fish-skin bioassay, skins become light in response to MCH due to melanosome aggregation within melanocytes; the skins can then be redarkened by the addition of MSH, which causes redispersion of melanosomes within melanocytes. Movement of melanosomes within melanocytes results in color changes that can be monitored by a Photovolt reflectometer. Changes in skin color (reflectance) are recorded as percent changes from the initial base (zero) value. The frog (*Rana pipiens*) skin bioassay was utilized as previously described.[18,19]

Acknowledgment. This work was supported by grants from the U.S. Public Health Service (AM 17420), the National Science Foundation, PCM-8412084 and DCB-8615706, and from Consello Nacional de Desenvolvimento Cientifico, Technológico 407196/87, Brazil.

Registry No. 1, 112794-06-6; 2, 112794-07-7; 3, 112794-08-8; 4, 112794-09-9; 5, 112794-10-2; 6, 112794-11-3; 7, 112794-12-4; 8, 112794-13-5; 9, 112794-14-6; BOC-Glu(OBzl), 13574-13-5; BOC-Trp(For), 47355-10-2; BOC-Cys(4-Me-Bzl), 61925-77-7; BOC-Pro, 15761-39-4; BOC-Arg(Tos), 13836-37-8; BOC-Tyr(2,6-Cl_2-Bzl), 40298-71-3; BOC-Val, 13734-41-3; BOC-Met, 2488-15-5; BOC-Ala, 15761-38-3; BOC-Thr(Bzl), 15260-10-3; BOC-Asp(OBzl), 7536-58-5; BOC-Gly, 4530-20-5.

Nonsteroidal Antiandrogens. Synthesis and Structure-Activity Relationships of 3-Substituted Derivatives of 2-Hydroxypropionanilides

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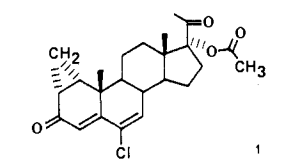
A series of 3-(substituted thio)-2-hydroxypropionanilides and some corresponding sulfones and sulfoxides of general structure **7**, in which R′ is methyl or trifluoromethyl, were prepared and tested for antiandrogen activity. Members of the trifluoromethyl series (**7**, R′ = CF_3) generally exhibited partial androgen agonist activity whereas the members of the methyl series (**7**, R′ = CH_3) were pure antagonists. Lead optimization in the methyl series has led to the discovery of novel, potent antiandrogens, which are peripherally selective. One of these, (*RS*)-4′-cyano-3-[(4-fluorophenyl)sulfonyl]-2-hydroxy-2-methyl-3′-(trifluoromethyl)propionanilide, **40** (ICI 176334), is being developed currently for the treatment of androgen-responsive benign and malignant disease.

Cancer of the prostate is the second most common cause of death in American males with about 25 000 deaths from this condition being recorded in 1974.[1] The growth of the prostate tumors is stimulated by androgens, the male sex hormones. Since the pioneering work of Huggins and Hodges in 1941,[2] which showed the hormone dependence of this tumor, the mainstay of treatment for prostate cancer has been the withdrawal of androgens either by castration (orchidectomy) or estrogen therapy. Both of these therapies have disadvantages. Orchidectomy is unpopular with the patients and can result in psychological problems, and it exposes the elderly patients to the trauma of surgery. Estrogen therapy is effective in most patients but is accompanied by severe side effects including cardiovascular complications (cardiac failure, edema, and thromboembolism), painful gynecomastia, impotence, and loss of libido.[3] Both these approaches lead to the withdrawal of the androgens produced by the testes; however, the adrenal glands also produce androgens, so a more effective therapeutic agent would be a compound that prevented the natural androgens from interacting with their receptors, i.e., an antiandrogen.

There are two antiandrogens currently available commercially, cyproterone acetate (**1**) and the nonsteroidal anilide flutamide (**2**), whose active form in vivo is the hydroxylated metabolite, hydroxyflutamide (**3**). In addition to its antiandrogen activity, cyproterone acetate is also a potent progestin and inhibits gonadotrophin secretion.[4] It is effective in the treatment of prostate cancer but among its side effects may be listed loss of libido, gynecomastia, fluid retention, and thrombosis. Flutamide is a pure antiandrogen; that is, it does not exhibit other



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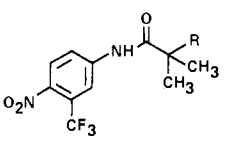

2 R = H

3 R = OH

hormonal activities. It is effective in the treatment of prostate cancer, the main side effect reported being gynecomastia.[5] One consequence of its pure antiandrogenic profile is that it prevents androgens from exerting their negative feedback mechanism on the hypothalamus, which results in an increased pituitary secretion of, interalia, luteinizing hormone (LH), which stimulates androgen production by the testes.[6] The antagonist, therefore, brings about the increased production of the natural agonist, which effectively diminishes its efficacy at the target organ. Our objective was to find a pure antiandrogen that was selective for the accessory sex organs, that is, had little or no effect on pituitary LH and consequently testosterone secretion. This paper describes our successful preparation of such a selective antiandrogen, which should have clinical advantages over existing antiandrogens in the treatment of prostate cancer.

Chemistry

Two general synthetic routes were used to prepare the anilides listed in Tables I–III. The route outlined in Scheme I was used for the preparation of anilides **7**. This involved coupling of an α-hydroxy acid chloride, prepared in situ by treating the α-hydroxy acid **5** with thionyl

