of three doses was used with four rabbits per dose.

Avoidance Response Acquisition. Old retired male breeder rats (Long-Evans, 600–800 g) were trained for avoidance response acquisition in the shuttle box (Lehigh Valley Electronics, LVE 28). Each trial (60-s duration) consisted of a 5-s avoidance period (light present in the opposite end of the box) during which the subject had to jump over the divider ("hurdle") to the other side of the box. A 5-s foot shock (0.6 mA) was delivered if no avoidance response was made and repeated until the animal escaped. A maximum of 50 avoidance trials at 60-s intervals was presented and the number of trials required to achieve eight avoidance responses in ten consecutive trials was obtained. The drugs were administered 30 min prior to the tests. Results were evaluated statistically using the Wilcoxon rank sum test.[36]

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Aryl-*s*-tetrazines with Antiinflammatory Activity

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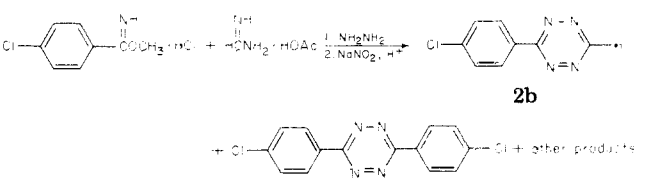
Various aryl-*s*-tetrazines and benzyl-*s*-tetrazines displayed aspirin-like activity when tested against carrageenan-induced edema in the rat, uv-induced erythema in guinea pigs, and adjuvant-induced arthritis in rats. These agents also displayed analgesic activity in the mouse writhing and paw pain tests but also lowered the red blood cell count in normal healthy rats.

From random screening 3-(*p*-chlorophenyl)-6-(1-methylhydrazino)-*s*-tetrazine (1) was found active in the carrageenan-induced edema assay in the rat. 3-(*p*-Chlorophenyl)-*s*-tetrazine (**2b**) was prepared as a potential precursor to 1 and this chemical was also active in the carrageenan test as well as the uv-induced erythema assay in the guinea pig and adjuvant-induced arthritis assay in the rat. This led to a chemical[1] and biological investigation of aryl-*s*-tetrazines as potential antiarthritic agents.

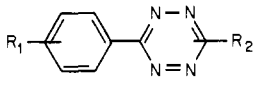
Chemistry. Aryl-*s*-tetrazines were prepared by the reaction of benzimidates and amidines with hydrazine hydrate[1-3] followed by oxidation (Scheme I). The reaction gave mixtures of the desired *s*-tetrazines, bis(aryl- and alkyl)-*s*-tetrazines, plus a number of hydrazine products.[1] The tetrazines were separated by chromatography on silica gel, eluting with methylene chloride.

Scheme I



Benzimidate intermediates, which could not be easily prepared by Pinner conditions,[1,4] were prepared from amides or nitriles and methyl fluorosulfonate (Scheme II). The highly reactive intermediates were mixed first with amidines and then with hydrazine hydrate with extreme

Table I. Carrageenan-Induced Edema Results



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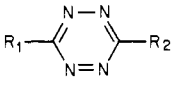

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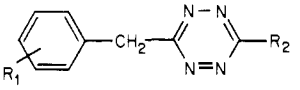


