reflux.

Results and Discussion

All compounds were screened for their ability to compete with [^{3}H]-R5020[13] using uterine progesterone receptors obtained from estrogen-primed rabbits.[14] Many were also tested for agonist and antagonist activity in

cultured T47D human breast carcinoma cells.[15] Biological activities for tetrahydroindazole-3-carboxaldehydes (**4**) are shown in Table 1. Variation of the aromatic substituent (R^1) on the benzyl group resulted in a wide range of binding affinities. For the most part, both electron-withdrawing and electron-donating substituents at the ortho- and meta- positions are tolerated in the binding assay. However, affinity is lower in analogs having larger para- substituents (**4i–n**). The weaker affinity of the beta-naphthyl (**4i**) vs. the alpha-naphthyl analog (**4c**) is also consistent with this observation. Cell proliferation is most potent in analogs having smaller R^1 substituents such as fluorine and hydrogen (**4b,d**). Substituents (R^2) on the N-phenyl group were also examined in a series of alpha-naphthyl analogs (R^1 = 2,3-benzo). Once again, the electronic character of the substituent has relatively little effect on affinity, at least in the para- position (**4c,o–r**); however, only the unsubstituted and fluorine-containing compounds are effective as agonists in the proliferation assay. The 4-carboxy analogs (**4s–u**) and the phenol (**4v**) are uniformly weak or inactive in the binding assay.

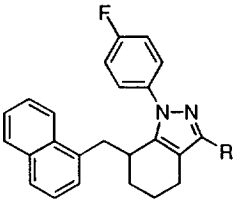
Table 1



cpd	R^1	R^2	rabbit progesterone receptor binding affinity, IC_{50} (nM)	T47D cell proliferation SC_{200} (nM)
4a	3,4-di-F	4-F	2	81
4b	H	4-F	3	22
4c	2,3-benzo	4-F	3.2	105
4d	4-F	4-F	6.5	27
4e	4-Me	4-F	7.7	275
4f	2-Cl	4-F	10.5	100
4g	4-Cl	4-F	11.9	na[16]
4h	3-MeO	4-F	16.8	417
4i	3,4-benzo	4-F	56.4	na
4j	4-MeO	4-F	163	
4k	4-t-Bu	4-F	1000	
4l	3,4-di-MeO	4-F	1891	
4m	4-i-Pr	4-F	2195	
4n	4-Ph	4-F	10000	
4o	2,3-benzo	H	6	56
4p	2,3-benzo	4-Cl	9	na
4q	2,3-benzo	4-MeO	12.7	na
4r	2,3-benzo	3-Cl	69	
4s	2,3-benzo	4-CO_2-n-Bu	851	na
4t	2,3-benzo	4-CO_2-n-C_8H_{17}	>10000	na
4u	2,3-benzo	4-CO_2Me	na[16]	
4v	2,3-benzo	4-OH	na	

In addition to the primary assays, a number of examples were screened for in vivo progestational and anti-progestational activity. Unfortunately, none of the compounds tested showed any biological activity in rat or rabbit models using a variety of administration routes.[17] Because of the lack of in vivo activity, we attempted to replace the potentially reactive aldehyde functionality with an isosteric group or a derivative we hoped would act as a prodrug (Table 2). In the isosteric replacement series, only the nitrile (**11**) and the primary alcohol derivatives (**5, 6a–c**) retain modest receptor affinity, albeit at the expense of proliferative activity. Of the potential pro-drugs, the easily hydrolyzed di-acetoxy (**7**) and phenylimino (**10a**) derivatives exhibit high receptor affinity and are potent in the proliferation assay; unfortunately, both are inactive in vivo.

Table 2



cpd	R	rabbit progesterone receptor binding affinity, IC_{50} (nM)	T47D cell proliferation SC_{200} (nM)
4c	CHO	3.2	105
5	CH_2OH	52	na[16]
6a	CH_2OAc	73	na
6b	$CH_2O(C=O)OEt$	90	na
6c	$CH_2O(C=O)Ph$	182	na
6d	$CH_2O(C=O)$-n-C_7H_{15}	4,010	na
7	$CH(OAc)_2$	5.3	72
8	$CH(-OCH_2CH_2O-)$	97	
9	$CH=CH_2$	147	na
10a	CH=N-Ph	2.3	185
10b	CH=NNHMe	49	na
10c	$CH=NNMe_2$	547	na
10d	CH=NOH	628	
10e	CH=NNH(C=O)Me	>10,000	
10f	CH=NNH(C=O)Ph	>10,000	2265
10g	$CH=NNHSO_2Ph$	>10,000	na
11	CN	41	na
14a	(C=O)Me	1,756	
14b	(C=O)Et	1,000	
15a	CH(OH)Me	236	
15b	CH(OH)Et	690	
16a	$(C=O)N(Et)_2$	3,000	
16b	$(C=O)NHCH_2Ph$	>10,000	
17a	$CH_2N(Et)_2$	1,377	
17b	CH_2NHCH_2Ph	1,381	
18	$NHCO_2Me$	1,574	

In summary, we have discovered a series of 1-aryl-7-benzyl-4,5,6,7-tetrahydro-1H-indazole-3-carboxaldehydes that exhibit high affinity for the rabbit uterine progestin receptor and behave as progestin agonists in a cultured T47D human breast carcinoma cell proliferation assay. Two potent compounds (**4c** and **4d**) bind selectively to rat and rabbit progesterone receptors vs. other steroid receptors and bind with roughly the same affinity as progesterone in human T47D cell receptors.[18] Compounds **4c, 4d**, and other examples were evaluated in several in vivo models for progestational and anti-progestational activity but were found to be inactive.

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10. In all cases, the 2-aryl isomers were found to have significantly lower affinity for the progesterone receptor than the 1-aryl isomers (IC_{50} > 1000 nM).
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13. R5020 is promegestone, 17α-methyl-17-propionylestra-4,9-dien-3-one (IC_{50} = 0.8 nM against [^{3}H]-R5020 in rabbit uterus progesterone receptor).
14. Phillips, A.; Demarest, K.; Hahn D. W.; Wong, F.; McGuire J. L. *Contraception* **1990**, *41*, 399.
15. In this assay, incorporation of [^{3}H]-thymidine into cellular DNA was measured after treatment with compound alone (for potential agonists) or treatment with compound in the presence of R5020 (for potential antagonists). Results for agonists are reported as SC_{200}, defined as the concentration at which incorporation of [^{3}H]-thymidine is 200% of control. With the exception of **4q**, **4r**, and **9**, which were weak antagonists (ED_{50} range 690–2250 nM), all compounds tested in the antagonist assay were inactive.
16. Not active at the highest concentration tested (10 μM for binding assay, 1 or 3 μM for cell proliferation assay).
17. Compounds were tested orally or as subcutaneous injections in the rabbit endometrial proliferation (Clauberg, C. *Zentr. Gynakol.* 1930) and rat contragestational assays; certain compounds were also administered directly into the endometrium.
18. Compounds **4c** and **4d** bind strongly to rabbit uterine, rat uterine, and human T47D cell progesterone receptors (IC_{50} < 10 nM) and poorly to rabbit uterine estrogen, rat and rabbit prostate androgen, and rat liver glucocorticoid receptors (IC_{50} > 1870 nM), with one exception (**4c**, rabbit estrogen receptor IC_{50} = 376 nM).

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