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(2) Huggins, C.; Hodges, C. V. *Cancer Res.* **1941**, *1*, 293.
(3) Byar, D. P. *Bull. N. Y. Acad. Med.* **1972**, *48*, 751.
(4) Neumann, F. In *Pharmacology and clinical uses of inhibitors of hormone secretion and action*; Furr, B. J. A., Wakeling, A. E., Eds.; Bailliere Tindall: London, 1987; p 132.
(5) Neri, R. O.; Florance, K.; Koziol, P.; van Cleave, S. *Endocrinology* **1972**, *91*, 427.
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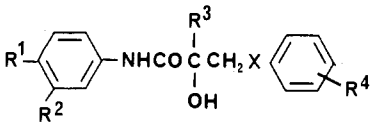
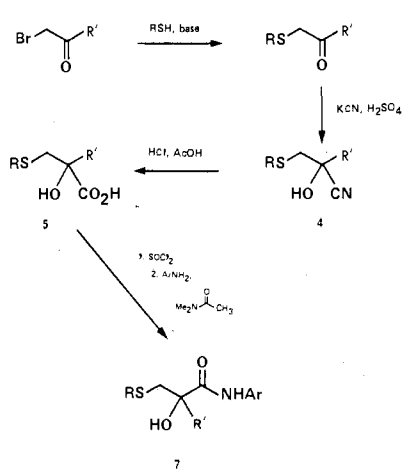
Table I



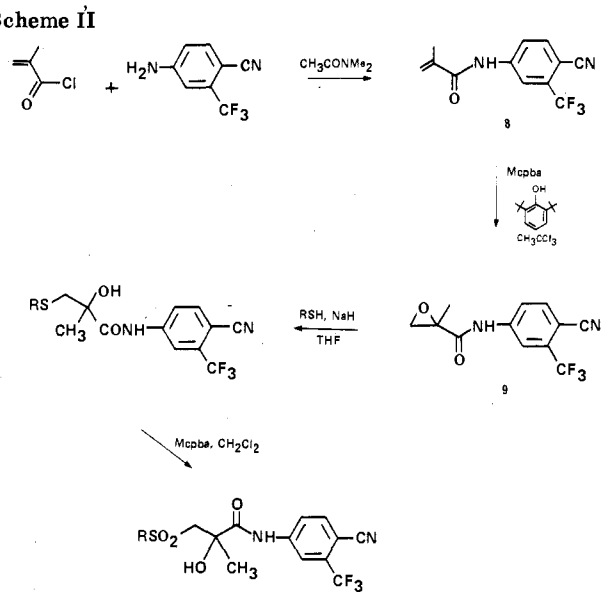
compd	R^1	R^2	R^3	R^4	X	synth route[a]	mp, °C	formula	anal.	ED_{50}	agonism[b]
10	NO_2	CF_3	CF_3	H	S	1	139–140	$C_{17}H_{12}F_6N_2O_4S$	C, H, N	33% at 2.5[c]	34
11	CN	CF_3	CF_3	H	S	1	144	$C_{18}H_{12}F_6N_2O_2S$	C, H, N	0.7	30
12	CN	CF_3	CF_3	H	SO	1	175–176	$C_{18}H_{12}F_6N_2O_3S$	C, H, N	0.3	
13	CN	CF_3	CF_3	H	SO_2	1	175–176	$C_{18}H_{12}F_6N_2O_4S$	C, H, N	0.5	32
14	CN	Cl	CF_3	H	S	1	152	$C_{17}H_{12}ClF_3N_2O_2S$	C, H, N	0.9	21
15	Cl	Cl	CF_3	H	S	1	104	$C_{16}H_{12}Cl_2F_3NO_2S{\cdot}0.5H_2O$	C, H, N	15	
16	NO_2	H	CH_3	H	S	1	110–112	$C_{16}H_{16}N_2O_4S$	C, H, N	11% at 50[c]	
17	CN	CF_3	CH_3	H	S	1	81.5–83	$C_{18}H_{15}F_3N_2O_2S$	C, H, N	1.7	NA
18	CN	CF_3	CH_3	H	SO	1	164–165	$C_{18}H_{15}F_3N_2O_3S$	C, H, N	1.4	NA
19	CN	CF_3	CH_3	H	SO_2	1	172–173.5	$C_{18}H_{15}F_3N_2O_4S$	C, H, N	1.8	NA
20	NO_2	CF_3	CH_3	H	S	1	103–105	$C_{17}H_{15}F_3N_2O_4S$	C, H, N	1.0	
21	NO_2	CF_3	CH_3	H	SO	1	126.5–127.5	$C_{17}H_{15}F_3N_2O_5S$	C, H, N	1.0	NA
22	NO_2	CF_3	CH_3	H	SO_2	1	149–151	$C_{17}H_{15}F_3N_2O_6S$	C, H, N	1.5	NA
23	CN	Cl	CH_3	H	S	1	60–62	$C_{17}H_{15}ClN_2O_2S$	C, H, N	2.5	NA
24	Cl	Cl	CH_3	H	S	1	85–86	$C_{16}H_{15}Cl_2NO_2S$	C, H, N	44% at 25[c]	
25	NO_2	Cl	CH_3	H	S	1	88–90	$C_{16}H_{15}ClN_2O_4S$	C, H, N[d]	0.5	
26	Cl	NO_2	CH_3	H	S	1	77–78	$C_{16}H_{15}ClN_2O_4S$	C, H, N[e]	36% at 50[c]	NA
27	CN	CH_3	CH_3	H	S	1	98–99	$C_{18}H_{18}N_2O_2S$	C, H, N	50	
28	CN	CF_3	CF_3	4-Cl	S	1	178–179	$C_{18}H_{11}ClF_6N_2O_2S$	C, H, N	11	
29	NO_2	CF_3	CF_3	4-Cl	S	1	147–148	$C_{17}H_{11}ClF_6N_2O_4S$	C, H, N	23% at 2.5[c]	43
30	NO_2	CF_3	CH_3	4-Cl	S	1	101–103	$C_{17}H_{14}ClF_3N_2O_4S$	C, H, N	1.6	NA
31	NO_2	CF_3	CH_3	3-Cl	S	11	132–133	$C_{17}H_{14}ClF_3N_2O_4S$	C, H, N	18	NA
32	NO_2	CF_3	CH_3	2-Cl	S	11	98–99.5	$C_{17}H_{14}ClF_3N_2O_4S$	C, H, N	5	
33	NO_2	CF_3	CH_3	4-F	S	11	112–113	$C_{17}H_{14}F_4N_2O_4S$	C, H, N	1.1	NA
34	NO_2	CF_3	CH_3	4-F	SO_2	11	188–189	$C_{17}H_{14}F_4N_2O_6S$	C, H, N	0.4	
35	NO_2	CF_3	CH_3	4-NO_2	S	11	139–141	$C_{17}H_{14}F_3N_3O_6S$	C, H, N[f]	20	NA
36	NO_2	CF_3	CH_3	4-CN	S	11	108–111	$C_{18}H_{14}F_3N_3O_4S$	C, H, N[g]	10	
37	NO_2	CF_3	CH_3	4-CH_3O	S	11	120–121	$C_{18}H_{17}F_3N_2O_5S$	C, H, N	16	NA
38	NO_2	CF_3	CH_3	4-CH_3S	S	11	111–112	$C_{18}H_{17}F_3N_2O_4S_2$	C, H, N	5	
39	CN	CF_3	CH_3	4-F	S	11	116–117	$C_{18}H_{14}F_4N_2O_2S$	C, H, N	0.5	NA
40	CN	CF_3	CH_3	4-F	SO_2	11	191–193	$C_{18}H_{14}F_4N_2O_4S$	C, H, N	0.5	NA
41	CN	CF_3	CH_3	4-Cl	S	1	137–138	$C_{18}H_{14}ClF_3N_2O_2S$	C, H, N	4	NA
42	CN	CF_3	CH_3	4-CH_3S	S	11	125–126	$C_{19}H_{17}F_3N_2O_2S_2$	C, H, N	25	

