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Antiimplantation Agents: Part I—1-Arylthiosemicarbazides[a,b]

K NAGARAJAN[c], P K TALWALKER, C L KULKARNI, A VENKATESWARLU, S S PRABHU & G V NAYAK
Hindustan CIBA-GEIGY Ltd Research Centre, Bombay 400 063

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Several 1-arylthiosemicarbazides, 2-arylhydrazinothiazolines and 2-arylhydrazinodihydrothiazines have been examined for their antiimplantation activity in rats. Among the active compounds, 4-methyl-1-(3,5-bistrifluoromethylphenyl)thiosemicarbazide (**3**, C 2696-Go) and the corresponding 4,4-dimethyl (**47**), ethyl (**4**), *n*-butyl (**5**) and allyl (**6**) derivatives completely inhibit implantation at doses of 10, 3, 20, 20 and 30 mg/kg respectively. The 3,4-dichlorophenyl analogue (**32**) is effective at a dose of 30 mg/kg. 2-(3,5-Bistrifluoromethylphenyl)hydrazinothiazoline (**51**) and the corresponding dihydrothiazine (**63**) show a weaker activity. The biological profile of C 2696-Go has been investigated in detail. It appears to prevent implantation by its antiuterotropic activity and ability to inhibit desiduoma formation.

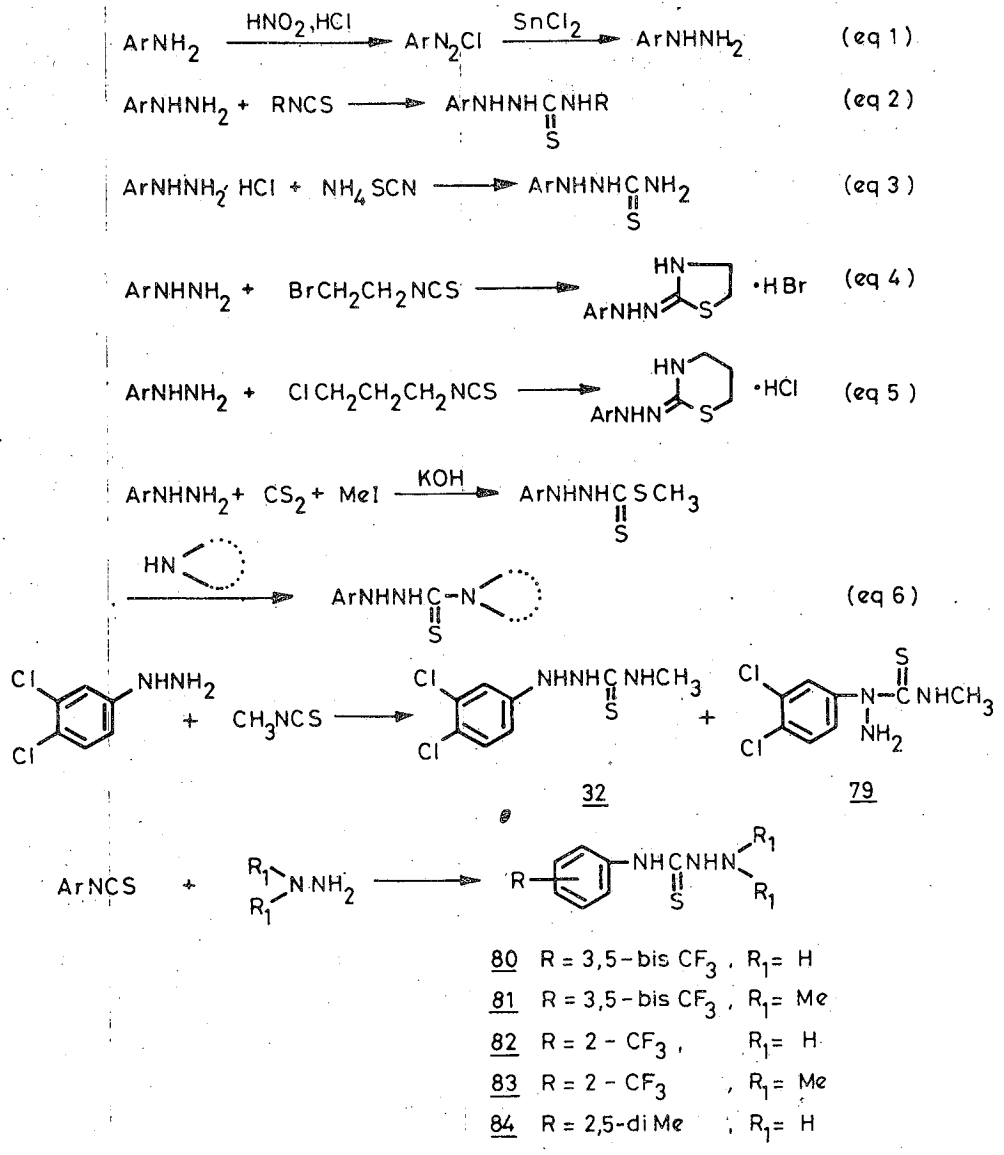
The explosive growth of human population and continuous depletion of earth's nonreplenishable resources require that family planning and control of its size has to be viewed more as an urgent need of the whole society and not of individuals alone and that all available measures should be marshalled towards this end. Antiovulatory steroids have had a remarkable degree of success as oral contraceptives[1], but this has been in general limited to the developed countries of the West and to the affluent elite of the developing ones, with the exception, perhaps of China. A major drawback in the use of antiovulatory steroids has been the need for swallowing the 'pill' for a greater period, if not whole, of the menstrual cycle. Although depot preparations are available which circumwent this difficulty, they have not found universal acceptability[1]. Further, there have been recurrent debates and persistent doubts about the side effects resulting from the continuous use of hormonal contraceptives[2]. Hence, efforts have been made during the last two decades from many research groups to discover post-coital and other types of antifertility agents not depending on the control of ovulation. One of the possible mechanisms of action of such an agent would be the inhibition of implantation. There have been very few successful breakthroughs and the challenge and seriousness of the problem continue to attract considerable chemical and biological efforts. For several years, we have been interested in the elaboration of antiimplantation agents and now we would like to report our results in this paper.

In our preliminary work, we found 4-allyl-1-(3,5-bistrifluoromethylphenyl)thiosemicarbazide (**6**) to show moderate antiimplantation activity. This led to the synthesis and evaluation of a large number of its analogues, which form the subject matter of this paper.

Chemistry

1-Aryl-4-monosubstituted-thiosemicarbazides were obtained by the action of isothiocyanates on arylhydrazines (Eq. 2) which in turn were prepared generally from anilines by diazotisation followed by stannous chloride reduction of the diazonium compounds (Eq. 1). It was considered that attack of the isothiocyanate would occur on the less substituted nitrogen in the case of arylhydrazines since the other one is expected to be less basic. In a large scale synthesis of 1-(3,4-dichlorophenyl)-4-methylthiosemicarbazide (**32**; Table 1) from hydrazine and methyl isothiocyanate, an isomer was obtained in low yield, which is considered to be **79**. In accordance with the structure proposed, 2-aryl-4-methylthiosemicarbazide (**79**) with a free NH_2 group formed a benzylidene derivative, while the 1-aryl isomer (**32**) and the analogous 4-methyl-1-(3,5-bistrifluoromethylphenyl)-thiosemicarbazide (**3**) were recovered unchanged from treatment with benzaldehyde. The mass spectra of **3**, **32** and **79** exhibited strong fragments corresponding to the loss of CH_3NCS, followed by further loss of NH_2, whereas only **79** showed a fragment corresponding to successive losses of CH_3NH and NH_2 groups. Compound **77** was synthesised by the action of methyl isothiocyanate on 1-ethyl-2-(3,5-bistrifluoromethylphenyl)hydrazine which in turn was obtained by LAH reduction of the acetyl derivative of **76**. 1-Aryl-4-unsubstituted-thiosemicarbazides were prepared by the reaction of hydrazine hydrochlorides with ammonium thiocyanate in hot ethanol (Eq. 3). A few selected 4-arylthiosemicarbazides (**80-83**) were obtained from hydrazines and aryl isothiocyanates.

