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Synthesis and Antihypertensive Activity of Novel 3-Hydrazino-5-phenyl-1,2,4-triazines

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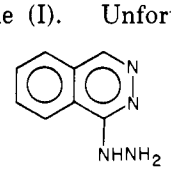
In an effort to develop antihypertensive agents with peripheral vasodilator activity, a series of 40 novel 3-hydrazino-5-phenyl-1,2,4-triazines (II) were synthesized and evaluated in the spontaneously hypertensive rat assay (SHR assay). Based on the performance of the structurally related standard, hydralazine (I), 15 triazines were active. Thirteen of these hypotensive triazines possessed LD_{50} values in the mouse greater than I (LD_{50} = 100 mg/kg); only one active triazine had an LD_{50} value greater than 300 mg/kg (**11d**). Four asymmetric triazines had moderate antihypertensive activity and LD_{50} values greater than 300 mg/kg (**6b**, **7c**, **8f**, and **9g**). Based on the relationship between toxicity and antihypertensive activity, three triazines (**8f**, **9g**, and **11d**) were chosen for dose–response studies in the SHR assay. None were as efficacious as I, but all three were less toxic, resulting in similar therapeutic indices relative to I.

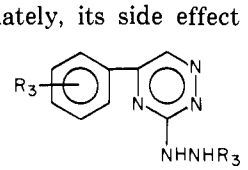
It has been estimated that approximately 15% of the adult American population is hypertensive.[1] Yet less than 10% of those considered hypertensive receive adequate hypertensive control through appropriate drug therapy.[1] Both the major Veterans Administration[2] and Framingham[3] studies demonstrated that blood reduction, through the use of drug therapy, decreases the incidence of most cardiovascular complications of hypertension. The critical need in hypertensive drug therapy is for a safe and effective antihypertensive drug, devoid of undesirable side effects, which can be used in the treatment of moderate levels of essential hypertension.

In most patients with chronic essential hypertension, abnormally high peripheral vascular resistance is the proximate cause of elevated arterial pressure.[4-6] Their cardiac output is generally within the normal range. Yet most antihypertensive drugs that elicit their hypotensive effect by depressing the sympathetic nervous system decrease cardiac output. While blood pressure decreases, the abnormally low cardiac output results in decreased tissue perfusion to the heart, brain and kidneys, a hemodynamic situation which is hardly desirable nor chronically tolerated. The hemodynamic goal in most chronic essential hypertension therapy should be to specifically reduce peripheral vascular resistance, without sympathodepression.

An antihypertensive drug presently marketed which acts directly on constricted arteriolar smooth muscle is hydralazine (I). Unfortunately, its side effect liability



I



II

(including the production of a lupus erythematosus-like syndrome) has limited its widespread use in recent years.[7] Also, the decrease in vascular resistance caused by therapy with I activates the baroreceptors, resulting in a reflex increase in sympathetic discharge which increases heart rate, stroke volume, and cardiac output.[8] Therefore, I is normally administered in combination with β-adrenergic blockers which eliminate the reflex action. A diuretic is also often added to offset the sodium and water retention caused by I. Hypertensive control is then achieved with no signs of postural hypotension, depression of cardiac output, impairment of renal or sexual function, or other adverse symptomatology.

In an effort to develop agents which act directly on arteriolar smooth muscle but do not possess the side effect liability associated with I, we synthesized a series of asymmetric triazines (II) which differ from I in the following ways: (1) One nitrogen atom has been added to the heterocyclic ring. (2) The nitrogen-containing ring is no longer fused to the phenyl ring but merely attached by a single bond. The addition of the nitrogen atom to the ring will decrease the electron density of the ring which should effect the distribution, metabolism, and intrinsic activity of the molecule. The lack of fusion between the two rings enables the aromatic rings, depending on ring substitution, to assume a nonplanar conformation, which is not possible with I. We also have investigated the effect of various substituents on the phenyl ring (R_5) and hydrazino side chain (R_3).

The antihypertensive effect of these compounds was evaluated in the spontaneously hypertensive rat assay (SHR assay), and LD_{50} values in the mouse were determined in a standard, multidimensional observational assay and calculated according to the method of Litchfield and Wilcoxon.[9]

Synthetic Aspects. The syntheses of 3-hydrazino- (**6a–g**), 3-(methylhydrazino)- (**7a**, **7c**, **7d**, and **7f**), 3-(acetylhydrazino)- (**8a**, **8d**, **8e**, **8f**, and **8g**), and 3-[(trifluoroacetyl)hydrazino]-5-(substituted phenyl)-1,2,4-triazines (**9a**, **9c**, **9d**, **9f**, and **9g**) are shown in Scheme I. Melting points and solvents of recrystallization are shown in Tables I and II.