[a] In the case of sulfoxides and sulfones, the synthetic route refers to the preparation of the parent sulfide. [b] The number quoted is the weight of the ventral prostate (mg). Control values are in the range 10–14 mg. NA indicates no statistically significant increase in ventral prostate weight compared to controls. Testosterone propionate when dosed at 0.5 mg/kg sc in this test gave a prostate weight of 48 mg. [c] Percentage inhibition at the dose stated. [d] C: calcd, 52.4; found, 51.9. [e] C: calcd, 52.4; found, 52.9. [f] N: calcd, 9.4; found, 8.9. [g] H: calcd, 3.3; found, 2.8.

Scheme I

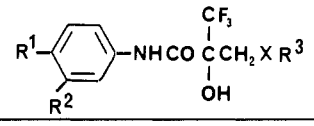


Scheme II



chloride in dimethylacetamide at −20 °C, with an aniline at the same temperature. The α-hydroxy acids **5** were prepared by acid hydrolysis of cyanohydrins **4**, which were obtained from the corresponding thio ketones by standard means. The hydrolysis of the cyanohydrins **4** with concentrated hydrochloric acid in Carius tubes at 110 °C was problematic, giving a 3:2 mixture of the required acid **5** and the intermediate amide **6**. Hydrolysis could be effected by using a mixture of glacial acetic acid (ca 20% by volume) and concentrated HCl and by heating the mixture on a steam bath overnight to give a 4:1 mixture of **5** and **6** in higher overall yield. The thio ketones were

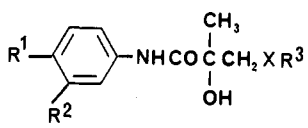
Table II



compd	R^1	R^2	R^3	X	synth route	mp, °C	formula	anal.	% inhibn at 2.5 mg/kg	agonism
43	CN	Cl	CH_3	S	1	121–122.5	$C_{12}H_{10}ClF_3N_2O_2S$	C, H, N	95	36
44	CN	Cl	n-C_3H_7	S	1	89–90	$C_{14}H_{14}ClF_3N_2O_2S$	C, H, N	98	26
45	Cl	Cl	C_2H_5	S	1	57–59	$C_{12}H_{12}Cl_2F_3NO_2S$	C, H, N	75	28
46	Cl	Cl	n-C_3H_7	S	1	60–61	$C_{13}H_{14}Cl_2F_3NO_2S$	C, H, N	80	32
47	CN	CF_3	CH_3	S	1	120.5–122	$C_{13}H_{10}F_6N_2O_2S$	C, H, N	68	50
48	CN	CF_3	C_2H_5	S	1	119–120	$C_{14}H_{12}F_6N_2O_2S$	C, H, N	74	31
49	CN	CF_3	C_2H_5	SO_2	1[a]	164–165	$C_{14}H_{12}F_6N_2O_4S$	C, H, N	68	
50	CN	CF_3	i-C_3H_7	S	1	107–109	$C_{15}H_{14}F_6N_2O_2S$	C, H, N	77	32
51	CN	CF_3	n-C_3H_7	S	1	88–90	$C_{15}H_{14}F_6N_2O_2S$	C, H, N	72	33
52	NO_2	CF_3	n-C_3H_7	S	1	67–68	$C_{14}H_{14}F_6N_2O_4S$	C, H, N	30	42

[a] Refers to synthesis of parent sulfide.