[a] Contribution No. 722 from Research Centre.
[b] Dedicated to Dr Nitya Anand on his 60th birth anniversary.
[c] Present address: Director, Searle R & D Centre, Thane-Belapur Road, Thane 400 601, Maharashtra.



2-Arylhydrazinothiazolines and 2-arylhydrazinodihydrothiazines were obtained as hydrobromide and hydrochloride salts respectively from arylhydrazines by reaction with 2-bromoethyl isothiocyanate (Eq. 4) and 3-chloropropyl isothiocyanate (Eq. 5). Compounds **48** and **49** (Table 2) were obtained from 3,5-bistrifluoromethylphenylhydrazine (**76**) via the dithiocarbamic ester (Eq. 6) while **47** was obtained by the action of dimethylthiocarbamoyl chloride on **76**.

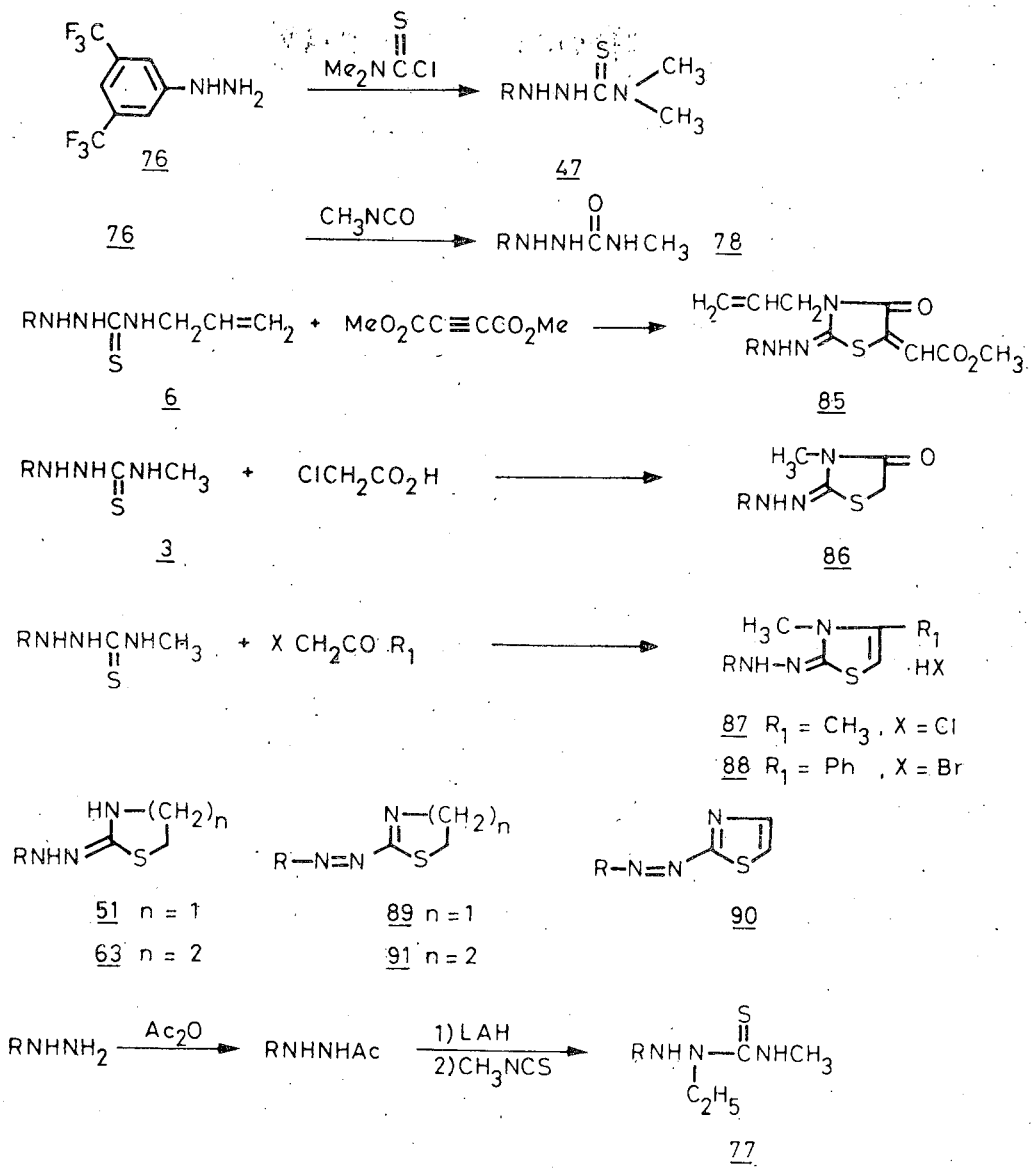
Treatment of the allylthiosemicarbazide (**6**) with dimethyl acetylenedicarboxylate gave the thiazolidinone **85**, while the methyl thiosemicarbazide (**3**) and chloracetic acid gave **86**. Similar reactions of **3** with chloracetone and phenacyl bromide gave respectively the iminothiazolines **87** and **88** as their salts. Silver oxide treatment[4] of the hydrazinothiazoline base (**51**; Table 3) gave the arylazothiazoline **89** as a crystalline compound with poor stability. This on keeping at room temperature for a few days underwent disproportionation into the starting material (**51**) and the arylazothiazole **90**. The latter was obtained directly from **51** by aerial oxidation in ethanol in the presence of sodium carbonate[4]. Silver oxide oxidation of dihydrothiazine (**66**; Table 4) gave the azo derivative (**91**; Table 5).

Biological activity

The basic screening assessed the potency of compounds in inhibiting implantation in the rat. Other tests were carried out to characterize the highly active preparation **3**.

Test 1: *Inhibition of implantation*

Proestrus rats of Charles Foster strain (150-200 g) were placed with males of proven fertility. The day sperm was identified in the vaginal smeal was



76

47

76 78

6

85

3

86

87 R_1 = CH_3, X = Cl

88 R_1 = Ph, X = Br

51 n = 1

63 n = 2

89 n = 1

91 n = 2

90

77

(R = 3,5-bistrifluoromethyl phenyl)

considered day 1 of pregnancy. On day 10, females were sacrified and uteri examined for implantation sites and gross abnormalities. Test compounds were administered as agar suspensions p.o. on days 1-3 or 4-6, the routine screening dose being 100 mg/kg. Minimum doses inhibiting implantation in 100% of the rats were determined. Compounds inactive at 100 mg/kg are marked φ.

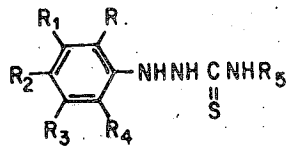
Test 2: *Estrogenic activity—Allen Doisy test*[5]

Adult female rats (120-140 g) were castrated. One week later the test compound was administered orally or estradiol in oil subcutaneously (s.c.) for 3 consecutive days. Vaginal smears were taken 24 and 48 hr after last administration. The smears were read according to the published method and the absence of leukocytes was considered as a positive response. 3 μg of estradiol s.c. elicited the response in all the treated rats.

Test 3: *Uterotropic activity—Immature castrated rat uterus*[5]

Immature female rats (about 50 g) were castrated. Three days later, rats were treated daily with the test substance orally or with estradiol in oil s.c. for 4 days. The day after last treatment, animals were sacrificed, uteri removed, cleaned, blotted on a filter paper and weighed. Dry weights of uteri were also recorded after overnight drying in an oven and compared with those of untreated control. 3 μg estradiol caused 300% increase in uterine weight.