4

No.	R_1	R_2	Mean C/T[a,b]	Formula	Mp, °C
2a	H	H	2.93	$C_8H_6N_4$	125-127[d]
2b	*p*-Cl	H	3.07	$C_8H_5ClN_4$	164-167[e]
2c	*p*-Cl	CH_3	2.60	$C_9H_7ClN_4$	143-145[e]
2d	*p*-CH_3	H	2.75	$C_9H_8N_4$	79-81[e]
2e	*m*-CH_3	H	2.56	$C_9H_8N_4$	75-78[e]
2f	*p*-F	H	2.79	$C_8H_7FN_4$	144-146[e]
2g	*p*-CH_3	CH_3	2.31	$C_{10}H_{10}N_4$	115-118[e]
2h	*p*-CF_3	H	2.52	$C_9H_5F_3N_4$	138-141[c]
2i	*p*-CH_3O	H	2.88	$C_9H_8N_4$	152-154[e]
2j	*m*-CF_3	H	2.50	$C_9H_5F_3N_4$	46-48[e]
2k	*p*-CF_3	CH_3	1.96	$C_{10}H_7F_3N_4$	164-165[c,f]
2l	*m*-Cl	H	3.71	$C_8H_5ClN_4$	73-74[c]
2m	*m*-CH_3	CH_3	5.15	$C_{10}H_{10}N_4$	43-45[e]
2n	3,4-Cl_2	CH_3	1.83	$C_9H_6Cl_2N_4$	162-165[c]
2o	*m*-CH_3O	H	2.91	$C_9H_8N_4O$	96-98[e]
2p	3,4,5-$(CH_3)_3$	H	2.15	$C_{11}H_{12}N_4O_3$	138-140[e]
2q	*m*-CH_3O	CH_3	2.47	$C_{10}H_{10}N_4O$	131-133[c]
2r	*p*-I	H	1.82	$C_8H_5IN_4$	168-171[c]
2s	*o*-F	H	2.17	$C_8H_5FN_4$	36-38[c]
3a	2-Naphthyl	H	1.96	$C_{12}H_8N_4$	198-201[e]
3b	2-Furyl	H	2.79	$C_6H_4N_4O$	126-130[e,f]
3c	2-Thienyl	CH_3	3.65	$C_7H_6N_4S$	132-135[e]
3d	2-Thienyl	H	3.39	$C_6H_4N_4S$	103-105[e]
3e	5-Methyl-2-thienyl	H	2.73	$C_7H_6N_4S$	113-114[c]
3f	3-Furyl	H	2.23	$C_6H_4N_4O$	112-114[c,f]
3g	2-Benzofuranyl	H	2.77	$C_{10}H_6N_4O$	185-188[c]
3h	3-Pyridyl	H	2.41	$C_7H_5N_4$	92-94[e]
3i	3-Pyridyl	CH_3	2.78	$C_8H_7N_5$	99-102[e]
3j	Cyclohexyl	H	2.14	$C_8H_{12}N_4$	Red oil[e]
3k	Cycloheptyl	H	2.24	$C_9H_{14}N_4$	Red oil[e]
4a	*p*-Br	H	2.94	$C_9H_7BrN_4$	53-56[e]
4b	*p*-F	H	6.39	$C_9H_7FN_4$	35-37[c]
4c	*p*-Br	CH_3	3.23	$C_{10}H_9BrN_4$	65-67[e]
4d	H	CH_3	2.23	$C_{10}H_{10}N_4$	Red oil[e]
4e	*p*-F	CH_3	2.34	$C_{10}H_9FN_4$	Red oil[e]
4f	*p*-F	*p*-$FC_6H_4CH_2$	1.79	$C_{16}H_{12}F_2N_4$	Red oil[e]
4g	H	H	2.23	$C_9H_8N_4$	Red oil[e]
Controls			1.00		
Aspirin (50 mg/kg)			2.80		

[a] Both controls and treated animals were injected with carrageenan but only treated animals received drugs; active compounds have C/T greater than 1.43. [b] Dosage 250 mg/kg by gavage. [c] All new compounds have correct elemental analysis and supporting spectral data. [d] Lit.[6] mp 126-129 °C. [e] Reported in ref 1. [f] Active at 125 mg/kg.

caution due to the high exothermic nature of the reaction.

Pharmacology. The compounds were initially evaluated for acute antiinflammatory activity against carrageenan-induced edema, using Royal Hart, Wistar strain rats as described in previous communications.[5]

The differences in edema were considered to be due to drug efficacy and are expressed as a control (C)/treated (T) (untreated/treated) efficacy ratio (the ratio of mean edema of eight control animals which did not receive drugs over the mean edema of two drug-treated rats). If the C/T ratio is equal to or greater than 1.41, the test was repeated with two additional rats. If the mean C/T in four rats is equal to or greater than 1.43, the compound was accepted as active. The results are summarized in Table I.

The carrageenan-induced edema screen is a general test for the discovery of antiarthritic agents[7] but is nonspecific.[8] Two other more specific tests for measuring antiinflammatory activity are erythema inhibition in guinea pigs and a chronic inflammation test, the adjuvant-induced arthritis in rats.