The 3-(methylthio)-5-phenyl-1,2,4-triazines **5a–h** were synthesized according to the method of Paudler and Chen.[10] The appropriately substituted acetophenone (**3a–g**) undergoes selenium dioxide oxidation to afford the corresponding phenylglyoxals **4a–g**.[11] Unpurified glyoxal **4** undergoes facile cyclization with methylthiosemi-

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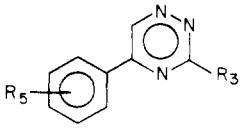
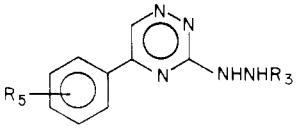
Table I. Melting Points and Solvents of Recrystallization of 3-(Methylthio)-5-phenyl-1,2,4-triazines 5a-h and 3-Hydrazino-5-phenyl-1,2,4-triazines 6a-6h[a]



no.	substituent R_3	substituent R_5	mp, °C	solv of recrystn
5a	SCH_3	H	99-100[b]	hexane
5b	SCH_3	3-CF_3	123-124	hexane
5c	SCH_3	4-Cl	165-166	methanol
5d	SCH_3	4-CH_3	160-161	methanol
5e	SCH_3	4-OCH_3	118-119	hexane
5f	SCH_3	3-Cl	79-81	methanol
5g	SCH_3	3,4-Cl	160-161	benzene
5h	SCH_3	4-OH	118-119	hexane
6a	$NHNH_2$	H	148-149[b]	methanol
6b	$NHNH_2$	3-CF_3	140-142	benzene
6c	$NHNH_2$	4-Cl	183-184[c]	methanol
6d	$NHNH_2$	4-$CH_3 \cdot H_2O$	183-184	ethanol
6e	$NHNH_2$	4-OCH_3	192-293	methanol
6f	$NHNH_2$	3-Cl	164-166	methanol
6g	$NHNH_2$	3,4-Cl	193-195	2-propanol

[a] C, H, N analyses were all within ±0.4%. [b] Identical with lit. mp; W. W. Paudler and T. K. Chen, *J. Heterocycl. Chem.*, 7, 767 (1970). [c] R. Fusco and R. Trave, *Rend. Ist. Lomb. Sci. Lett. A*, **91**, 202 (1957).

Table II. Melting Points and Solvents of Recrystallization of 3-(Methylhydrazino)triazines 7a-d, 3-(Acetylhydrazino)triazines 8a-e, and 3-[(Trifluoroacetyl)hydrazino]triazines 9a-e[a]



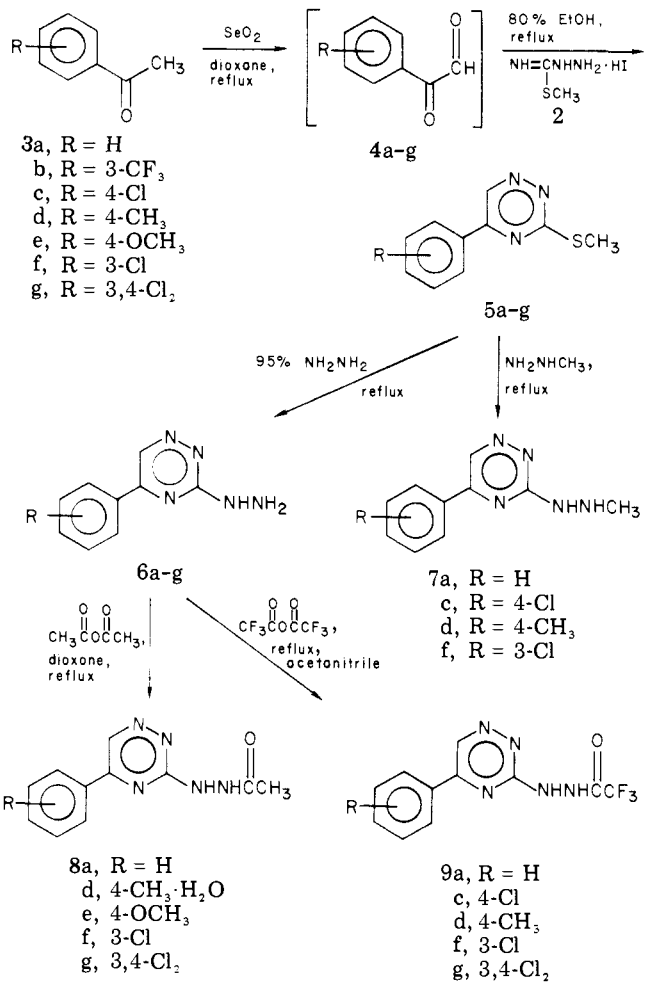
no.	substituents R_3	substituents R_5	mp, °C	solv of recrystn
7a	$-CH_3$	H	112-113	heptane
7c	$-CH_3$	4-Cl	156-157	heptane
7d	$-CH_3$	4-CH_3	124-125	heptane
7f	$-CH_3$	3-Cl	115-116	heptane
8a	$-C(=O)CH_3$	H	182-183	dioxane
8d	$-C(=O)CH_3$	4-$CH_3 \cdot H_2O$	183-184	ethanol
8e	$-C(=O)CH_3$	4-OCH_3	172-173	EtOAc
8f	$-C(=O)CH_3$	3-Cl	228-229	dioxane
8g	$-C(=O)CH_3$	3,4-Cl	258-259	MeOH/DMF
9a	$-C(=O)CF_3$	H	193-194	$CHCl_3$
9c	$-C(=O)CF_3$	4-Cl	210-211	$CHCl_3$
9d	$-C(=O)CF_3$	4-CH_3	224-225	$CHCl_3$/EtOAc
9f	$-C(=O)CF_3$	3-Cl	181-182	$CHCl_3$
9g	$-C(=O)CF_3$	3,4-Cl	221-222	$CHCl_3$