Table 1—1-Arylthiosemicarbazides



Compd	R	R_1	R_2	R_3	R_4	R_5	Mol. formula	m.p. °C	Crystallized from[a]	Antiferitility activity[b]		Found (%) (Calc.)		
										1-3 days	4-6 days	C	H	N
1	H	H	H	H	H	Me	$C_8H_{11}N_3S$	172-74	L	φ	φ	53.4 (53.0	6.3 6.1	23.3 23.2)
2[c]	H	CF_3	H	CF_3	H	H	$C_9H_7F_6N_3S$	195	G+C	φ	φ	35.6 (35.7	2.4 2.3	14.1 13.9)
3	H	CF_3	H	CF_3	H	CH_3	$C_{10}H_9F_6N_3S$	207-8	H+E	10	10	38.2 (37.9	3.1 2.9	12.9 13.2)
4	H	CF_3	H	CF_3	H	Et	$C_{11}H_{11}F_6N_3S$	203-4	J+E	20	20	40.0 (39.9	3.6 3.4	12.9 12.7)
5	H	CF_3	H	CF_3	H	n-Bu	$C_{13}H_{15}F_6N_3S$	166-68	F+E	20	20	43.5 (43.5	4.2 4.2	11.8 11.7)
6	H	CF_3	H	CF_3	H	$-CH_2CH=CH_2$	$C_{12}H_{11}F_6N_3S$	182-83	F+E	30	20	42.3 (42.0	3.4 3.2	12.7 12.3)
7	H	CF_3	H	CF_3	H	$-CHCH=CH_2$ (Me)	$C_{13}H_{13}F_6N_3S$	105-7	F+E	100	100	44.0 (43.7	3.6 3.7	11.6 11.8)
8	H	CF_3	H	CF_3	H	Me $-CH_2C=CH_2$	$C_{13}H_{13}F_6N_3S$	132-34	F+E	>100	>100	43.6 (43.7	3.8 3.7	11.3 11.8)
9	H	CF_3	H	CF_3	H	Ph	$C_{15}H_{11}F_6N_3S$	158-59	F+E	φ	φ	47.9 (47.5	3.1 2.9	10.7 11.1)
10	H	CF_3	H	CF_3	H	$PhCH_2-$	$C_{16}H_{13}F_6N_3S$	164-66	B+C	>100	>100	48.6 (48.9	3.3 3.3	10.5 10.7)
11	H	CF_3	H	CF_3	H	$PhCH_2CH_2-$	$C_{17}H_{15}F_6N_3S$	133-35	D+E	φ	φ	50.1 (50.1	3.5 3.7	10.2 10.3)
12	H	CF_3	H	CF_3	H	3,5-bis$CF_3-C_6H_3$	$C_{17}H_9F_{12}N_3S$	149-51	J+E	φ	φ	39.5 (39.6	1.7 1.8	8.3 8.2)
13	H	CF_3	H	CF_3	H	2-Ph$-C_6H_4$	$C_{21}H_{15}F_6N_3S$	175-76	J+E	φ	φ	55.5 (55.4	3.5 3.3	9.1 9.2)
14	H	CF_3	H	CF_3	H	C_6H_{11}	$C_{15}H_{17}F_6N_3S$	165-67	I+E	φ	φ	47.0 (46.8	4.3 4.5	11.0 10.9)

(Contd)

Table 1—1-Arylthiosemicarbazides—*Contd*

Compd	R	R_1	R_2	R_3	R_4	R_5	Mol. formula	m.p. °C	Crystallized from[a]	Antifertility activity[b] 1-3 days	4-6 days	Found (%) (Calc.) C	H	N
15[d]	H	CF_3	H	CF_3	H	$-CH_2CH_2NEt_2$	$C_{13}H_{16}F_6N_3S$	145	I+E	>100	>100	42.0	4.3	15.5
												(41.7	4.3	15.0)
16	H	CF_3	H	CF_3	H	PhCO	$C_{16}H_{11}F_6N_3OS$	122-23	J+K	φ	φ	47.3	2.6	10.4
												(47.2	2.7	10.3)
17	H	CF_3	H	CF_3	H	CO_2Et	$C_{12}H_{11}F_6N_3O_2S$	161-62	D+E	not tested		38.4	2.7	10.8
												(38.4	3.0	11.1)
18	H	CF_3	H	H	H	CH_3	$C_9H_{10}F_3N_3S$	151-52	F+E	>100	>100	43.9	4.0	17.1
												(43.7	4.1	16.9)
19	H	CF_3	H	H	H	$-CH_2C(CH_3)=CH_2$	$C_{12}H_{14}F_3N_3S$	111-13	D+E	φ	φ	49.7	4.7	14.3
												(49.8	4.9	14.5)
20	H	CF_3	H	H	H	Ph	$C_{14}H_{12}F_3N_3S$	153	F+E	φ	φ	53.9	4.2	13.4
												(54.0	3.9	13.5)
21	H	CF_3	H	H	H	$PhCH_2$	$C_{15}H_{14}F_3N_3S$	121-23	D+E	φ	φ	55.6	4.6	12.8
												(55.4	4.3	12.9)
22	H	H	CF_3	H	H	CH_3	$C_9H_{10}F_3N_3S$	185-87	J+E	>100	>100	43.6	3.8	16.7
												(43.4	4.1	16.9)
23	CF_3	H	H	H	H	CH_3	$C_9H_{10}F_3N_3S$	114-15	H+E	φ	φ	43.3	4.1	17.2
												(43.4	4.1	16.9)
24	H	CF_3	H	Cl	H	CH_3	$C_9H_9ClF_3N_3S$	188-89	H+E	100	100	38.1	3.0	15.2
												(38.1	3.2	14.8)
25	H	CF_3	H	H	Cl	CH_3	$C_9H_9ClF_3N_3S$	188-89	K+E	>100	100	38.3	3.4	14.7
												(38.1	3.2	14.8)
26	H	CF_3	Cl	H	H	CH_3	$C_9H_9ClF_3N_3S$	160-62	H+E	100	100	38.2	3.0	14.8
												(38.1	3.2	14.8)
27	CF_3	H	Cl	H	H	CH_3	$C_9H_9ClF_3N_3S$	143-45	K+E	100	100	38.5	3.0	14.8
												(38.1	3.2	14.8)
28	H	CF_3	H	CO_2Et	H	CH_3	$C_{11}H_{12}F_3N_3O_2S$	243(d)	K+E	φ	φ	43.0	3.8	13.4
												(43.0	3.9	13.7)
29[e]	H	Cl	H	Cl	H	H	$C_7H_7Cl_2N_3S$	200	D+E	φ	φ	35.3	3.2	17.7
												(35.6	3.0	17.8)
30	H	Cl	H	Cl	H	CH_3	$C_8H_9Cl_2N_3S$	208-9	K	>100	>100	38.4	3.7	16.6
												(38.4	3.6	16.8)
31	H	Cl	Cl	H	H	H	$C_7H_7Cl_2N_3S$	208-9	A+C	φ	φ	35.7	3.0	17.8
												(35.6	3.0	17.8)
32	H	Cl	Cl	H	H	CH_3	$C_8H_9Cl_2N_3S$	202-3	I	30	30	38.8	3.8	17.0
												(38.4	3.6	16.8)

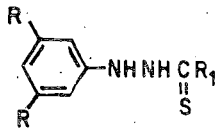
(*Contd*)