In order to test for erythema inhibition, albino guinea pigs (Lederle breeding colony) were depilitated on their flanks the evening before testing with a standard mixture of barium sulfide and gum acacia. On the morning of testing, groups of four guinea pigs were dosed by gavage at a dosage of 250 mg/kg 1 h prior to ultraviolet exposure

Scheme II

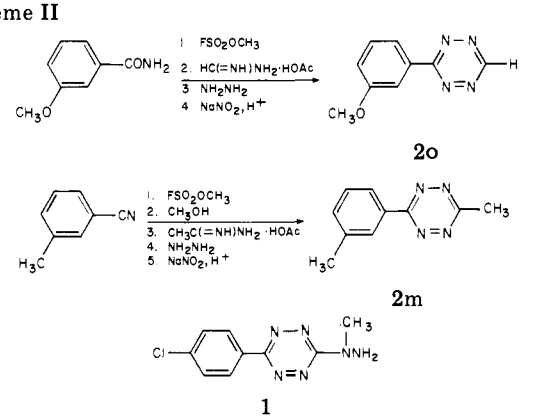
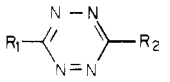


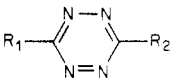
Table II. Results from Ultraviolet-Induced Erythema of the Guinea Pig[a]



No.	R_1	R_2	1-h score	4-h score	Decision[b]
Controls	(Historical)		2.1	2.8	
2a	C_6H_5	H	0.0	1.0	A
2b	p-ClC_6H_4	H	0.6	2.6	A[c]
2d	p-$CH_3C_6H_4$	H	0.5	2.9	A
2f	p-FC_6H_4	H	0.0	1.9	A[c]
2g	p-$CH_3C_6H_4$	CH_3	0.3	1.8	A
2o	m-$CH_3OC_6H_4$	H	0.1	1.5	A
2q	m-$CH_3OC_6H_4$	CH_3	0.1	1.9	A
3b	2-Furyl	H	0.1	1.6	A
3d	2-Thienyl	H	0.2	2.3	A[d]
3c	2-Thienyl	CH_3	0.1	2.2	A[c]
4a	p-$BrC_6H_4CH_2$	H	0.1	2.3	A
4c	p-$BrC_6H_4CH_2$	CH_3	0.6	2.9	A
4d	$C_6H_5CH_2$	CH_3	0.3	2.11	A
Aspirin (125 mg/kg)			0.1	1.9	A

[a] Dosage 250 mg/kg. [b] A is active by discriminant function analysis. [c] Active at 125 mg/kg. [d] Active at 62.5 mg/kg.

Table III. Adjuvant-Induced Arthritis Results



No.	R_1	R_2	Dosage, mg/kg	Mean RSA[a]
2a	C_6H_5	H	50	0.53
2b	p-ClC_6H_4	H	50	0.54
2d	p-$CH_3C_6H_4$	H	100	0.57
2f	p-FC_6H_4	H	50	0.46
2k	p-$CF_3C_6H_4$	CH_3	50	0.59
2g	p-$CH_3C_6H_4$	CH_3	50	0.68
2q	m-BrC_6H_4	CH_3	50	0.62
2s	o-FC_6H_4	H	50	0.65
3c	2-Thienyl	CH_3	25	0.66
3d	2-Thienyl	H	25	0.54
3e	5-Methyl-2-thienyl	H	50	0.61
4c	p-$BrC_6H_4CH_2$	CH_3	50	0.62
3g	5-Bromo-2-furyl	H	50	0.58
4f	p-$FC_6H_4CH_2$	p-$FC_6H_4CH_2$	50	0.60
Control				1.00
Aspirin (400 mg/kg/day)				0.55

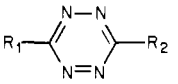
[a] Mean relative surface area; compounds with a mean RSA less than 0.753 are accepted as active.

(−1 h). At 0 h they were restrained in a plastic container which allows exposure of three-circular spots. They were exposed to ultraviolet irradiation from a "Hanovia" Kormayer Lamp Model 10 for 60 s. At +1 and +4 h the degree of erythema for each of the three sites was assessed according to the following scoring system: 0, no erythema; 0.5, incomplete circle or faint erythema; and 1.0, complete circle of distinct erythema. Thus, the maximum score for each animal was 3.0. Table II summarizes the results of this test.