[a] C, H, N analyses were all within ±0.4%.

carbazide hydrogen iodide (**2**) to afford the desired 3-(methylthio)-1,2,4-triazine (**5a–g**). In a few cases, small quantities (<5%) of the corresponding 3-(methylthio)-6-phenyl-1,2,4-triazines were formed. This minor product was easily removed by fractional recrystallization or column chromatography on silica gel. The 3-(methylthio)-5-phenyltriazines **5a–g** were utilized in the synthesis of all 3-hydrazino-1,2,4-triazines shown in Schemes I and II.

The methylthiotriazine **5** undergoes nucleophilic displacement when refluxed with methylhydrazine to yield 3-(methylhydrazino)-5-phenyl-1,2,4-triazines **7a**, **7c**, **7d**, and **7f**. Reaction of **5** with 95% hydrazine hydrate affords high yields of the corresponding 3-hydrazino-5-phenyl-1,2,4-triazines **6a–g**. The unsubstituted hydrazinotriazines **6** undergo reaction with a wide variety of acid chlorides, anhydrides, and acids to form 3-hydrazido-1,2,4-triazines **8a**, **8d**, **8e**, **8f**, **8g**, **9a**, **9c**, **9d**, **9f**, **9g**, **10a–e**, and **11a–n**.

Scheme I



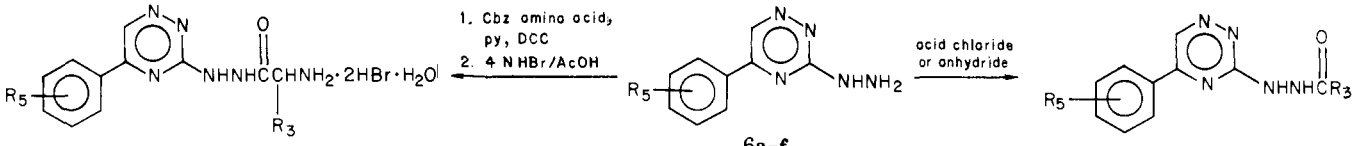
Acetylation of **6** with acetic anhydride or trifluoroacetic anhydride at reflux affords 3-(acetylhydrazino)-1,2,4-triazines **8a**, **8d**, **8e**, **8f**, and **8g** and 3-[(trifluoroacetyl)-hydrazino]-1,2,4-triazines **9a**, **9c**, **9d**, **9f**, and **9g**, respectively. The acetylhydrazinotriazines **8** were of particular interest, since they are likely metabolites of the parent hydrazinotriazines **6**. Hydralazine has been shown to undergo rapid acetylation in man, followed by cyclization to 3-methyl-*s*-triazolo[3,4-*a*]phthalazine.[12] The structural similarities between hydralazine and the hydrazinotriazines **6** suggest the potential of a similar metabolic pathway.

The 3-(amino acid)hydrazido-5-(substituted phenyl)-1,2,4-triazines **10a–e** and 3-(acylhydrazino)-5-(substituted phenyl)-1,2,4-triazines **11a–n** were synthesized according to Scheme II. Melting points, solvents of recrystallization and the chemical reagents used are shown in Table III.

Reaction of **6** with Cbz-protected amino acids in the presence of DCC and pyridine affords the corresponding Cbz-amino acid hydrazidotriazine. Hydrolysis of the Cbz group with 4 N HBr in acetic acid affords the desired water-soluble amino acid hydrazido hydrobromide salts **10a–e**. Reaction of **6** with various acid chlorides or anhydrides results in high yields of the corresponding 3-(acylhydrazino)-5-phenyl-1,2,4-triazines **11a–n** (see Table III for reagents used).

Biological Results. The effect of the hydrazinotriazines on systolic blood pressure was evaluated in a pri-

Scheme II

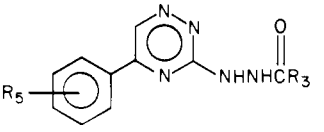


	R_3	R_5
10a	H	H
b	Bz	H
c	i-Pr	H
d	CH_3	4-Cl
e	CH_3	3-Cl

6a-f

	R_3	R_5
11a	CH_2Cl	H
b	$CHCl_2$	H
c	Ph	H
d	c-$C_{10}H_{15}$·HCl·H_2O	H
e	c-C_6H_{11}	H
f	$C_6H_5CH_2$·HCl	H
g	i-C_3H_7·HCl	H
h	n-C_5H_{11}·HCl	H
i	C_2H_5	4-Cl
j	$(CH_2)_3Cl$	4-Cl
k	$(CH_3)_2Cl$	4-Cl
l	CCl_3·HCl	4-Cl
m	c-$C_{10}H_{15}$·HCl	4-CH_3
n	$C_6H_5CH_2$	4-CH_3