Table III



compd	R^1	R^2	R^3	X	synth route[a]	mp, °C	formula	anal.	ED_{50}	agonism[b]
53	CN	CF_3	C_2H_5	SO_2	1	118–119	$C_{14}H_{15}F_3N_2O_4S$	C, H, N	1.1	NA
54	CN	CF_3	C_2H_5	SO	1	110–112	$C_{14}H_{15}F_3N_2O_3S$	C, H, N	1.3	NA
55	CN	CF_3	CH_3	S	1	108.5–109.5	$C_{13}H_{13}F_3N_2O_2S$	C, H, N	2.5	NA
56	CN	CF_3	i-C_3H_7	S	1	98–100	$C_{15}H_{17}F_3N_2O_2S$	C, H, N	<2.5	NA
57	CN	CF_3	i-C_3H_7	SO_2	1	117.5–119	$C_{15}H_{17}F_3N_2O_4S$	C, H, N	10	
58	NO_2	CF_3	CH_3	S	1	109–110	$C_{12}H_{13}F_3N_2O_4S$	C, H, N	1.7	NA
59	NO_2	CF_3	C_2H_5	SO_2	1	135–136	$C_{13}H_{15}F_3N_2O_6S$	C, H, N	1.4	NA
60	NO_2	CF_3	i-C_3H_7	S	1	66–68	$C_{14}H_{17}F_3N_2O_4S$	C, H, N	5.4	NA
61	NO_2	CF_3	n-C_3H_7	SO_2	1	118–119	$C_{14}H_{17}F_3N_2O_6S$	C, H, N[c]	6	NA
62	NO_2	CF_3	n-C_5H_{11}	SO_2	11	104–105	$C_{16}H_{21}F_3N_2O_6S$	C, H, N	40	
63	NO_2	CF_3	t-C_4H_9	SO_2	11	136.5–138	$C_{15}H_{19}F_3N_2O_6S$	C, H, N	NA	
64	NO_2	CF_3	2-thiazolyl	S	11	131–132	$C_{14}H_{12}F_3N_3O_4S_2$	C, H, N	0.6	NA
65	NO_2	CF_3	2-pyridyl	S	11	155–157	$C_{16}H_{14}F_3N_3O_4S$	C, H, N	0.9	
66	CN	CF_3	2-pyridyl	S	11	137–139	$C_{17}H_{14}F_3N_3O_2S$	C, H, N[d]	2.0	NA
67	NO_2	CF_3	3-pyridyl	S	11	149–150	$C_{16}H_{14}F_3N_3O_4S$	C, H, N	10	
68	CN	CF_3	3-pyridyl	S	11	135–136	$C_{17}H_{14}F_3N_3O_2S$	C, H, N	7	
69	NO_2	CF_3	4-pyridyl	S	11	193–195	$C_{16}H_{14}F_3N_3O_4S$	C, H, N	4	NA
70	CN	CF_3	2-pyrimidyl	S	11	120–121	$C_{16}H_{13}F_3N_4O_2S$	C, H, N	9	
71	CN	CF_3	2-thienyl	S	11	101–103	$C_{16}H_{13}F_3N_2O_2S_2$	C, H, N	4	NA
72	NO_2	CF_3	5-methyl-2-1,3,4-thiadiazolyl	S	11	109–111	$C_{14}H_{13}F_3N_4O_4S_2$	C, H, N	1.4	NA
73	CN	CF_3	2-benzothiazolyl	S	11	178–180	$C_{19}H_{14}F_3N_3O_2S_2$	C, H, N	1.8	NA
74	NO_2	CF_3	4-methyl-2-thiazolyl	S	11	160–162	$C_{15}H_{14}F_3N_3O_4S_2$	C, H, N	2.0	NA
2	flutamide								2.0	NA

[a] In the case of sulfoxides and sulfones, the synthetic route refers to the preparation of the parent sulfide. [b] NA indicates no statistically significant increase in ventral prostate weight. [c] C: calcd, 42.2; found, 42.7. [d] C: calcd, 53.5; found, 54.0.

prepared from bromoacetone or 3-bromotrifluoroacetone by a literature route.[7]

The route outlined in Scheme II provided a more convenient and highly convergent means of preparing anilides **7** in which R′ is a methyl group. The methacrylamide **8** was epoxidized following a literature route,[8] and the subsequent reaction of the epoxide **9** with thiols was straightforward and is fully exemplified in the Experimental Section.

The sulfoxides were obtained cleanly by sodium metaperiodate oxidation of the sulfides in aqueous methanol. Oxidation of the sulfides with *m*-chloroperbenzoic acid gave sulfones as the sole products.

Results and Discussion

In our search for a selective antiandrogen, the hydroxylated metabolite of the nonsteroidal agent flutamide was a more attractive starting point than the steroid cyproterone acetate with its multihormonal profile of activity. Previous work from these laboratories on hydroxyflutamide analogues[9] had confirmed that the structural elements required for antiandrogen activity were an electron-deficient aromatic ring separated from the tertiary carbinol by an amide link. In addition, it was found that the biological profile was influenced greatly by the nature of the two other substituents attached to the carbon atom bearing the tertiary hydroxy group. In this paper we describe the structure–activity relationships of substituted methylthio derivatives of general structure **7** in which R′ is a methyl or trifluoromethyl group.

Antiandrogen activity was optimum when there were two electron-withdrawing groups present in the 3- and 4-positions of the anilide ring, and this is exemplified by the relative potencies of **11**, **17**, **20**, **23**, and **25** compared with those of **27** and the monosubstituted analogue **16**. In practice the best antiandrogen activity was found in compounds where the 4-substituent in the anilide ring was either cyano or nitro and the 3-substituent was chloro or

(7) Noskov, V. G.; Soborovskii, L. Z. *Zh. Org. Khim.* **1971**, *7*, 221.
(8) Kishi, Y.; Aratani, M.; Tanino, H.; Fukuyama, T.; Goto, T.; Inoue, S.; Sugiura, S.; Kakoi, H. *J. Chem. Soc., Chem. Commun.* **1972**, 64.
(9) Glen, A. T.; Hughes, L. R.; Morris, J. J.; Taylor, P. J. *Proceedings of the Third SCI-RSC Medicinal Chemistry Symposium*; Lambert, R. W., Ed.; Royal Society of Chemistry: London, 1986; p 345.

trifluoromethyl. This is illustrated by comparing the superior potency of **11** and **14** with **15**, **17** and **20** with **24**, and **25** with its positional isomer **26**. However, there were interseries variations. In the trifluoromethyl series the 4-nitro-3-trifluoromethyl analogues were less potent as shown by a comparison of **10** with **11** and **14** and **52** with **44**, **46**, and **51**. For the methyl series, the 4-nitro-3-trifluoromethyl analogues were essentially equipotent with or more potent than analogues with other patterns of electron-withdrawing groups in the anilide ring, e.g., compare **20** with **17**, **23**, and **25**.