Tests to show activity against chronic inflammation in adjuvant arthritis were carried out. Groups of three Royal Hart, Wistar strain rats, weighing 200 ± 10 g each, were injected intradermally in the right hind paw with Freunds's adjuvant (dried human tubercle bacilli in a mineral oil vehicle) at a dose of 2 mg/kg of body weight. Test compounds were administered orally in a 1.5% starch vehicle at the indicated dosage in mg/kg of body weight once daily on days 0–13 postchallenge. Control rats were treated in a similar manner but given starch vehicle instead of the test compound. On the 14th day postchallenge the diameter of the injected paw (primary lesions) was measured by micrometer caliper. From these measurements of inflammed paws a determination was made of the relative surface area (RSA). This is a ratio expressed as (mean surface area of paws of three treated rats)/(mean surface area of paws of 60 control rats). If the relative surface area was equal to or less than 0.76, the compound was tested again. After the second test the mean relative surface area (RSA) for the rat paws from both tests was calculated and if the mean RSA was equal to or less than 0.736, the compound was tested a third time and if the mean RSA of all three tests was less than 0.753, the compound was accepted as active. The results are shown in Table III.

These compounds are active analgesics when measured by the "writhing syndrome" test for analgesic activity as described by Siegmund.[9] This method is based upon the reduction of the number of writhes following the intraperitoneal injection of 1 mg/kg of body weight of phenyl-p-quinone in male Swiss albino mice weighing 15–25 g/mouse. The syndrome is characterized by intermittent contractions of the abdomen, twisting and turning of trunk, and extention of the hind legs beginning 3–5 min after

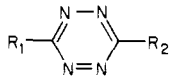
Table IV. "Writhing Syndrome" Results



No.	R_1	R_2	No. of writhes per 3-min period: Pair 1	Pair 2
	Aspirin (historical)		3	3
	Controls (historical)		30	30
2a	C_6H_5	H	6	1
2b	p-ClC_6H_4	H	16	5
2d	p-$CH_3C_6H_4$	H	10	4
2e	m-$CH_3C_6H_4$	H	6	3
2f	p-FC_6H_4	H	2	0[a]
2h	p-$CF_3C_6H_4$	H	0	7[a]
2i	p-$CH_3OC_6H_4$	H	2	0
2j	m-$CF_3C_6H_4$	CH_3	6	7
3c	2-Thienyl	CH_3	3	5
3d	2-Thienyl	H	6	3
3b	2-Furyl	H	10	3[a]
4b	p-$FC_6H_4CH_2$	H	10	16[b]
4a	p-$BrC_6H_4CH_2$	H	2	8[a]
3h	3-Pyridyl	CH_3	12	15[a]
4c	p-$BrC_6H_4CH_2$	CH_3	10	10[a]
4h	$C_6H_5CH_2$	H	3	14[a]
3k	Cycloheptyl	H	8	14[a]

[a] Active at 100 mg/kg level. [b] Active at 50 mg/kg level.

Table V. Rat Paw Pain Test



No.	R_1	R_2	T/C ratio
	Starch (control)		1.0
	Aspirin		2.2
2a	C_6H_5	H	2.6
2b	p-ClC_6H_4	H	3.1
2d	p-$CH_3C_6H_4$	H	1.9
3b	2-Furyl	H	2.3
3c	2-Thienyl	CH_3	1.7
3d	2.Thienyl	H	1.9
4c	p-$BrC_6H_4CH_2$	CH_3	1.5[a]

[a] Active at 50 mg/kg.

injection of the phenyl-p-quinone. The test compounds are administered orally at dosage of 200 mg/kg to groups of two mice each 30 min before injection of the phenyl-p-quinone. The total number of writhes exhibited by each group of mice is recorded for a 3-min period commencing 15 min after injection of the phenyl-p-quinone. A compound is considered active if it reduces the total number of writhes in two test mice from a control value of approximately 30 per pair to a value of 18 or less. Table IV summarizes the activity of the present compounds as active analgesics.