Table III. Melting Points, Solvents of Recrystallization, and Chemical Reactants of 3-[(Amino acid)hydrazido]triazines 10a-e and 3-(Acylhydrazino)triazines 11a-n[a]



	substituents				
no.	R_3	R_5	mp, °C	solv of recrystn	chem reactant
10a	-CH_2NH_2·2HBr·H_2O	H	220-221	acetic acid	Cbz-Gly
10b	-$CHCH_2Ph$ | NH_2·2HBr·H_2O	H	264-265	acetic acid	Cbz-DL-Phe
10c	-$CHCH(CH_3)_2$ | NH_2·2HBr·H_2O	H	274-275	acetic acid	Cbz-DL-Val
10d	-$CHCH_3$ | NH_2·2HBr·H_2O	4-Cl	252-254	acetic acid	Cbz-DL-Ala
10e	-$CHCH_3$ | NH_2·2HBr·H_2O	3-Cl	214-216	acetic acid	Cbz-DL-Ala
11a	-CH_2Cl	H	171-172	$CHCl_3$	chloroacetic anhydride
11b	-$CHCl_2$	H	222-223	EtOAc	dichloroacetic anhydride
11c	-Ph	H	249-250	MeOH	benzoyl chloride
11d	c-$C_{10}H_{15}$·HCl·H_2O	H	218-220	MeOH/EtOAc	adamantane-1-carboxylic acid chloride
11e	c-C_6H_{11}	H	208-209	MeOH/H_2O	cyclohexanecarboxylic acid chloride
11f	$C_6H_5CH_2$·HCl	H	197-198	dioxane	phenylacetyl chloride
11g	i-C_3H_7·HCl	H	198-200	dioxane	isobutyryl chloride
11h	n-C_5H_{11}·HCl	H	198-200	dioxane	hexanoyl chloride
11i	C_2H_5	4-Cl	213-214	MeOH/EtOAc	propionic anhydride
11j	-$(CH_2)_3Cl$	4-Cl	178-179	DMF/EtOAc	4-chlorobutyryl chloride
11k	-$(CH_2)_2Cl$	4-Cl	199-100	DMF/EtOAc	3-chloropropionyl chloride
11l	-CCl_3·HCl	4-Cl	204-205	EtOAc	trichloroacetyl chloride
11m	c-$C_{10}H_{15}$·HCl	4-CH_3	254-256	dioxane	cyclohexanecarboxylic acid chloride
11n	$C_6H_5CH_2$	4-CH_3	228-230	MeOH	phenylacetyl chloride

[a] C, H, N analyses are all within ±0.4%.

mary SHR assay. Genetically hypertensive rats, having a systolic blood pressure of 170 mmHg or greater, were administered test compound orally with 0.25% methylcellulose as the vehicle. Groups of five rats were used per test. Systolic blood pressure was determined by a tail cuff method, utilizing capacitance transducers for the detection of blood pressure. A predose measurement of blood pressure was made to establish the control level. Compounds were administered at a dose of 100 mg/kg, unless otherwise noted, and blood pressure readings were taken at intervals of 4, 24, 28, and 48 h postdose 1. A second dose of compound was administered immediately after the 24-h reading. The mean drop in systolic blood pressure was recorded for each of the four reading intervals. Summation of the mean systolic blood pressure drops for each active compound provides a value for semiquantitative comparison with other compounds. The predose levels of blood pressure were compared statistically to each of the four postdose levels utilizing the Student's t test. Compounds having a level of significance of $p < 0.05$ were regarded active. A zero indicates that the compound was not statistically active at that reading interval, and compounds

Table IV. Antihypertensive Activity in the Spontaneously Hypertensive Rat Assay (SHR) and LD_{50} Values of 3-Hydrazino-1,2,4-triazines 6a-h, 3-(Methylhydrazino)-1,2,4-triazines 7a-d, and 3-(Acetylhydrazino)-1,2,4-triazines 8a-e and 9a-e

no.	dose, mg/kg	mean syst BP drop, mmHg (sum of mean syst BP drops)	LD_{50},[b] mg/kg
6a	50	-30, -43, -58, -60 (191)	56
6b	100	-26, -0, -48, -40 (114)	316
6c	100	-52, -61, -90, -87 (290)	237
6d	100	-62, -28, -75, -38 (203)	224
6e	100	-48, -26, -38, -22 (134)	56
6f	100	-0, -0, -55, -51 (106)	237
6g	100	inactive	>300
7a	100	-0, -49, -56, -92 (197)	178
7b	100	-0, -12, -39, -53 (115)	316
7c	100	inactive	316
7d	100	-28, -35, -63, -51 (177)	237
8a	100	-40, -94, -106, -104 (344)	178
8d	100	inactive	>300
8e	100	inactive	237
8f	100	-27, -29, -50, -55 (161)	>300
8g	100	inactive	237
9a	50	-51, -29, -70, -88 (238)	178
9c	100	-30, -46, -92, -74 (242)	237
9d	100	inactive	>300
9f	100	-0, -0, -64, -59 (123)	237
9g	100	-30, -31, -51, -26 (138)	>300
hydralazine, I	30	-88, -59, -47, -14 (208)	100

[a] These values represent the mean drops in blood pressure observed between predose levels and determinations made at 4, 24, 28, and 48 h. Oral dosing of compound was performed immediately after the 0- and 24-h readings. Inactives did not have statistically significant activity (student's t test; $p < 0.05$) at any of the determinations, while other determinations were statistically significant unless designated by 0. [b] LD_{50} values were determined in the mouse and calculated according to the method of Litchfield and Wilcoxon.[9]

denoted as "inactive" were not statistically active at any of the four reading intervals.