Table 1—1-Arylthiosemicarbazides—*Contd*

Compd	R	R_1	R_2	R_3	R_4	R_5	Mol. formula	m.p. °C	Crystallized from[a]	Antifertility activity[b]		Found (%) (Calc.)		
										1-3 days	4-6 days	C	H	N
33	H	Cl	Cl	H	H	Et	$C_9H_{11}Cl_2N_3S$	160-62	H+E	100	100	41.3 (40.9	4.2 4.2	16.1 15.9)
34	H	Cl	Cl	H	H	$CH_2CH=CH_2$	$C_{10}H_{11}Cl_2N_3S$	118-19	I+E	100	>100	43.3 (43.5	4.2 4.0	15.4 15.2)
35[e]	Cl	H	Cl	H	H	H	$C_7H_7Cl_2N_3S$	193-94	G	>30	>30	35.9 (35.6	3.1 3.0	17.5 17.8)
36	Cl	H	Cl	H	H	CH_3	$C_8H_9Cl_2N_3S$	183-84	K+E	>100	>100	38.7 (38.4	4.0 3.6	17.0 16.8)
37	Cl	Cl	H	H	H	CH_3	$C_8H_9Cl_2N_3S$	164-65	H+E	φ	φ	38.5 (38.4	3.3 3.6	17.1 16.8)
38	Cl	H	H	Cl	H	CH_3	$C_8H_9Cl_2N_3S$	197-99	H+E	φ	φ	38.4 (38.4	3.8 3.6	16.7 16.8)
39	Cl	H	H	H	Cl	CH_3	$C_8H_9Cl_2N_3S$	221(d)	K+E	φ	φ	38.3 (38.4	3.6 3.6	16.7 16.8)
40	H	OCH_3	H	OCH_3	H	CH_3	$C_{10}H_{15}N_3O_2S$	191-92	L	φ	φ	49.9 (49.8	6.4 6.3	15.7 17.4)
41	H	CH_3	H	CH_3	H	CH_3	$C_{10}H_{15}N_3S$	186-88	B+C	φ	φ	57.5 (57.4	6.9 7.2	19.7 20.1)
42	H	Cl	H	H	H	CH_3	$C_8H_{10}ClN_3S$	177-78	H+E	φ	φ	44.9 (44.6	4.9 4.7	19.5 19.5)
43	H	H	Cl	H	H	CH_3	$C_8H_{10}ClN_3S$	195-96	K	100	100	44.3 (44.6	4.6 4.7	19.5 19.5)
44	Cl	H	H	H	H	CH_3	$C_8H_{10}ClN_3S$	147-49	H+E	φ	φ	45.3 (44.6	4.8 4.7	19.6 19.5)
45	Ph	H	H	H	H	CH_3	$C_{14}H_{15}N_3S$	165	K+E	>100	> 100	65.3 (65.4	5.7 5.9	16.6 16.3)
46	Ph	H	H	H	H	$CH_2CH=CH_2$	$C_{16}H_{17}N_3S$	140-42	B	>100	> 100	68.2 (67.8	5.8 6.1	15.3 14.8)

[a] A = DMF; B = MeOH; C = water; D = Et_2O; E = hexane; F = $CHCl_2$; G = EtOH; H = acetone; I = benzene; J = $CHCl_3$; K = EtOAc;
[b] In rat; MED_{100} in mg/kg/day p.o, postcoital.
[c] Toxic at 100 mg; tested at 20 mg.
[d] Maleate.
[e] Toxic at 100 mg; tested at 30 mg.

Table 2—1-Aryl-4,4-disubstituted-thiosemicarbazides



Compd	R	R_1	Mol. formula	m.p. °C	Crystallized from[a]	Antifertility activity[b]		Found (%) (Calc.)		
						1-3 days	4-6 days	C	H	N
47	CF_3	Me_2N	$C_{11}H_{11}F_6N_3S$	162-64	D+E	3	10	39.9	3.7	12.6
								(39.9	3.4	12.7)
48	CF_3	Piperidino	$C_{14}H_{15}F_6N_3S$	103-4	D+E	φ	φ	45.7	4.3	11.4
								(45.3	4.1	11.3
49	H	Piperidino	$C_{12}H_{11}N_3S$	119-20	H+E	φ	φ	61.5	7.4	17.8
								(61.3	7.3	17.9)
50	H	Morpholino	$C_{11}H_{15}N_3OS$	146-47	H+E	Not tested		55.5	6.6	17.5
								(55.7	6.4	17.7)

[a] D = Et_2O; E = hexane; H = acetone
[b] In rat; *MED* 100 in mg/kg/day p.o. post-coital

Test 4: *Uterotropic activity—Immature mouse*[5]

Immature albino Swiss mice (8-10 g) were treated orally with test substance or with estradiol in oil s.c. for 3 days. The day after last treatment, animals were sacrificed and the uteri removed, cleaned, blotted on a filter paper and their wet weights compared with those of untreated controls. Estradiol at 0.1 μg per mouse caused an increase of 400% in uterine weight.

Test 5: *Estrogenic activity—Vaginal opening in the immature mouse*[5]

Vaginal opening was also measured in the previous experiment and results from treated mice compared with those of control. All the mice treated with estradiol gave a positive response.

Test 6: *Estrogenic activity—Withdrawal bleeding in the immature rhesus monkey*[6]

Immature rhesus monkeys (*Macaca mullata*) weighing 1.5-2.0 kg were given daily s.c. injection of estradiol or oral administration of the test substance for 20 days. Beginning on the 21st day, saline vaginal lavage was used to determine the onset of withdrawal bleeding; this procedure was continued until bleeding was completed or until 1 month. The interval between cessation of treatment and onset of bleeding was recorded. On the 21st day, vaginal smears were taken, stained and examined under a microscope. Estradiol at a dose of 2.5 μg/kg/day consistently and uniformly induced withdrawal bleeding in all the treated animals. The dose of the test substance giving similar effects was ascertained.

Test 7: *Antiestrogenic activity in castrated rats—Vaginal smears test*[7]

Mature female rats (about 150 g) were castrated. Two weeks later, four groups of 6 rats each were continuously treated daily s.c. with 0.4 μg/kg/day of estradiol in oil for 14 days. Beginning from day 6 of estradiol treatment, three groups were treated orally daily with test substance at doses of 5, 10 and 20 mg/kg/day for 8 days. A fourth group served as controls. Daily vaginal smears were taken and read. The presence of leukocytes or inhibition of cornification was considered as positive response. Within 3 days all the animals treated with estradiol came into estrus.

Test 8: *Antiuterotropic activity—Immature mouse*[7]

Immature albino Swiss mice (8-10 g) were treated orally with test substance at 5, 10 or 20 mg/kg/day together with 0.1 μg/mouse/day estradiol in oil s.c. for 3 days. The day after last treatment, animals were killed with $CHCl_3$. The uteri were removed, cleaned, blotted on filter paper and their wet weights recorded and compared with those of controls.

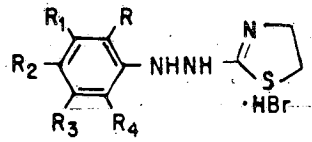
Test 9: *Antiestrogenic activity in immature mice—Inhibition of vaginal opening*[7]

Vaginal opening in the mice from the previous experiment was determined. Inhibition of estradiol induced opening by test substance was assessed.