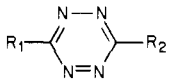
A more definitive test for analgesics was the experiments conducted to determine analgesia by a modification of the method of Randall and Selitto[10] in rats whose paws were made sensitive to pressure by the injection of a 20% aqueous suspension (0.1 ml) of Brewer's yeast into the plantar surface of the left hind paw. Constantly increasing force (16 g/s) was applied to the swollen paw using an Analgesy Meter, Ugo Basile. The pressure is stopped when the animal responds (sudden struggle or vocalization). Control rats treated with the starch vehicle respond to a pressure or force of about 200 g. Pressure–pain thresholds are recorded 2 h after Brewer's yeast injection. The agents being tested are given orally at the same time as the yeast at a dosage of 200 mg/kg. The ratios of treated (T)/control (C) reaction thresholds are calculated as estimates of analgesic efficacy and are recorded in Table V. A compound is considered an active analgesic if its T/C ratio = 1.5 or greater.

Table VI. Results of s-Tetrazines on GI Tract[a]

No.	Gi hemorrhage	Gastric ulcers	Intestinal ulcers	Anemia
2a	−	+	−	+
2b[b]	−	+	−	+
2f	−	+	−	+
2g	−	−	−	±[c]
2o[b]	−	±[c]	±[c]	±[c]
3b	−	+	−	+
3c[b]	+	+	−	+
3d	+	+	−	+
2e	−	+	−	+
Aspirin[d]	+	±[c]	−	−
Controls	−	−	−	−

[a] Dosage 250 mg/kg. [b] Sc drug administration gave identical results. [c] Results were considered borderline. [d] 50 mg/kg.

Table VII. Blood Studies in Normal Rats



No.	R_1	R_2	Dose, mg/kg	RBC[a]	WBC[b]
	Controls			5.0	21.3
2a	C_6H_5	H	50	3.1	45.9
2b	p-ClC_6H_4	H	50	2.9	43.0
2d	p-$CH_3C_6H_4$	H	100	3.4	33.7
2f	p-FC_6H_4	H	25	3.9	32.2
2o	m-$CH_3OC_6H_4$	H	50	4.6	22.1
3b	3-Furyl	H	50	3.4	23.1
3c	2-Thienyl	CH_3	50	4.3	26.1
3d	2-Thienyl	H	25	3.1	34.7
2j	m-$CF_3C_6H_4$	H	50	4.8	21.5
2m	m-$CH_3C_6H_4$	CH_3	50	4.5	21.2
2n	3,4-$(CH_3)_2$-C_6H_3	H	50	4.5	26.3
2p	2,4,5-$(CH_3)_3$-OC_6H_2	H	50	4.3	20.9
3f	3-Furyl	CH_3	50	3.7	32.8
3i	3-Pyridyl	H	50	4.2	24.7
2h	p-$CF_3C_6H_4$	H	50	4.5	35.9
4a	p-$BrC_6H_4CH_2$	H	50	4.1	73.2
4b	p-$FC_6H_4CH_2$	H	50	2.7	95.1
4c	p-$BrC_6H_4CH_2$	CH_3	50	4.5	51.6
4d	$C_6H_4CH_2$	CH_3	100	5.2	24.5
2t[c]	p-$CH_3SO_3C_6H_4$	H	25	3.9	49.0

[a] RBC is red blood cell count × 10^6/mm^3. [b] WBC is white blood cell count × 10^3/mm^3. [c] Mp 121–124 °C; all new compounds have correct elemental analysis and supporting spectral data.

Discussion

A few of the tetrazines that possessed a broad spectrum of antiinflammatory and analgesic activity were tested in rats for their ability to induce gastric or intestinal lesions. Two rats were dosed orally at 250 mg/kg twice a day (morning and evening) for 2 days. On the third day, the animals were dosed in the morning and sacrificed in the afternoon. The conditions of the gi tract were compared against a group of five control animals. The results are summarized in Table VI.

A number of 3-aryl-s-tetrazines were found to inhibit prostaglandin synthesis but because of solubility problems in the test media, a quantitative comparison with known inhibitors was not possible.

The observed anemia was related to lowering of the red blood cell count in the test rats. Table VII summarizes

the blood studies in normal rats.