In order to determine the relative toxicity of the compounds, the approximate ip LD_{50} value for each derivative was determined in the mouse, utilizing a standard, multidimensional observational assay. The LD_{50} value was calculated according to the method of Litchfield and Wilcoxon.[9] The antihypertensive results and LD_{50} values are shown in Tables IV and V.

Of the 40 hydrazinotriazines evaluated, 28 are statistically active in the SHR assay at various reading intervals. Five of the active triazines possess LD_{50} values greater than 300 mg/kg. Although the SHR assay is not quantitative in nature, semiquantitative comparisons of hypotensive activity can be made by comparing the sum of the mean systolic blood pressure drops. Hydralazine, at a dose of 30 mg/kg, has a sum of mean systolic blood pressure drops of 208 mmHg. When compared to I, 15 triazines at a dose of 100 mg/kg have sums of systolic blood pressure drops comparable to or greater than I (**6c**, **6d**, **8a**, **9a**, **9c**, **10a**, **10b**, **10d**, 11a, **11b**, **11d**, 11e, **11g**, **11h**, and **11i**). Hydralazine has an LD_{50} value in the mouse of approximately 100 mg/kg; 13 of the above-mentioned triazines have LD_{50} values in excess of 100 mg/kg. However, this class of compounds was not devoid of overt toxicity. Of the 15 most active asymmetric triazines, only one (**11d**) has an LD_{50} value greater than 300 mg/kg.

Within the unsubstituted hydrazinotriazine class (**6a–g**, Table IV), all compounds are statistically active, except **6g**. However, only **6c** and **6d** have levels of activity comparable to I. In the methylhydrazine class (**7a**, **7c**, **7d**, and **7f**; Table IV), three of the four derivatives are statistically active, but none have levels of activity comparable to I. Hypotensive activity was variable in the acetylhydrazino class (**8a**, **8d**, **8e**, **8f**, and **8g**; Table IV), with only two of the five derivatives possessing statistically significant activity. Triazine **8a**, with an LD_{50} value of 178 mg/kg, is one of the most active derivatives evaluated, with a sum of systolic blood pressure drops of 344 mmHg. Among the trifluoroacetylhydrazinotriazines (**9a**, **9c**, **9d**, **9f**, and **9g**; Table IV), four of the five compounds are statistically active; **9a** and **9c** have levels of activity greater than I. The amino acid hydrazidotriazines **10a–e** (Table V) have excellent hypotensive activity, with three of the five analogues possessing activity greater than I (**10a**, **10b**, and **10d**). However, toxicity was also prevalent in this class with LD_{50} values in the 75–237 mg/kg range. The miscellaneous acylhydrazinotriazines 11a–n (Table V) have nine statistically active analogues, with seven of the nine possessing levels of activity greater than I (**11a**, **11b**, **11d**, 11e, **11g**, 11h, and **11i**). Three of these active triazines have LD_{50} values of 237 mg/kg or greater (**11d**, 11e, and **11i**).

Since the improvement of the safety of vasodilator therapy was one of our synthetic goals, we also evaluated the data in terms of overt toxicity. Among those triazines which were statistically active, but not at levels comparable to I, four derivatives had LD_{50} values greater than 300 mg/kg (**6b**, **7c**, **8f**, and **9g**). These high LD_{50} values represent the potential for reasonably high therapeutic indices, in subsequent dose–response studies. Based on the general relationship between overt toxicity and primary antihypertensive activity within this class of compounds, three candidates were selected for subsequent dose–response studies (**8f**, **9g**, and **11d**).

The results of the dose–response study in the SHR assay are shown in Table VI. Blood pressure and heart rate were measured at doses of 100, 30, 10, and 3 mg/kg. All three triazines were statistically active at 100 and 30 mg/kg, and trifluoroacetyltriazine **9g** demonstrated hypotensive activity at 10 mg/kg. Hydralazine possessed statistically significant activity at all doses, with only limited activity at 3 mg/kg. The equiactive dose needed to produce a hypotensive response similar to that of I at