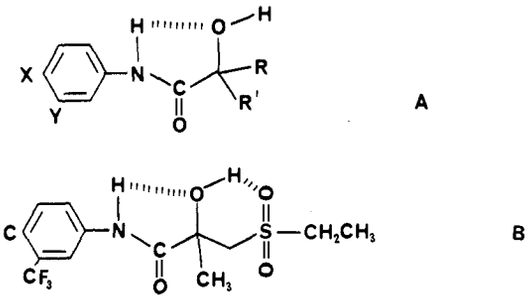
Interesting antiandrogen activity, which was better than that of flutamide, was found in the trifluoromethyl series, e.g., compounds **11–14**, **43–51**. However androgen agonism was an additional, undesirable property in some of these compounds. During a preliminary toxicological study in the dog,[10] it was found that the sulfide **11** was metabolized extensively to the sulfone **13** together with trace amounts of the sulfoxide **12**. Since the antiandrogenic potencies of **11–13** were essentially the same, it was concluded that the sulfone was the active biological entity. This pattern of activity was seen also with other sets of sulfides, sulfoxides, and sulfones, e.g., **17–19**; **20–22**; **39**, **40**; **53**, and **54**. There was no further interest in **11** because of its partial agonist activity.

In the case of the methyl series (structure **7**, R′ = CH_3), the most surprising and exciting finding here was that no agonist activity was seen in any of the compounds measured, even for compounds **30**, **55**, and **61**, whose trifluoromethyl analogues **29**, **47**, and **52** exhibited high levels of androgen agonism. Good antiandrogen activity was found across the arylthio, alkylthio, and heterocyclic thio analogues. In the case of the arylthio analogues, para substituents in the arylthio ring decreased activity (compounds **35–38**), except for chloro, which had little effect on potency (compounds **30** and **41**), and fluoro, which in one case increased potency (compare **17** and **19** with **39** and **40**). In the one set studied, the ortho and meta analogues were less potent than the para analogue (compounds **30–32**).

In the alkylthio series (Table III), potency was optimum for the ethylthio analogues and decreased with increasing size of the alkyl group (compare **53** with **55–57**; **59** with **58**, **60–63**).

The attraction of introducing the heterocyclic thio groups was that while any aromatic ring interactions were retained, the lipophilicity of this aromatic ring could be varied widely, e.g., the log *P* values (octanol) for the pyrimidine and thiophene rings are −0.40 and 1.81, respectively. This was of interest at the time since it was hypothesized that hydrophilic groups at this end of the molecule could contribute to selectivity for the accessory sex organs. Although potency was retained with a number of heterocyclic rings (e.g., **64**, **65**, **72**, and **73**), this was no better than that found in the aryl and alkylthio analogues.

A physicochemical study had shown that the dominant conformation in the antiandrogenic anilides was that in which the amide NH eclipsed the OH group (Figure 1, structure A).[11] This alignment between the NH and OH groups confers additional proton-donor ability to the OH group. It has been argued that this effect, which is influenced by electron-withdrawing groups in the anilide ring and the electronic properties of R and R′ in structure A, was a crucial factor in receptor interactions and hence the antiandrogen activity of these compounds.[9] An infrared



49 R′ = CF_3

53 R′ = CH_3

Figure 1.

study[12] on the two sulfones **49** and **53** has shown that the dominant (>95%) conformation in nonpolar solvents is B (Figure 1), in which the OH group is bound intramolecularly to one of the sulfonyl group oxygen atoms. The implication of this is that the OH group would not be free to participate in receptor interactions so that these sulfones would interact with the receptor via a different mechanism. However, a NMR study in which the chemical shift of the OH proton was plotted against solvent polarity indicated that the hydrogen bond to the sulfonyl group was disrupted in proton acceptor solvents such as deuterioacetone. This led to the conclusion[12] that since the moderately basic deuterioacetone could effect this disruption, then the aqueous environment of the receptor inself would certainly disrupt this internal hydrogen bond enabling the OH group to interact with the receptor in the manner proposed earlier.

On the question of androgen agonism, if we consider pairs of compounds that differ structurally only in whether R′ (structure **7**) is a methyl or trifluoromethyl group (e.g., **11** and **17**, **14** and **23**, **29** and **30**, and the alkylthio analogues of Tables II and III), the main biological difference is the androgen agonism found in the trifluoromethyl series. We have already discussed that one of the consequences of introducing this trifluoromethyl group is its enhancement of the proton–donor ability of the tertiary OH group. It is reasonable, therefore, to attribute the observed agonism to a tighter binding of the trifluoromethyl-substituted compounds to the receptor.

Potent compounds that did not exhibit androgen agonist activity were tested for their selectivity of action for the accessory sex organs over the hypothalamic–pituitary axis in rats. A sex organ selective antiandrogen would not prevent the natural agonists from exerting their negative feedback actions on the hypothalamic–pituitary axis so that there would be no stimulation of LH and testosterone production. This selectivity of action was measured essentially by using the primary test system described, except that dosing was carried out over a period (14 days) at three different dose levels (25, 5, and 1 mg/kg). A compound was considered to be selective if there was no elevation in hormone levels compared to untreated controls when dosed at a high multiple (10–25 times) of its ED_{50}. A selection of results is presented in Table IV, and it is clear that **17**, **20**, **33**, **40**, **65**, and **72** are selective antiandrogens. Minor structural changes can affect the selectivity (e.g., **65** and **66**) in some cases, whereas similar changes in other analogues have no effect (e.g., **17** and **20**; **33** and **40**). This would not appear to be merely a difference between arylthio and heterocyclic thio analogues since we have

(10) We thank G. F. Plummer of the Pharmacokinetics Section for providing this data.

(11) Dr. J. J. Morris of the Physical Chemistry Section, unpublished work. See also ref 9.

(12) Dr. J. J. Morris, unpublished work.

Table IV. Effects on Hormone Levels on 14-Day Dosing in Intact Rats

compd	testosterone[a]	LH[a]
17	NS	NS
20	NS	NS
33	NS	NS
40	NS	NS
53	<0.01	NS
64	<0.10	NS
65	NS	NS
66	<0.10	<0.05
72	NS	NS
flutamide	<0.01	<0.01

[a] Figures in the columns are the statistical significances for the hormone levels of the treated animals compared to a control group, which received vehicle only. NS indicates no statistically significant difference from controls.