A cursory view of the phenyl- and benzyl-*s*-tetrazines shows that the broadest activity appears to be centered mainly in the halogen, pseudohalogen (CF_3), H, and CH_3 residues and, to a greater extent, when the 6 (3) position of the tetrazine has an H substituent. Compounds **2a,b,d,f** and **4c** are those with the broadest activity in these tests. These compounds also show comparable activity in the analgesic test system. All, however, lower the red blood cell count.

A similar view of heterocyclic *s*-tetrazines demonstrates the broadest degree of activity in the three antiinflammatory tests and the two analgesic tests to be centered in the furyl- and thienyl-*s*-tetrazines, in particular, compounds **3b–d**. Again, however, these structures caused a lowering in the red blood cell count.

The aryl-*s*-tetrazines displayed activity in the range of 125–250 mg/kg against carrageenan-induced edema in the rat, uv-induced erythema in the guinea pig, and adjuvant-induced arthritis in rats. These agents are also active analgesics as measured by the writhing syndrome in mice and rat paw pain tests.

Experimental Section

All melting points are uncorrected and were observed on a Mel-Temp. NMR were recorded on a Varian HA-100 and are reported as parts per million from Me_4Si. Ir were recorded on a Perkin-Elmer 137 and, unless otherwise noted, were recorded as a potassium bromide pellet. All solvents were dried before use, and all reagents, unless otherwise noted, were used as received. For NMR solvents, Me_2SO refers to Me_2SO-d_6. The silica gel used for chromatography was 200–325 mesh (grade 922) obtained from the Grace Division Chemical Co.

General Preparation A. A suspension of 10 g of benzimidate hydrochloride and 15 g of formamidine (acetamidine) acetate in 35 ml of hydrazine hydrate was stirred at room temperature for 2–3 h. The suspension was poured into 250 ml of water and the resulting solid was collected by filtration and sucked as dry as possible. (When an oil resulted, this was extracted with chloroform and the volatiles removed in vacuo.) The solid was suspended in 80 ml of glacial acetic acid and placed in a cooling bath at 5–10 °C. Sodium nitrite (~5 g) was added with vigorous stirring. After addition of all of the sodium nitrite, the dark purple solution was poured into water (300 ml) and the solid was collected and air-dried (oils were isolated by extraction with chloroform and removal of the volatiles in vacuo). The components of the mixture were separated by chromatography on silica gel, eluting with methylene chloride.

3-(*p*-Chlorophenyl)-*s*-tetrazine (2b). A suspension of 10 g (0.053 mol) of ethyl *p*-chlorobenzimidate hydrochloride and 15 g (0.15 mol) of formamidine acetate in 35 ml of hydrazine hydrate was stirred at room temperature for 2–3 h. The now yellow suspension was poured into 250 ml of water and the resulting solid was collected by filtration and sucked as dry as possible. The damp solid was dissolved in 80 ml of glacial acetic acid and placed in a cooling bath at 5–10 °C. Sodium nitrite (~5 g) was slowly added with vigorous stirring. After addition (~10–15 min), the dark purple solution was poured into water (300 ml) and the solid was collected and air-dried.

The solid was chromatographed on silica gel, eluting with methylene chloride. The initial fraction yielded 3,6-bis(*p*-chlorophenyl)-*s*-tetrazine: mp 198–201 °C (220 mg) (lit.[4] mp 192 °C). The second fraction yielded the desired material, 3-(*p*-chlorophenyl)-*s*-tetrazine (**2b**): 4.5 g (44.1%); mp 164–167 °C (lit.[1] mp 164–167 °C).

Further elution (methylene chloride–5% methanol) gave a yellow solid which was recrystallized from chloroform, mp 180–183 °C with loss of gas, which resolidified, and then melted at 278–281 °C. This was identified as *sym*-1,2-bis(*p*-chlorobenziminoyl)-hydrazine[1] (lit.[1] mp 278–281 °C).

In other experimental runs, a white material was eluted with 5% methanol–methylene chloride. This material, mp 278–281 °C, was identified as 3,5-di(*p*-chlorophenyl)-1,2,4-triazole (lit.[1] 278–281 °C).