Test 10: *Progestational (Clauberg) and progesterone antagonistic activities*[8]

Groups of 3 to 5 New Zealand immature female rabbits (1-1.2 kg) were primed daily with s.c. injection

Table 3—2-Arylhydrazinothiazoline Hydrobromides

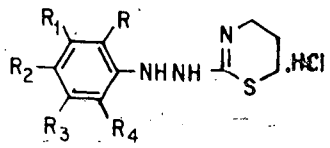


Compd	R	R_1	R_2	R_3	R_4	Mol. formula	m.p. °C	Crystallised from[a]	Antifertility activity[b]		Found (%) (Calc.)		
									1-3 days	4-6 days	C	H	N
51	H	CF_3	H	CF_3	H	$C_{11}H_{10}BrF_6N_3S$	288-91	B+D	50	50	2.1 (32.2	2.3 2.5	10.4 10.3)
52	Cl	H	H	CF_3	H	$C_{10}H_{10}BrClF_3N_3S$	232-34	B+D	φ	φ	31.9 (31.9	2.7 2.7	11.3 11.2)
53	CF_3	H	Cl	H	H	$C_{10}H_{10}BrClF_3N_3S$	249-51	B+D	φ	φ	32.3 (31.9	3.0 2.7	11.6 11.2)
54	H	Cl	H	Cl	H	$C_9H_{10}BrCl_2N_3S$	239-41	B+D	φ	φ	31.8 (31.5	3.2 2.9	12.5 12.3)
55	H	Cl	Cl	H	H	$C_9H_{10}BrCl_2N_3S$	218-20	G+D	>100	>100	31.7 (31.5	3.3 2.9	11.9 12.3)
56	Cl	H	Cl	H	H	$C_9H_{10}BrCl_2N_3S$	234-36	B+D	φ	φ	31.9 (31.5	3.4 2.9	12.5 12.3)
57	Cl	H	H	Cl	H	$C_9H_{10}BrCl_2N_3S$	248-50 (d)	B+D	φ	φ	31.9 (31.5	3.1 2.9	12.6 12.3)
58	Cl	H	H	H	Cl	$C_9H_{10}BrCl_2N_3S$	240-42	B+D	φ	φ	31.8 (31.5	2.7 2.9	12.4 12.3)
59	Cl	Cl	H	H	H	$C_9H_{10}BrCl_2N_3S$	251-53	B+D	φ	φ	31.8 (31.5	3.3 2.9	12.2 12.3)
60	CH_3	H	H	H	CH_3	$C_{11}H_{16}BrN_3S$	230-31 (d)	B+D	not tested		43.8 (43.7	5.3 5.3	13.9 13.9)
61	H	H	Cl	H	H	$C_9H_{11}BrClN_3S$	234-36	H+E	φ	φ	35.2 (35.0	4.0 3.6	14.2 13.6)
62	CH_3	H	H	H	H	$C_{10}H_{14}BrN_3S$	219-23	B+D	not tested		41.9 (41.7	5.2 4.9	14.4 14.6)

[a] B = MeOH; D = Et_2O; E = hexane; G = EtOH; H = acetone

[b] In rat; MED_{100} in mg/kg/day p.o. post-coital.

Table 4—2-Arylhydrazino-4,5-dihydro-6*H*-thiazine Hydrochlorides



Compd	R	R_1	R_2	R_3	R_4	Mol. formula	m.p. °C	Crystallised from[a]	Antifertility activity[b]		Found (%) (Calc.)		
									1-3 days	4-6 days	C	H	N
63	H	CF_3	H	CF_3	H	$C_{12}H_{12}ClF_6N_3S$	278-79	B+D	100	30	38.2 (38.0	3.4 3.2	11.2 11.1)
64	Cl	H	H	CF_3	H	$C_{11}H_{12}Cl_2F_3N_3S$	214-16	B+D	not tested		38.5 (38.2	3.7 3.5	12.1 12.1)
65	H	Cl	H	Cl	H	$C_{10}H_{12}Cl_3N_3S$	253-55	B+D	100	100	38.4 (38.4	3.8 3.9	13.2 13.4)
66	H	Cl	Cl	H	H	$C_{10}H_{12}Cl_3N_3S$	230-31	B+D	φ	φ	38.3 (38.4	4.1 3.9	13.6 13.4)
67	Cl	H	H	H	Cl	$C_{10}H_{12}Cl_3N_3S$	230-32	B+D	not tested		38.7 (38.4	4.2 3.9	13.3 13.4)
68	Cl	Cl	H	H	H	$C_{10}H_{12}Cl_3N_3S$	230-32	B+D	φ	φ	38.8 (38.4	3.9 3.9	13.0 13.4)
69	Cl	H	Cl	H	H	$C_{10}H_{12}Cl_3N_3S$	196-98	B+D	100	100	38.5 (38.4	3.9 3.9	13.1 13.4)
70	Cl	H	H	Cl	H	$C_{10}H_{12}Cl_3N_3S$	250-52	B+D	not tested		38.7 (38.4	4.1 3.9	13.8 13.4)
71	CH_3	H	H	H	CH_3	$C_{12}H_{18}ClN_3S$	202-4	G+E	not tested		52.7 (53.0	6.8 6.7	15.4 15.5)
72	H	Cl	H	H	H	$C_{10}H_{13}Cl_2N_3S$	207-9	B+D	φ	φ	43.2 (43.2	5.0 4.7	15.0 15.1)
73	H	H	Cl	H	H	$C_{10}H_{13}Cl_2N_3S$	239-40	B+D	not tested		43.1 (43.2	4.8 4.7	15.0 15.1)
74	H	H	Br	H	H	$C_{10}H_{13}BrClN_3S$	217-19	B+D	not tested		37.5 (37.2	4.1 4.2	12.7 13.0)
75	H	H	NO_2	H	H	$C_{10}H_{13}ClN_4O_2S$	284-85	B+D	φ	φ	41.8 (41.6	4.7 4.5	19.4 19.4)

[a] B = MeOH; D = Et_2O; E = hexane; G = EtOH.
[b] In rat; MED_{100} in mg/kg/day p.o. post-coital.

Table 5—Miscellaneous Thiosemicarbazides, Thiazolines, Thiazoles and Thiazines

Compd	Mol. formula	m.p. °C	Crystallized from[a]	Antifertility activity[b]		Found (%) (Calc.)		
				1-3 days	4-6 days	C	H	N
76	$C_8H_6F_6N_2$	78-80	E	φ	φ			
77	$C_{12}H_{13}F_6N_3S$	183-84	D+E	>100	>100	41.5	3.4	12.5
						(41.7	3.8	12.2)
78	$C_{10}H_9F_6N_3O$	212-14	K+E	φ	φ	39.7	3.1	14.2
						(39.9	3.0	14.0)
79	$C_8H_9Cl_2N_3S$	155-56	I	φ	φ	38.6	3.4	17.2
						(38.4	3.6	16.8)
80	$C_9H_7F_6N_3S$	156-58	B+C	φ	φ	36.0	2.7	13.6
						(35.7	2.3	13.9)
81	$C_{11}H_{11}F_6N_3S$	140-41	D+E	>100	>100	40.3	3.4	12.9
						(39.9	3.4	12.7)
82	$C_8H_8F_3N_3S$	160-62	G+B	φ	φ	41.2	3.8	18.2
						(40.9	3.4	17.9)
83	$C_{10}H_{12}F_3N_3S$	178-79	H+E	φ	φ	45.5	4.9	15.9
						(45.6	4.6	16.0)
84	$C_9H_{13}N_3S$	146-48	B	φ	φ	55.3	7.1	21.8
						(55.4	6.7	21.5)
85	$C_{17}H_{13}F_6N_3O_3S$	149-51	H+B	φ	φ	45.1	3.2	9.4
						(45.0	2.9	9.3
86	$C_{12}H_9F_6N_3OS$	174-75	D+E	30	30	40.6	2.8	11.8
						(40.3	2.5	11.8)
87	$C_{13}H_{12}ClF_6N_3S$	250-52	B+D	φ	φ	40.1	3.2	11.1
						(39.9	3.2	10.7)
88	$C_{18}H_{14}BrF_6N_3S$	248-49	B+D	100	100	43.7	3.1	8.8
						(43.4	2.8	8.4)
89	$C_{11}H_7F_6N_3S$	50-2	E	<100[c]		40.7	2.4	12.8
						(40.4	2.2	12.8)
90	$C_{11}H_5F_6N_3S$	105-7	D+E	<100[c]		40.7	1.7	13.0
						(40.6	1.6	12.9)
91	$C_{12}H_9F_6N_3S$	104-6	D+E	<100[c]		42.7	3.2	12.3
						(42.2	2.7	12.3)

[a] B = MeOH; C = water; D = Et_2O; E = hexane; G = EtOH; H = acetone I = benzene; K = EtOAc.
[b] In rat; MED_{100} in mg/kg/day p.o. post-coital.
[c] Administered days 1-6; smaller doses not tried.

of estradiol in oil (5 μg/kg/day) for 6 consecutive days. Following 5 days, they were treated with progesterone (in oil s.c.) or test substance (p.o.) or progestrone together with test substance. One day after the last administration, animals were sacrificed, uteri removed and weighed; a segment of each uterus was fixed and prepared for histological examination. Degree of glandular arborization was determined and graded following the method of McPhail. Maximum uterine arborization (McPhail score 3-4) was obtained following s.c. administration of 0.2 mg/day of progesterone. The ability of the test substance to induce uterine arborization or to antagonise progesterone-induced uterine arborization was determined.