Table V. Comparison of the Effects of Compound **40** and Flutamide on Hormone Levels following 14-Day Dosing to Intact Rats at 25 mg/kg[a]

compd	ED_{50}[b]	testosterone, ng/mL	LH, ng/mL
flutamide	7.0	7.61 ± 1.87[c]	9.00 ± 1.72[c]
40	1.3	3.97 ± 1.29	1.04 ± 0.25
control		1.90 ± 0.62	1.19 ± 0.19

[a] Values shown are means ± SEM, and there were five rats per group. [b] ED_{50} values measured in this test system are usually lower than those measured in the standard test system. [c] $P < 0.01$ compared with control.

found arylthio analogues also that were not selective (unpublished data). In general, members of the alkylthio series tended to be nonselective. The selective antiandrogens were studied in more detail. The best of these was compound **40**.[13,14] The peripheral selectivity of **40** was not due to progestational activity since it was inactive in the Clauberg assay, which is commonly used to measure progestational activity. Table V presents comparative data for **40** and flutamide when dosed to rats over a period of 14 days and clearly demonstrates that **40** is more potent than flutamide, and, when dosed at 19 times its ED_{50}, it does not elicit any elevation in testosterone or LH levels, whereas flutamide significantly elevates both at 3.5 times its ED_{50}.

Summary

Our studies on the effects of introducing substituted-methylthio groups onto the tertiary hydroxy-bearing carbon atom of hydroxyflutamide has led to a series of potent antiandrogens, a number of which exhibit selectivity for the accessory sex organs. The best of these novel, selective antiandrogens, compound **40** (ICI 176334), is being developed currently for the treatment of prostate cancer.

Experimental Section

All melting points were obtained with an Electrothermal capillary melting point apparatus and are uncorrected. NMR spectra were recorded either on a JEOL FX90Q or on a Varian EM390 instrument. Spectra were run on all isolated intermediates, and final products and were consistent with the structural assignments.

3-(Phenylthio)-1,1,1-trifluoropropan-2-one Cyanohydrin (4, R = Ph, R′ = CF_3). 3-(Phenylthio)-1,1,1-trifluoropropan-2-one[7] (17.95 g, 81.5 mmol) was added to a cooled solution of potassium cyanide (6 g, 92 mmol) in water (20 mL) over 20 min at such a rate that the internal temperature of the reaction mixture was kept between 5 and 10 °C. Sulfuric acid (20.7 mL of 25% v/v) was added over 15 min, while the internal temperature was kept below 5 °C. The mixture was allowed to warm to room temperature and was stirred for 16 h. The reaction mixture was extracted with diethyl ether (5 × 60 mL), and the combined ether extracts were washed with aqueous sodium bicarbonate and brine and dried over magnesium sulfate. Removal of the solvent gave a quantitative yield of 3-(phenylthio)-1,1,1-trifluoropropan-2-one cyanohydrin as an oil, which was used for the next stage without further purification.

2-Hydroxy-2-(trifluoromethyl)-3-(phenylthio)propionic Acid (5, R = Ph, R′ = CF_3). A mixture of 3-(phenylthio)-1,1,1-trifluoropropan-2-one cyanohydrin (20.9 g, 85 mmol) in concentrated HCl (300 mL) and glacial acetic acid (50 mL) was heated on a steam bath for 24 h with vigorous stirring.

The reaction mixture was cooled, diluted with water (250 mL), and extracted with diethyl ether (4 × 150 mL). The combined ether extracts were washed with 10% sodium bicarbonate solution (8 × 50 mL). After drying and evaporation of the ether extracts, the amide of 2-hydroxy-2-(trifluoromethyl)-3-(phenylthio)-propionic acid was obtained as an oil.

The sodium bicarbonate extracts were acidified with concentrated HCl to pH 1–2 and extracted with diethyl ether (5 × 100 mL). The ether extracts were dried ($MgSO_4$) and evaporated to dryness to give 2-hydroxy-2-(trifluoromethyl)-3-(phenylthio)-propionic acid as an oil (17.69 g), which solidified on trituration with petroleum ether (bp 40–60 °C), yield 13.65 g (61%), mp 82–4 °C. Anal. ($C_{10}H_9F_3O_3S$) C, H, N.

4′-Cyano-2-hydroxy-3-(phenylthio)-2,3′-bis(trifluoromethyl)propionanilide (11). Thionyl chloride (1.3 mL, 18 mmol) was added to a cooled (−15 °C), stirred solution of 2-hydroxy-2-(trifluoromethyl)-3-(phenylthio)propionic acid (4.8 g, 18 mmol) in *N,N*-dimethylacetamide (60 mL) at such a rate as to maintain the temperature of the reaction mixture at −15 °C. The mixture was stirred at −15 °C for 15 min, and 4-cyano-3-(trifluoromethyl)aniline[15] (3.35 g, 18 mmol) was added all at once. The mixture was stirred at −15 °C for 15 min and then at room temperature for 16 h and was then poured into water (500 mL) and extracted with diethyl ether (6 × 75 mL). The combined ether extracts were washed with saturated brine and dried ($MgSO_4$). Removal of the solvent gave an oily product, which was flash chromatographed on silica gel (Merck type 9385) with a 3:2 v/v mixture of petroleum ether (bp 60–80 °C) and ethyl acetate as eluant. Compound **11** was crystallized from a mixture of toluene and petroleum ether (bp 60–80 °C), yield 3.54 g (45%), mp 144 °C. Anal. ($C_{18}H_{12}F_6N_2O_2S$) C, H, N.

2-Methyl-*N*-[4-nitro-3-(trifluoromethyl)phenyl]-2-propenamide. A solution of 4-nitro-3-(trifluoromethyl)aniline[16] (16.7 g, 81 mmol) in *N,N*-dimethylacetamide (40 mL) was added dropwise to a cooled stirred solution of freshly distilled methacryloyl chloride (12.5 mL, 128 mmol) while the temperature of the reaction mixture was maintained below 5 °C. The yellow solution was allowed to warm to room temperature and was stirred for 4 h. The reaction mixture was poured into water (500 mL) and extracted with diethyl ether (5 × 100 mL). The combined ether extracts were washed with saturated sodium bicarbonate solution (3 × 50 mL) and brine (50 mL) and dried ($MgSO_4$). Evaporation of the ether and crystallization of the residue from a petroleum ether (bp 60–80 °C)–ethyl acetate mixture gave 2-methyl-*N*-[4-nitro-3-(trifluoromethyl)phenyl]-2-propenamide, yield 13.45 g, mp 105–106 °C. An additional 5.7 g of product was recovered from the mother liquors. The total yield was 19.15 g (86%). Anal. ($C_{11}H_9F_3N_2O_3$) C, H, N.