General Preparation B. To a suspension of 10 g of substituted benzamide in 50 ml of methylene chloride was added 35 ml of methyl fluorosulfonate and the solution was stirred overnight at room temperature. (With ortho-substituted benzamides, the solution was refluxed overnight.) The excess solvent and reagent were removed in vacuo.

The remaining residue was mixed well with formamidine (acetamidine) acetate and placed in an ice bath. Hydrazine hydrate was cautiously added and after the exotherm had passed, the ice bath was removed and the mixture stirred for 30 min. Water was added and the residue was collected and heated as in method A.

3-(*m*-Methoxyphenyl)-*s*-tetrazine (2o). A suspension of *m*-methoxybenzamide (25 g, 0.17 mol) in 50 ml of methylene chloride and 35 ml (excess) of methyl fluorosulfonate was allowed to stir at room temperature overnight. The excess solvent and reagent were removed in vacuo.

The remaining residue was mixed well with formamidine acetate and placed in an ice bath. Hydrazine hydrate (60 ml) was cautiously added and after the exotherm the ice bath was removed and the mixture was stirred for 30 min. Water was added and the oil was collected by extraction with chloroform. After oxidation with sodium nitrite, the residue was chromatographed on silica gel, eluting with methylene chloride, yielding 5.4 g (19%) of 3-(*m*-methoxyphenyl)-*s*-tetrazine, mp 96–98 °C (lit.[1] mp 96–98 °C).

General Preparation C. A solution of *m*-toluonitrile (10 g) in 50 ml of methylene chloride and 15 ml of methylfluorosulfonate was stirred at room temperature overnight (*o*-benzonitriles were refluxed overnight). The solvent and excess reagent were removed in vacuo and the residue was dissolved in methylene chloride. The solution was stirred at 5–10 °C and 10 ml of methanol was added dropwise. The volatiles were removed in vacuo. Formamidine (acetamidine) acetate (20 g) was added and the components were mixed well. The flask was placed in an ice bath and hydrazine hydrate was added with extreme caution as the reaction was highly exothermic. After all the hydrazine hydrate was added, the ice bath was removed and stirring continued for 30 min. Water was added and the oily residue extracted with chloroform. The chloroform was removed and the residue treated as in method A.

3-Methyl-6-*m*-tolyl-*s*-tetrazine (2m). A solution of *m*-toluonitrile (10 g, 0.085 mol) in 50 ml of CH_2Cl_2 and 15 ml (excess) of methyl fluorosulfonate was stirred at room temperature overnight. The solvent and excess reagent were removed in vacuo and the residue was dissolved in CH_2Cl_2. Methanol (10 ml) was added slowly with cooling and the excess solvent and reagent were removed in vacuo.

Acetamidine acetate (20 g) was added and components were mixed well. The flask was placed in an ice bath and hydrazine hydrate (35 ml) was added with extreme caution as the reaction was highly exothermic. After all the hydrazine hydrate was added, the ice bath was removed and stirring was continued for 30 min. Water was added and the oily residue was extracted with chloroform. Oxidation of the isolate gave a semisolid which was chromatographed on silica gel to give 500 mg (2.5%) of product, mp 43–45 °C (lit.[1] mp 43–45 °C).

3-(*o*-Fluorophenyl)-*s*-tetrazine (2s). A solution of 25 g of *o*-fluorobenzamide and 30 ml of methyl fluorosulfonate in 100 ml of chloroform was refluxed for 5 h. The solvent and excess reagent were removed in vacuo and the resultant residue was mixed well with 50 g of formamidine acetate and placed in an ice bath. Hydrazine hydrate (100 ml) was slowly added and after complete addition, the ice bath was removed and the suspension stirred for 30 min and then poured into 500 ml of water. Oxidation in acetic acid, aqueous work-up, and extraction with chloroform gave a purple oil which was chromatographed on silica gel, eluting with methylene chloride. The first eluting material was bis-(3,6-*o*-fluorophenyl)-*s*-tetrazine. The second eluting material, a deep red oil, was 3.2 g of 3-(*o*-fluorophenyl)-*s*-tetrazine. The oil has spectral constants and analytical data identical with that cited in the literature.[1]

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Potential Antitumor Agents. 20. Structure–Activity–Site Relationships for the 4'-(9-Acridinylamino)alkanesulfonanilides