Test 11: *Inhibition of Gonadotropin secretion—Compensatory ovarian hypertrophy in hemicastrated rat*[9]

The method used was essentially the same as reported by Peterson *et al*[9]. Adult female rats (120-140 g) were hemicastrated (left ovary). Treatment began immediately after operation and continued for 14 days. At autopsy, the right ovary was removed and weighed. One group of rats served as intact controls and their right ovaries were removed and weighed on the first day of treatment. Percent change in ovarian weights of treated groups was expressed on the basis of comparison with hemicastrated control group. The dose of the test substance which significantly inhibited increase (45% in the right ovary of control rats) following hemicastration was determined.

Test 12: *Inhibition of gonadotropin secretion in immature male rats*[9]

Five groups of 30 day old immature male rats (60-65 g) (10 rats per group) were treated daily for 14 days. At autopsy, seminal vesicles, ventral prostate, testes, thymus, adrenals and thyroid were removed and their weights recorded. The weight of the test substance inhibiting increase in organ weights significantly was noted.

Test 13: *Effect on desiduoma formation in the pregnant or pseudopregnant rat*[10]

One of the horns of the uteri of a group of 5 rats was taumatized on day 4 of pregnancy or pseudopregnancy. The treatment was given from days 1-3. The latter was induced by electrical stimulation of uterine cervix. At autopsy, on day 9, both the horns were weighed separately. The dose of test substance inhibiting significantly desiduoma formation in the rat was recorded.

Test 14: *Effect on blastocyst in the Provera-delayed implantation in rat*[11]

Implantation of blastocyst was delayed in pregnant rats by administering Provera (medroxyprogesterone acetate) (10 mg/kg/day s.c.) from days 1-20. Groups of rats were given the test substance orally at different doses from days 9-11. Estrone was injected from days 16-20 for inducing implantation. Prevention of implantation in Provera- and estrone-treated animals was noted.

Discussion

The parent compound 4-methyl-1-phenylthiosemicarbazide (**1**) exhibited no antifertility activity at a dose of 100 mg/kg p.o. administered post-coital 1-3 days or 4-6 days. Introduction of a single chlorine substituent in the *ortho*- or *meta*-position gave inactive compounds **44** and **42** respectively, while the *p*-chloro analogue (**43**) was effective at 100 mg/kg dose. 4-Methyl-1-phenylthiosemicarbazides with a trifluoromethyl substituent at *meta*- (**18**) or *para*-position (**22**) were weakly active, and even this activity was lost when the substituent was moved to the *ortho*-position (**23**). In this series, the optimal substitution at position-4 was a methyl group (**18**), since β-methallyl (**19**), phenyl (**20**) and benzyl (**21**) substituted derivatives were inactive at 100 mg/kg dose. A phenyl substituent at *ortho*-position of **1** (compound **45**) was responsible for weak antifertility activity which persisted even when the methyl group was replaced by an allyl group (**46**).

Consistent antifertility activity was seen in the group of 1-(3,5-bistrifluoromethyl)phenylthiosemicarbazides, although the starting material 3,5-bistrifluoromethylphenylhydrazine (**76**) was devoid of this property. The 4-unsubstituted derivative **2** was toxic and found to be inactive at a dose of 20 mg/kg, but the 4-methyl derivative **3** (Go 2696) was highly active. The potency decreased as the alkyl side chain was lengthened, in the order methyl (**3**) > ethyl (**4**) = *n*-butyl (**5**) > allyl (**6**) > α-methallyl (**7**) > β-methallyl (**8**) = β-diethylaminoethyl (**15**) = benzyl (**10**). Inactive compounds (at 100 mg/kg) resulted in when the substituent at position-4 was an aryl (9, 12, 13) or β-phenethyl (**11**), cyclohexyl (**14**) or benzoyl (**16**) group, but the activity on 1-3 days experiment increased with respect to that of **3** when position-4 carried two instead of one methyl group (**47**). However, it is interesting to note that the replacement of dimethylamino group in **47** by a piperidino group resulted in **48** which was as inactive as the analogue **49** which lacked the two CF_3 groups. Most of the compounds of this group were considerably more active than the bisthiocarbamoylhydrazine (**93**)[12] which in our hands did not inhibit implantation even at 100 mg/kg p.o.

Replacement of one trifluoromethyl group in the most active compound **3** of the above series by a chlorine atom (**24**) caused a ten-fold decrease in activity, but the toll was heavier when the replacement was done by a carboethoxy group (**28**). Isomers **26** and **27** of **24** were still equiactive with **24**, but a further fall was observed in another isomer (**25**).

Replacement of the sulphur atom in **3** by oxygen gave the semicarbazide **78** which was devoid of antifertility activity; however, this compound was found to be an interesting anti-convulsant[3].

The antifertility activity of the two 4-methyl-1-phenylthiosemicarbazides with two substituents at positions 3 and 5 of the phenyl group prompted the synthesis of other 1-(3,5-disubstituted)arylthiosemicarbazides. The 3,5-dimethoxy (**40**) and 3,5-dimethyl (**41**) analogues showed no antifertility properties at 100 mg/kg. The 3,5-dichloro analogue was weakly active. Its desmethyl derivative **29**, like **2**, was toxic at 100/mg/kg and inactive at 30 mg/kg. The weak activity of **30** inspired the synthesis of analogues with the two chlorine atoms juxtaposed differently. It may be noted that chemically this was an easier exercise than to shuffle two CF_3 groups around the phenyl moiety in **3**. 1-(2,3-Dichlorophenyl)-4-methylthiosemicarbazide (**37**) and 2,5-dichloro (**38**) and 2,6-dichloro (**39**) isomers were inactive at 100 mg/kg, while the 2,4-dichloro derivative (**36**) was partially active. Again the desmethyl product (**35**) was toxic at 100 mg/kg, but exhibited some activity at 30 mg. 1-(3,4-Dichlorophenyl)-4-methylthiosemicarbazide (**32**) was the most potent member of this group with 100% antifertility activity at 30 mg/kg. This activity was diminished on lengthening the alkyl chain to two (**33**) or three carbon atoms (**34**), and was lost when the methyl group was

replaced by hydrogen (**31**). The 2-aryl isomer (**79**) of **32** was likewise inactive.

We turned our attention next to a series of thiazolines obtained by the reaction described in Eq. 4. In this series again, the 3,5-bistrifluoromethyl compound (**51**) turned out to be most active, although it was only one-fifth as potent as **3**. The activity extended to the 3,4-dichloro analogue (**55**) in an attenuated form, but other analogues carrying two chlorine atoms, or one chlorine atom and a trifluoromethyl group or a single chlorine atom were inactive at 100 mg/kg. Thiazolinone **86** was more active than thiazoline **51**, being one-third as active as **3**. Phenylthiazoline **88** was weaker and the activity was lost in the case of methylthiazoline (**87**). Thiazolidinone **85**, obtained from **6**, was also inactive at 100 mg/kg.