1,2-Epoxy-2-methyl-*N*-[4-nitro-3-(trifluoromethyl)-phenyl]propanamide. *m*-Chloroperoxybenzoic acid (7.4 g, 43 mmol) was added in portions to a refluxing solution of 2-methyl-*N*-[4-nitro-3-(trifluoromethyl)phenyl]-2-propenamide (5.7 g, 21 mmol) and 2,6-di-*tert*-butyl-4-methylphenol (70 mg) in 1,1,1-trichloroethane (130 mL). Heating was continued for 6 h, and the reaction mixture was allowed to cool. Methylene chloride (50 mL) was added, and the resulting solution was washed with saturated aqueous sodium sulfite (4 × 75 mL), saturated sodium bicarbonate (4 × 75 mL), and brine (75 mL) and dried ($MgSO_4$).

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The solvent was removed under pressure, and the solid residue obtained was crystallized from toluene–petroleum ether (bp 60–80 °C) to give 4.9 g (81%) of 1,2-epoxy-2-methyl-*N*-[4-nitro-3-(trifluoromethyl)phenyl]propanamide, mp 120–121 °C. Anal. ($C_{11}H_9F_3N_2O_4$) C, H, N.

4′-Nitro-2-hydroxy-2-methyl-2-(2-pyridylthio)-3′-(trifluoromethyl)propionanilide (65). A solution of 2-mercaptopyridine (0.42 g, 3.8 mmol) in THF (5 mL) was added dropwise to a stirred suspension of sodium hydride (0.19 g, 4 mmol, 50% dispersion in oil) in THF (10 mL) under argon at 0 °C, and the mixture was stirred at this temperature for 15 min. A solution of 1,2-epoxy-2-methyl-*N*-(4-nitro-3-trifluoromethyl)propanamide (1.1 g, 3.8 mmol) in THF (10 mL) was added. The reaction mixture was allowed to warm to room temperature, and stirring was continued for 16 h. Water (50 mL) was added, and the mixture was extracted with diethyl ether (2 × 50 mL). The ether extracts were dried ($MgSO_4$). Removal of the solvent under reduced pressure and crystallization of the solid residue from toluene–petroleum ether (bp 60–80 °C) gave **65**, 1.15 g (76%), mp 159–60 °C. Anal. ($C_{16}H_{14}F_3N_3O_4S$) C, H, N.

4′-Cyano-2-hydroxy-3-(phenylsulfinyl)-2,3′-bis(trifluoromethyl)propionanilide (12). A solution of sodium metaperiodate (430 mg, 2 mmol) in water (15 mL) was added to a solution of compound **11** (700 mg, 1.6 mmol) in methanol (35 mL), and the mixture was stirred at room temperature for 48 h. The reaction mixture was filtered, and the filter cake was washed with methanol (10 mL). The filtrate was evaporated to dryness, the residue was dissolved in ethyl acetate (100 mL), and the ethyl acetate solution was washed with water (25 mL). The solvent was removed under reduced pressure, and the solid obtained was crystallized from a toluene–petroleum ether (bp 60–80 °C) mixture to give **12**, yield 0.53 g (73%), mp 175–6 °C. Anal. ($C_8H_{12}F_6N_2O_3S$) C, H, N.

4′-Cyano-3-[(4-fluorophenyl)sulfonyl]-2-hydroxy-2-methyl-3′-(trifluoromethyl)propionanilide (40). *m*-Chloroperoxybenzoic acid (2.8 g, 16.2 mmol of 85% strength material) was added in portions to a stirred solution of **39** (2.5 g, 6.3 mmol) in methylene chloride (500 mL). The reaction mixture was stirred at room temperature for 16 h and then was washed with saturated sodium sulfite solution (2 × 50 mL), aqueous sodium carbonate (3 × 50 mL), and brine (75 mL) and dried ($MgSO_4$). The solid obtained on removal of the solvent was crystallized from ethyl acetate–petroleum ether (bp 60–80 °C) to give **40** yield 2.45 g (90%), mp 191–193 °C. Anal. ($C_{18}H_{14}F_4N_2O_4S$) C, H, N.

Pharmacology. Antiandrogen Activity in Intact Rats.[14] Groups of five male rats (170–190 g) were dosed orally with the test compound (ball-milled in 0.5% polysorbate) once daily for 4 days at various doses. Animals were killed 24 h after the last dose, and the seminal vesicles were dissected, blotted, and weighed. Each test had a control group, which received 0.5% polysorbate alone, and a group treated with the antiandrogen flutamide, dosed orally at 5 mg/kg. The percent inhibition was calculated with use of a cumulative castrate control group as the 100% effect. The ED_{50} values were calculated from the dose response curves.

Androgenic Activity. Groups of five male rats were castrated at 24 days of age and administered the test compound at a dose of 25 mg/kg (ball-milled in 0.5% polysorbate) once daily for 7 days. The animals were killed 24 h after the final dose, and the seminal vesicles and ventral prostate glands were dissected, cleaned, and weighed. Agonist activity was indicated by a statistically significant increase in seminal vesicle and ventral prostate gland weights compared to control castrated animals who received vehicle alone. The values quoted in the tables are the actual weights of the ventral prostate glands in milligrams.

Test for Selectivity.[13] This is essentially the same test as described for measuring antiandrogenic activity except that dosing is continued once daily for 14 days at three dose levels of 25, 5, and 1 mg/kg. The weights of the seminal vesicles are recorded, and blood samples are collected for the measurement of luteinizing hormone and testosterone blood levels 24 h after the final dose. A selective antiandrogen will exhibit no increase in LH or testosterone compared to a control group, which received vehicle only, when dosed as high multiples of its ED_{50}.