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A series of 87 L1210 active 4'-(9-acridinylamino)alkanesulfonanilides has been screened against L1210 cells (10^5) implanted at various sites (ip, sc, ic) employing early ip drug administration for a limited time. With each implantation site a different most active congener was selected. For good activity against tumor implanted remotely from the ip drug administration site, an agent should be more lipophilic than that found optimal for ip implanted tumor. An acridine 4-CH_3 group appears to assist drug translocation, possibly by sterically hindering binding to nonproductive sites. An unprotected NH_2 group on the acridine ring system is incompatible with activity against sc implanted tumor. Agents in which NH_2 is shielded by N-acetylation, N-monomethylation, or ortho substitution with a bulky group can inhibit sc implanted tumor.

Depending on the target disease process the chemotherapeutic agent which is selected from a range of congeners for clinical trial must have certain necessary properties. Inevitably reasonable efficacy in animal screening tests is demanded. Intrinsic physical, chemical, pharmacologic, or toxicologic properties of that selected agent may prove such as to force selection of a different congener which, although significantly less active in a screening test, has more desirable alternate features for clinical application. This is particularly true in cancer chemotherapy where, in striving for cure, we must aim for total tumor cell eradication.[1-3] To have curative potential against the advanced disease, agents must be able to distribute in effective concentration to all cancer cells. In analyzing the structure–activity relationships (SAR) of the acridylmethanesulfonanilide series, we have generated several hundred tumor-active congeners.[4-9] Screening in these studies has employed early intraperitoneal (ip) treatment of ip implanted L1210. We have now attempted to develop simple screening type tests which will demonstrate those molecular features which provide favorable drug disposition patterns, as well as those which contribute to high intrinsic selectivity toward target tumor cells. From such information it was hoped it would be possible to select structural components common to both sets and, from these, generate agents with desirable clinical attributes.

The research described examines the comparative effectiveness of certain earlier prepared agents against depots of L1210 leukemia implanted at different anatomic sites. Early drug dosage for a strictly limited period has been employed so that observed life extensions may reflect primarily effects against the initial tumor depot, rather than the widely disseminated disease that is combated when treatment is either late or protracted. To probe further our initial findings in this study, an extensive range of new congeners has been prepared and evaluated in these modified screening systems. Certain structure–activity–site relationships are presented.

Chemistry. Most synthetic steps necessary in agent generation have been dealt with earlier in full.[4-9] The overall route requires generation of an *N*-arylanthranilic acid[6] and, following ring closure ($POCl_3$, H_2SO_4, PPA, PPE)[9] to a 9(10*H*)-acridone, then conversion of this ($POCl_3$, $SOCl_2$–DMF)[6] to a 9-chloroacridine. Final mild acid catalyzed coupling of the 9-chloroacridines and a 4'-aminosulfonanilide component provides agents.[4-9] To avoid troublesome isomer separation following the ring closure step acridones have been prepared, where possible, by unequivocal routes. For example, Ullmann condensation[6,10] of 2,4-dichlorobenzoic acid and 4-nitroaniline provided a substituted *N*-arylanthranilic acid which on ring closure provided as sole product the 3-chloro-7-nitroacridone necessary for **65** (Table II). Similar steps from 2,4-dichloro-5-nitrobenzoic acid and aniline provided the isomeric 3-chloro-2-nitroacridone required for **67**. The formulas of the intermediary *N*-arylanthranilic acids quoted in the Experimental Section specify the 2-halobenzoic acid and aniline components utilized in Ullmann condensations.

Side-chain intermediates for **42–44** were prepared by acylation of *N*-(4-amino-2-methoxyphenyl)butanamide[7] with the requisite sulfonyl chloride and then hydrolytic (H^+) removal of the protecting butyryl function.

Acylation (*o*-phenylenephosphorochloridite–RCOOH)[6] of the corresponding 3-NH_2 compound provided **45** and **46**. Most variants containing primary amino groups (**57**, **66**, **68**, **70**) were prepared by terminal reduction (Fe/H^+) of the corresponding nitro compounds. Certain amine variants were more conveniently prepared from the acetylamino derivatives. This was the case with the 2-amino-3-trifluoromethyl analogue **70**; initial preparation