A series of 4,5-dihydro-6*H*-thiazines were also investigated among which the 3,5-bistrifluoromethyl derivative (**63**) exhibited some antifertility activity, although this was only one-third to one-tenth of that of **3**. Surprisingly, the 3,4-dichlorophenyl analogue (**66**) was inactive at 100 mg/kg, although the 2,4-(**69**)- and 3,5-(**65**)-dichloroisomers were active at 100 mg/kg. Some azo compounds were also screened; of these **89-91** were active at 100 mg/kg.

Among the few 4-arylthiosemicarbazides screened, it is interesting to mention that 1,1-dimethyl-4-(3,5-bistrifluoromethylphenyl)thiosemicarbazide (**8**) was only weakly active unlike its isomer **47** which was one of the most active compounds encountered in this study.

Antifertility properties of **3** (*C 2696-Go*)

C 2696-Go which was one of the most potent antiimplantation agents in this study was investigated in detail in the tests outlined earlier. The results are as follows:

(1) At oral doses of 20, 10 and 5 mg/kg/day, C 2696-Go did not exhibit any estrogenic activity as determined by tests 2-6.

(2) At oral doses of 20, 10 and 5 mg/kg/day, it showed antiestrogenic activity as determined by tests 7-9.

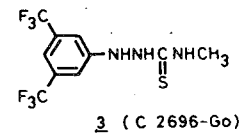
(3) It did not show any progestational or progesterone antagonistic activities in the rabbit at 30 or 10 mg/kg/day (test 10).

(4) It inhibited gonadotropin secretion in the rat at 20 mg/kg/day (test 11) and at 20, 10 or 5 mg/kg/day (test 12).

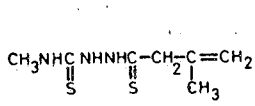
(5) It inhibited desiduoma formation significantly in the rat at 3 or 10 mg/kg/day (test 13).

(6) It did not exhibit blastolytic activity at 10 and 5 mg/kg/day (test 14).

(7) At 5, 10 and 20 mg/kg/p.o. doses, it did not show any androgenic or antiandrogenic activity in the rat as evident from the immature ventral prostate or seminal vesicle weight tests[15].



3 (C 2696-Go)



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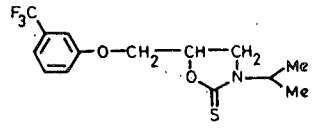

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The profile of C 2696-Go which resulted from these tests was found to be quite similar to that of the Upjohn preparation (**93**)[13].

In further studies in the rat, it was found that C 2696-Go at a single dose of 10 mg/kg administered on day 4 or day 5 post-insemination, was effective in inhibiting implantation. At 2.3 mg/kg/day given on days 1-3 or 4-6 post-fertilisation, conception was found to be prevented when the uteri were examined on day 21 rather than day 10. Doses of 10 and 25 mg/kg given to rats once every week were able to prevent conception without influencing estrus cycle and mating. While implantation in mice was inhibited at 2.5 mg/kg/day p.o. given on either 1-3 or 4-6 days post-coitus, a similar effect was not perceived in the hamster or in the rabbit at 100 mg/kg/day given 1-5 days post-coitus.

C 2696-Go at an oral dose of 5, 10 or 20 mg/kg/day for 4 days did not inhibit ovulation in the rat[15]. Given C 2696-Go orally 20 mg/kg/day for 10 days, beginning at a metestrus stage, cyclic activity in the rat as judged by vaginal smears ceased after first appearance of estrus, this persisting as long as dosing continued. When the treatment was stopped, vaginal cycles were resumed within 13-20 days. Treatment at 10 mg/kg/day had no effect on the estrus cycle. Subsequent fertility of C 2696-Go treated rats remained unimpaired.

C 2696-Go did not show any hypoglycaemic activity at an oral dose of 100 mg/kg in glucose loaded rats. At 9 mg/kg/day i.v., it had no marked effect on blood pressure in the dog. It did not exhibit any tranquilizing effect but provided 100% protection against electroshock convulsion in mice at an oral dose of 250 mg/kg. At this dose level it exhibited very slight analgesic activity in the rat. In acute toxicity studies, LD_{50} of C 2696-Go was 414 ± 12 mg/kg in the rat and 269 ± 25.5 mg/kg p.o. in the mouse. In a 30 day toxicity study, rats and dogs tolerated 5 and 10 mg/kg/day respectively. However, in reproductive studies, definite teratogenic effects were found in the rat at 2.5 mg/kg/day[16]. Hence the preparation was not pursued further.

Experimental Procedure

Melting points are uncorrected.

1-Arylhydrazines

A typical procedure is illustrated by the preparation of 3,5-bistrifluoromethylphenylhydrazine (**76**).

3,5-Bistrifluoromethylaniline (13.6 g) was added to conc. HCl (40 ml), when the HCl salt separated out. The mixture was cooled to 0° to −5° by ice and salt and treated with stirring during 1 hr with sodium nitrite (4 g) in water (20 ml). Stirring was continued for an additional 15 min and the solution treated with stannous chloride (30 g) in conc. HCl (30 ml) during 2 hr below 0°. The mixture was stirred further for 1 hr and filtered. The precipitate was treated with aq. sodium hydroxide and the liberated hydrazine extracted into ether. The ethereal layer was washed with water, dried (Na_2SO_4), solvent removed and the residue crystallised from hexane to give **76** (8.1 g), m.p. 75-77°.

1-Aryl-4-monosubstituted-thiosemicarbazides

These were prepared in 50-90% yields by the reaction of **76** with appropriate isothiocyanates in a low boiling solvent at reflux temperature. The synthesis of 4-methyl-1-(3,5-bistrifluoromethylphenyl)-thiosemicarbazide (**3**) is described below:

A mixture of hydrazine **76** and methyl isothiocyanate (3 g) was heated under reflux in dry benzene (50 ml) for 4 hr when the product crystallised out from the reaction. Hexane (50 ml) was then added and the mixture left at 0° for 1 hr. The resultant thiosemicarbazide (**3**) was filtered, washed with hexane, and recrystallized from acetone-hexane, yield 11.7 g, m.p. 205°

1-(3,4-Dichlorophenyl)-4-methylthiosemicarbazide (**32**) *and 2-(3,4-dichlorophenyl)-4-methylthiosemicarbazide* (**79**)

Reaction of 3,4-dichlorophenylhydrazine (58.7 g) with methyl isothiocyanate (24.1 g) in benzene (150 ml) under reflux for 2 hr and cooling gave a product (76 g) which was filtered, washed with hexane, and recrystallized from acetone-hexane to give **32** (66.5 g), m.p. 205-6°. The mother liquor on concentration gave a crop (4.7 g) which was recrystallized from benzene to afford **79** (3.9 g), m.p. 155-56°. Reaction of **79** with benzaldehyde in hot benzene for a short period gave the benzylidene derivative, m.p. 220-22°, M^+ at *m/z* 337, 339, 341 (Found: C, 53.8; H, 4.2; N, 12.3. $C_{15}H_{13}Cl_2N_3S$ requires C, 53.3; H, 3.9; N, 12.4%).

1-Arylthiosemicarbazides

As an example the synthesis of 1-(3,5-bistrifluoromethylphenyl)thiosemicarbazide (**2**) is described below:

A solution of **76** (24.5 g) and ammonium thiocyanate (15 g) in 2*N* HCl (60 ml) and ethanol (100 ml) was heated under reflux for 15 hr, ethanol removed *in vacuo* and the residue treated with water (100 ml). The product was filtered and crystallized from ethanol to give **2** (19.2 g), m.p. 194-95° (d).