Table V. Antihypertensive Activity in SHR Assay and LD_{50} Values of 3-[(Amino acid)hydrazido]-1,2,4-triazines 10a-e and 3-(Acylhydrazino)-1,2,4-triazines 11a-n

no.	dose, mg/kg	mean BP drop, mmHg[a] (sum of mean BP drops)	LD_{50},[b] mg/kg
10a	50	-41, -35, -105, -91 (272)	75
10b	100	-26, -38, -75, -82 (221)	178
10c	100	-23, -17, -17, -44 (121)	178
10d	100	-44, -58, -91, -103 (296)	237
10e	100	inactive	178
11a	100	-63, -74, -101, -55 (293)	100
11b	100	-33, -54, -106, -80 (273)	178
11c	100	inactive	>300
11d	100	-49, -86, -51, -36 (212)	>300
11e	100	-24, -51, -76, -79 (230)	237
11f	100	inactive	237
11g	100	-44, -46, -98, -83 (271)	178
11h	100	-53, -53, -81, -68 (255)	178
11i	100	-47, -79, -107, -111 (344)	237
11j	100	-0, -25, -48, -73 (146)	237
11k	100	inactive	237
11l	100	inactive	237
11m	100	-32, -0, -24, -30 (86)	216
11n	100	inactive	237
hydralazine, I	30	-88, -59, -47, -14 (208)	100

[a] See footnote *a* in Table IV. [b] LD_{50} values were determined in the mouse and calculated according to the method of Litchfield and Wilcoxon.[9]

Table VI. Antihypertensive Activity and Heart Rate in the SHR Assay of Triazine Developmental Candidates 8f, 9g, and 11d

no.	dose, mg/kg	mean BP drop, mmHg[a] (sum of mean BP drops)	mean heart rate,[b] beats/min
8f	100	−20, −23, −50, −55 (148)	465, 478, 482, 482, 490
	30	−16, −14, −19, −19 (68)	424, 400, 438, 460, 462
	10	inactive	463, 458, 460, 448, 448
	3	inactive	448, 422, 430, 436, 442
9g	100	−38, −29, −35, −25 (127)	460, 472, 462, 438, 438
	30	−18, −18, −18, −16 (70)	448, 444, 438, 435, 426
	10	−16, −12, −17, −15 (60)	424, 412, 438, 448, 400
	3	inactive	422, 430, 424, 428, 424
11d	100	−42, −70, −46, −35 (193)	454, 446, 428, 442, 458
	30	−20, −12, −18, −11 (51)	400, 410, 412, 380, 392
	10	inactive	462, 468, 446, 428, 422
	3	inactive	440, 445, 420, 418, 426
hydralazine	30	−88, −60, −47, −14 (209)	442, 492, 480, 496, 448
	10	−60, −38, −10, −10 (118)	420, 504, 468, 428, 448
	3	−13, −0, −11, −0 (24)	400, 448, 392, 444, 432

[a] See footnote a in Table IV. [b] These values are the mean heart rates observed predose, followed by readings at 4, 24, 28 and 48 h.

30 mg/kg was in excess of 100 mg/kg with all three triazines. While these developmental candidates were not as efficacious as I in terms of hypotensive activity, they possessed significant advantages over I in terms of toxicity.

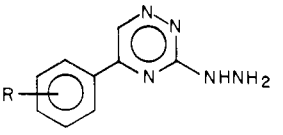
Each developmental candidate had an LD_{50} value threefold greater than that of I. If one defines the therapeutic index (TI) as the LD_{50} value in mice over the equiactive dose required to produce a hypotensive effect in the SHR assay similar to I at 30 mg/kg, our candidates are comparable to I in terms of TI. It is also interesting to note that the reflex tachycardia observed clinically and in this assay with I was not prevalent with triazine administration (see Table VI). Only triazine **8f** demonstrated marginal tachycardia at 100 mg/kg, while I produced this effect at all four doses.

Biological Discussion. The semiquantitative nature of the primary SHR assay limits the validity of extensive quantitative structure–activity studies. However, general consideration of relationships between physicochemical parameters and the level of overt toxicity observed within each chemical class reveals a basic pattern. The most toxic analogues within each chemical class are those with low lipid solubilities, relative to other compounds in that specific series. As derivatives with greater lipid solubilities were evaluated, toxicity generally decreased while activity was maintained. This correlation is not linear, and several exceptions are observed; however, the general trend is seen in all four triazine classes.

Since each hydrazinotriazine within its particular chemical class was identical to other members of the class, except for the substituents on the phenyl ring, hydrophobic substituent constants for aromatic rings (π)[13,14] were utilized to estimate the relative lipophilicity of compounds in each series. A general correlation between hydrophobicity (π value) and toxicity (LD_{50} value) was observed among the unsubstituted hydrazinotriazines **6a–g**. Table VII shows this correlation by comparison of π and LD_{50} values. As lipophilicity was increased by substitution with more lipophilic substituents, indicated by the greater π constant, LD_{50} values progressively increase from 56 mg/kg to greater than 300 mg/kg. No apparent correlation is observed between antihypertensive activity and π values or electronic (σ)[15] and steric (Es)[16] parameters.

Among the other triazine classes a similar trend was observed. In the methylhydrazino class (**7a**, **7c**, **7d**, and **7f**), the phenyl derivative **7a** had an LD_{50} value of 178 mg/kg and π constant of 0; it was the most toxic compound in the class and had the lowest π value. As lipophilicity was increased, LD_{50} values increased to the 237–316 mg/kg range. In the acetylhydrazino class (**8a**, **8d**, **8e**, **8f**, and **8g**), phenyl derivative **8a** had an LD_{50} value of 178 mg/kg and π constant of 0. It was the most toxic of the group. The 4-methoxyphenyl analogue **8e** had a slightly lower π value (−0.04) but is not as toxic (LD_{50} = 237 mg/kg). This was one of the few exceptions we observed in this correlation. In the trifluoroacetyl class (**9a**, **9c**, **9d**, **9f**, and **9g**), the phenyl derivative **9a** was the most toxic analogue in the class with an LD_{50} value of 178 mg/kg and a π value of 0. As substituents with high π values were substituted on the phenyl ring, toxicity decreased to the 237–300 mg/kg range.