Acknowledgment. We thank Dr. B. J. A. Furr, B. Valcaccia, B. Deegan, and C. Lunning for providing the biological data.

Registry No. **4** (R = Ph, R′ = Me), 112988-42-8; **4** (R = Ph, R′ = CF_3), 90357-42-9; **4** (R = 4-ClC_6H_4, R′ = Me), 112988-43-9; **4** (R = 4-ClC_6H_4, R′ = CF_3), 112988-44-0; **4** (R = *i*-Pr, R′ = Me), 112988-45-1; **4** (R = R′ = Me), 112988-46-2; **4** (R = Et, R′ = Me), 112988-47-3; **4** (R = Pr, R′ = Me), 112988-48-4; **5** (R = Ph, R′ = Me), 90357-22-5; **5** (R = Ph, R′ = CF_3), 90357-34-9; **5** (R = 4-ClC_6H_4, R′ = Me), 112988-49-5; **5** (R = 4-ClC_6H_4, R′ = CF_3), 90357-35-0; **5** (R = *i*-Pr, R′ = Me), 90357-29-2; **5** (R = R′ = Me), 90357-26-9; **5** (R = Et, R′ = Me), 90357-27-0; **5** (R = Pr, R′ = Me), 90357-28-1; **8** (R′ = CN, R^2 = CF_3), 90357-53-2; **8** (R′ = NO_2, R^2 = CF_3), 90357-52-1; **9** (R′ = NO_2, R^2 = CF_3), 90357-50-9; **9** (R′ = CN, R^2 = CF_3), 90357-51-0; **10**, 90356-28-8; **11**, 90356-35-7; **12**, 112988-36-0; **13**, 90356-98-2; **14**, 90356-46-0; **15**, 90356-41-5; **16**, 112988-37-1; **17**, 90356-00-6; **18**, 90356-93-7; **19**, 90357-05-4; **20**, 90356-01-7; **21**, 90356-92-6; **22**, 90356-99-3; **23**, 90356-13-1; **24**, 90357-19-0; **25**, 90356-16-4; **26**, 90356-15-3; **27**, 90356-20-0; **28**, 90356-36-8; **29**, 90356-29-9; **30**, 90356-59-5; **31**, 90356-58-4; **32**, 90356-57-3; **33**, 90356-60-8; **34**, 90357-00-9; **35**, 90356-62-0; **36**, 90356-61-9; **37**, 90356-63-1; **38**, 90356-64-2; **39**, 90356-78-8; **40**, 90357-06-5; **41**, 90356-77-7; **42**, 90356-79-9; **43**, 90356-47-1; **44**, 90356-49-3; **45**, 90356-43-7; **46**, 90356-44-8; **47**, 90356-37-9; **48**, 90356-38-0; **49**, 90357-09-8; **50**, 90356-40-4; **51**, 90356-39-1; **52**, 90356-33-5; **53**, 90357-07-6; **54**, 112988-38-2; **55**, 90356-10-8; **56**, 90356-09-5; **57**, 112988-39-3; **58**, 90356-03-9; **59**, 90357-02-1; **60**, 90356-06-2; **61**, 90357-03-2; **62**, 90357-04-3; **63**, 112988-40-6; **64**, 90356-74-4; **65**, 90356-70-0; **66**, 90356-80-2; **67**, 90356-71-1; **68**, 90356-81-3; **69**, 90356-72-2; **70**, 90356-88-0; **71**, 90356-83-5; **72**, 90356-76-6; **73**, 90356-87-9; **74**, 90356-75-5; PhSH, 108-98-5; *p*-ClC_6H_4SH, 106-54-7; *i*-PrSH, 75-33-2; MeSH, 74-93-1; PrSH, 107-03-9; EtSH, 75-08-1; *m*-ClC_6H_4SH, 2037-31-2; *o*-ClC_6H_4SH, 6320-03-2; *p*-FC_6H_4SH, 371-42-6; *p*-$O_2NC_6H_4SH$, 1849-36-1; *p*-NCC_6H_4SH, 36801-01-1; *p*-$MeOC_6H_4SH$, 696-63-9; *p*-$MeSC_6H_4SH$, 1122-97-0; *n*-$C_5H_{11}SH$, 110-66-7; *t*-BuSH, 75-66-1; $PhSCH_2COCF_3$, 34509-09-6; $PhSCH_2Ac$, 5042-53-5; *p*-$ClC_6H_4SCH_2COCF_3$, 92682-40-1; *p*-$ClC_6H_4SCH_2Ac$, 25784-83-2; *i*-$PrSCH_2Ac$, 112988-41-7; $MeSCH_2Ac$, 14109-72-9; $PrSCH_2Ac$, 17078-37-4; $EtSCH_2Ac$, 20996-62-7; *p*-$NH_2C_6H_4NO_2$, 100-01-6; 1-NH_2-3-CF_3-4-NO_2C_6-$_3$, 393-11-3; 1-NH_2-3-CF_3-4-CNC_6H_3, 654-70-6; 1-NH_2-3-Cl-4-CNC_6H_3, 20925-27-3; 1-NH_2-3,4-$Cl_2C_6H_3$, 95-76-1; 1-NH_2-3-NO_2-4-ClC_6H_3, 635-22-3; 1-NH_2-3-Me-4-CNC_6H_3, 72115-06-1; 1-NH_2-3-Cl-4-$NO_2C_6H_3$, 825-41-2; $BrCH_2Ac$, 598-31-2; $BrCH_2COCF_3$, 431-35-6; 2-thiazolylthiol, 5685-05-2; 2-pyridylthiol, 2637-34-5; 3-pyridylthiol, 16133-26-9; 4-pyridylthiol, 4556-23-4; 2-pyrimidylthiol, 1450-85-7; 2-thienylthiol, 7774-74-5; 5-methyl-2-1,3,4-thiadiazolylthiol, 29490-19-5; 2-benzothiazolylthiol, 149-30-4; 4-methyl-2-thiazolylthiol, 5685-06-3.