2-Ethyl-4-methyl-1-(3,5-bistrifluoromethylphenyl)-thiosemicarbazide (**77**)

A mixture of **76** (3.7 g), acetic anhydride (3 ml) and triethylamine (3 ml) was heated at 80° for 1 hr and poured into water to give the acetyl derivative (**4**.4 g) which was crystallized from tetrahydrofuran-ether, m.p. 200-201°. The acetyl derivative (8.5 g) was treated with lithium aluminium hydride (4 g) in tetrahydrofuran (400 ml) for 3 days at room temperature with stirring. The mixture was treated with water to decompose excess lithium aluminium hydride and the product complex. The tetrahydrofuran layer was dried (Na_2SO_4) and evaporated. The residue was dissolved in hexane and filtered to remove insoluble starting material (4.2 g). The filtrate was evaporated to give N-ethyl-N′-(3,5-bistrifluoromethylphenyl)hydrazine as an oil (2.5 g) which was heated with methyl isothiocyanate (0.7 g) in hexane (10 ml) under reflux for 1 hr. The precipitate was filtered and crystallized from ether-hexane to give **77** (0.7 g), m.p. 183-84°.

2-Arylhydrazinothiazolines

The synthesis of 2-(3,5-bistrifluoromethylphenyl)-hydrazinothiazoline (**51**) is typical of the general procedure. The details are as follows:

A solution of **76** (5.5 g) in dry ether (25 ml) was treated at room temperature while stirring with 2-bromoethyl isothiocyanate (3.7 g) in ether (15 ml) during 20 min, when a crystalline precipitate started separating out. After stirring further for 2 hr, the mixture was treated with hexane (30 ml) and filtered to give **51** (HBr salt) which was recrystallized from methanol-ether.

The free base was liberated by treating the salt with aq. ammonia and recovered by ether extraction, m.p. 140-42°.

2-Arylhydrazino-4,5-dihydro-6H-thiazines

These were synthesised by the following procedure used for 2-(3,5-bistrifluoromethylphenyl)-4,5-dihydro-6*H*-thiazine (**63**).

A solution of **76** (18.5 g) and 3-chloropropyl isothiocyanate (10.5 g) in benzene (10 ml) was heated under reflux for 6 hr, filtered and the precipitate crystallized from methanol-ether to give **63** (HCl salt) (23.6 g), m.p. 278-80° (d).

The free base was obtained as before, m.p. 156-58°.

4,4-*Dimethyl*-1-(3,5-*bistrifuloromethyl-phenyl)thiosemicarbazide* (**47**)

Hydrazine **76** (24.4 g), dimethylthiocarbamoyl chloride (12.4 g), and triethylamine (10.1 g) were heated together in benzene (100 ml) under reflux for 16 hr. Thereafter, ether (200 ml) was added to the mixture and the precipitated triethylamine hydrochloride filtered off. The filtrate was evaporated and the residue crystallized from ether-hexane to give **47** (5.5 g), m.p. 162-64° (ref. 6).

1-*Arylhydrazinothiocarbonyl-piperidines* (**48**, **49**) and morpholine (**50**)

A typical procedure used for **48** is described below:

Hydrazine **76** (48 g) in ethanol (160 ml) was treated with carbon disulphide (20 g), followed by addition of KOH (11 g) in water (50 ml). To the solution was then added methyl iodide (26.4 g) when an exothermic reaction occurred. After setting aside at room temperature for 1 hr, the solution was concentrated *in vacuo* to remove most of the alcohol and treated with water to give a sticky residue. This was crystallised from ether-hexane to afford methyl (3,5-bistrifluoromethyl)anilinodithiocarbamate (22 g), m.p. 141-43° (Found: C, 36.0; H, 2.5; N, 8.5. $C_{10}H_8F_6N_2S_2$ requires C, 35.9; H, 2.4; N, 8.4%).

The above carbamate (10 g), piperidine (5.2 g) and pyridine (20 ml) were mixed when an exothermic reaction occurred with the evolution of methylmercaptan. The solution was heated under reflux for 2 hr. Pyridine was then evaporated off *in vacuo*. The residue was treated with water and extracted with ether. The ether layer was washed well with water, dried, concentrated and treated with hexane to yield 1-(3,5-bistrifluoromethylphenyl)hydrazinothiocarbonyl-piperidine (**48**) which recrystallised from ether-hexane, 3.1 g, m.p. 103-4°.

3-*Allyl*-5-*carbomethoxymethylene*-2-(3,5-*bistrifluoromethyl-anilino)iminothiazolidin*-4-*one* (**85**)

Thiosemicarbazide **6** (3.4 g) and dimethyl acetylenedicarboxylate (1.4 g) were mixed together in methanol (25 ml) when an exothermic reaction took place. This was completed by heating on a steam-bath for 15 min. The mixture was cooled and the orange crystals were filtered and recrystallized from acetone-methanol to give **85** (4 g), m.p. 149-50°.

3-*Methyl*-2-(3,5-*bistrifluoromethylanilino)iminothiazolidin*-4-*one* (**86**)

Thiosemicarbazide **3** (3.2 g) and chloracetic acid (1.1 g) were heated together in ethanol (20 ml) under reflux for 16 hr. The solvent was evaporated off *in vacuo* and the residue treated with water to give a sticky solid which crystallized from ether-hexane to give **86** (1.1 g), m.p. 173-74°.

3,4-*Dimethyl*-2-(3,5-*bistrifluoromethylanilino)imino*-4-*thiazoline hydrochloride* (**87**)

A solution of thiosemicarbazide **3** (3.2 g) and chloracetone (1 g) in acetone (15 ml) was heated under reflux for 6 hr. The crystalline precipitate was filtered off and recrystallized from methanol-ether to afford **87** (2.4 g), m.p. 250-52° (d).

3-*Methyl*-4-*phenyl*-2-(3,5-*bistrifluoromethylanilino)imino*-4-*thiazoline hydrobromide* (**88**)

A solution of thiosemicarbazide **3** (3.2 g) and phenacyl bromide (2 g) in acetone (20 ml) was set aside for 2 days at room temperature. The precipitate was filtered off and recrystallized from methanol-ether to give **88** (3.5 g), m.p. 248-49° (d).

2-(3,5-*Bistrifluoromethylphenyl)azo*-2-*thiazoline* (**89**)

Hydrazinothiazoline base **51** (6.0 g) was dissolved in ethyl acetate (150 ml) and the solution stirred with silver oxide (2.2 g) for 18 hr at room temperature. The mixture was filtered and the solvent removed *in vacuo*. The residue was dissolved in hexane and the solution filtered through a column of silica gel (100 g). The product eluted with hexane solidified on cooling in a mixture of dry ice and acetone and was crystallized from hexane to give **89** (3.7 g) as orange crystals, m.p. 50-52°.

2-(3,5-*Bistrifluoromethyl)azothiazole* (**90**)

Hydrazinothiazoline base **51** (4.9 g) and sodium carbonate (5.5 g) were heated together in ethanol (200 ml) under reflux for 3 days. The mixture was filtered and the filtrate evaporated *in vacuo* to give a black gum (4.1 g). This was dissolved in chloroform and the solution passed through a column of silica gel (200 g) in the same solvent. The column was eluted with chloroform and 100 ml fractions were collected. The third fraction was evaporated to give a gum (1.4 g) which crystallized from hexane to afford **90** (0.7 g) as yellow crystals m.p. 102-4°. The same product was obtained along with **51** base on keeping **89** for some days at room temperature.

2-(3,5-*Bistrifluoromethylphenylazo*)-4,5-*dihydro*-6*H*-*thiazine* (**91**)

The free base (1.8 g) from the hydrochloride **63** was oxidised with silver oxide (0.8 g) in ethyl acetate (50 ml) for 20 hr at room temperature. The mixture was filtered, the filtrate treated with charcoal and again filtered. Concentration and addition of hexane gave **91** as orange crystals, m.p. 104-6°.

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