Table VII. Correlation between Relative Lipid Solubility (π Values) and Toxicity (LD_{50} Values) of 3-Hydrazino-5-(substituted phenyl)-1,2,4-triazines 6a–g



no.	R	LD_{50}, mg/kg	π[a]
6e	4-OCH_3	56	−0.04
6a	H	56	0.00
6d	4-CH_3	224	0.60
6c	4-Cl	237	0.70
6f	3-Cl	237	0.76
6b	3-CF_3	316	1.21
6g	3,4-Cl	300	1.46

[a] References 13 and 14.

Since the unsubstituted phenyl analogues were, in most cases, the most toxic, one could attempt to explain this trend in terms of differences in drug metabolism. The unsubstituted phenyl derivatives **6a**, **7a**, **8a**, and **9a** are more susceptable to para hydroxylation than other members of the series. Thus, if the corresponding *p*-hydroxyl or subsequent metabolites are toxic, those derivatives less susceptable to metabolism would be less toxic. Metabolic studies done in man with structurally related hydralazine demonstrate that the major metabolite of I is the acetate of the free hydrazino group.[12] Only 7% of the excreted material was identified as para-hydroxylated metabolites.[12] If one assumes a similar metabolic fate in the mouse and that the hydroxylated metabolites are solely responsible for these toxic effects, the observed correlation still would not extend throughout the entire series.

Therefore, it appears unlikely that metabolism in itself can explain this apparent correlation. Lipid solubility may be related to toxicity in terms of rates of distribution to various sites of toxic manifestations.

Experimental Section

Elemental analyses were performed by Diamond Shamrock Corp., Analytical Group, Painesville, Ohio. Infrared spectra were determined on a Perkin-Elmer Model 137 spectrophotometer with sodium chloride optics. Nuclear magnetic resonance spectra were recorded on a Varian A56/60D and a Bruker WH90 spectrometer. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected.

3-(Methylthio)-5-(substituted phenyl)-1,2,4-triazines 5a–g. In a 1-L round-bottom flask was placed the appropriate acetophenone **3** dissolved in dioxane (500 mL). To this solution was added an equal molar quantity of SeO_2 dissolved in dioxane/H_2O (4:1). The mixture was stirred at reflux for 4 h, forming a red oil and elemental selenium. The mixture was cooled, and the oil was decanted from the selenium solid and concentrated under reduced pressure. The viscous red oil (**4a–g**) was dissolved in 80% EtOH and added dropwise to a stirring equal molar solution of methylthiosemicarbazide hydrogen iodide (**2**) and $NaHCO_3$ in 80% EtOH. The mixture was stirred at ambient temperature for 10 h and diluted with H_2O to form a solid. The solid was collected, treated with activated charcoal, and recrystallized from the appropriate solvent (see Table I) to afford the corresponding 3-(methylthio)triazine (**5a–g**).

In several cases, a small amount of the corresponding 6-phenyltriazine was observed by NMR. The triazine ring proton has a significantly different chemical shift when in the 5 position of the ring (phenyl substituent in the 6 position) than when it is in the 6 position (phenyl ring in the 5 position). The C_6-H chemical shifts observed in the major products were in the vicinity of δ 9.8, and the C_5-H chemical shift observed in the isolated cases where the minor product was produced was in the vicinity of δ 9.0. In each case, they were not coupled and, therefore, appeared as singlets. The two isomers can be readily separated by column chromatography on silica gel (activity III, 30 mm), utilizing a chloroform/ethyl acetate (4:1) solvent system. Once isolated, the two isomers were unequivocally characterized by ^{13}C NMR.

Fractional recrystallization from the solvents listed in Tables I–III also eliminated the small quantities of minor isomer produced.

3-Hydrazino-5-(substituted phenyl)-1,2,4-triazines 6a–g. The desired 3-(methylthio)triazine **5** was placed in a one-necked round-bottom flask, dissolved in dioxane. To this solution was added a 2 M excess of 95% hydrazine, and the mixture was refluxed for 15 h. Upon cooling, the mixture was poured over ice, forming a solid precipitate. The solid was collected and recrystallized from the appropriate solvent (see Table I) to yield the desired 3-hydrazinotriazine **6a–g**.

3-(Methylhydrazino)-5-(substituted phenyl)-1,2,4-triazines 7a, 7c, 7d, and 7f. In a 1-L round-bottom flask was placed the desired 3-(methylthio)triazine **5** dissolved in acetonitrile. To this solution was added a 2 M excess of methylhydrazine, and the mixture was stirred at reflux for 2 h, cooled, concentrated under reduced pressure, and diluted with H_2O. The resulting solid precipitate was collected and recrystallized from the appropriate solvent (see Table II) to form the desired methylhydrazinotriazine **7a**, **7c**, **7d**, or **7f**.

The formation of the terminal methylhydrazine product, rather than the $N(CH_3)NH_2$ product favored by nucleophilicity, was verified by proton NMR, IR, and chemical methods. The NMR spectra of **7a**, **7c**, **7d**, and **7f** all possessed singlets in the δ 9 ($-NHNHCH_3$) and 5 regions ($-NHNHCH_3$). Infrared spectra showed no absorption bands in the primary amine region of 3400 to 3500 cm^{-1}. Weak bands were observed in the 3300-cm^{-1} region characteristic of secondary amines. All methylhydrazines gave a negative Hinsberg test for primary amines.

3-(Acetylhydrazino)-5-(substituted phenyl)-1,2,4-triazines 8a, 8d, 8e, 8f, and 8g. In an Erlenmeyer flask was placed the desired 3-hydrazino-5-phenyl-1,2,4-triazine (**6**) dissolved in DMF/acetonitrile. To this stirring solution at ambient temperature was added dropwise an equal molar quantity of acetic anhydride. The mixture was stirred at ambient temperature for 4 h and then heated on a steam bath for 1 h. Upon cooling, a solid precipitate formed, which was collected and recrystallized from the appropriate solvents (see Table II) to afford the desired 3-(acetylhydrazino)triazine **8a**, **8d**, **8e**, **8f**, or **8g**.

3-[(Trifluoroacetyl)hydrazino]-5-(substituted phenyl)-1,2,4-triazines 9a, 9c, 9d, 9f, and 9g. In an Erlenmeyer flask was placed 3-hydrazino-5-phenyl-1,2,4-triazine (**6**) dissolved in DMF/acetonitrile. To this stirring solution was added dropwise at ambient temperature an equal molar quantity of trifluoroacetic anhydride. The reaction mixture was stirred at ambient temperature for 4 h and then heated on a steam bath for 1 h. Upon cooling, a solid precipitate formed, which was collected and recrystallized from the appropriate solvent (see Table II) to yield the desired trifluoroacetylhydrazinotriazine **9a**, **9c**, **9d**, **9f**, or **9g**.

3-[(Amino acid)hydrazido]-5-(substituted phenyl)-1,2,4-triazines 10a–e. In a three-necked round-bottom flask equipped with condenser, addition funnel, and mechanical stirrer was placed the desired 3-hydrazino-5-phenyl-1,2,4-triazine (**6**) dissolved in pyridine. To this stirring solution at −10 °C was added dropwise an equal molar quantity of the desired Cbz-amino acid (see Table III) and DCC dissolved in pyridine. The mixture was stirred for 10 h with slow warming to ambient temperature. The precipitate which formed was collected and washed several times with hot CH_3OH to remove the product from the dicyclohexylurea byproduct. The CH_3OH washings were collected, concentrated, and cooled to afford the Cbz-protected amino acid hydrazide triazine.

The desired free amine salts **10a–e** were obtained by treatment of the Cbz-amino acid dissolved in acetic acid with 4 N HBr in acetic acid. The mixture was stirred for 1 h at ambient temperature, forming a white precipitate. The reaction mixture was diluted with anhydrous ether to precipitate additional product. The solid was collected, washed numerous times with ether/acetone, and dried under reduced pressure to afford the desired water-soluble 3-[(amino acid)hydrazido]-5-phenyl-1,2,4-triazine hydrobromide salt (**10a–e**).

3-(Acylhydrazino)-5-(substituted phenyl)-1,2,4-triazines 11a–n. In an Erlenmeyer flask equipped with magnetic stirrer was added the desired 3-hydrazino-5-phenyl-1,2,4-triazine (**6**) dissolved in dioxane. An equal molar quantity of the desired acid chloride or anhydride (see Table III) was added, and the mixture was stirred at ambient temperature for 6 h and then heated on a steam bath for 1 h. The mixture was cooled, forming a precipitate which was collected and recrystallized from the appropriate solvent (see Table III) to afford the desired acylhydrazinotriazine **11a–n**.

Antihypertensive SHR Assay. Spontaneously hypertensive rats, 12 to 16 weeks of age, were used in this assay. Systolic blood pressures were determined by the tail cuff method, utilizing capacitance transducers for the detection of pressure, an aneroid manometer for measuring pressure, and an oscilloscope for visualizing the disappearance and/or appearance of the pressure pulse. Heart rate was measured by a biotachometer. Groups of five rats having systolic blood pressure of 170 mmHg or greater were chosen, and the test compound was administered at 100 mg/kg po as a solution or suspension in 0.25% methylcellulose (MC) at a volume of 5 mL/kg.

Systolic blood pressure and heart rate were measured before dosing of compound to establish the control level. Additional readings were taken at 4, 24, 28, and 48 h postdose 1. A second dose of compound was administered immediately following the 24-h reading.

The systolic blood pressures were compared statistically using the Student's t test. Compounds having a level of significance $p < 0.05$ were regarded as active.

Neuropharmacological Profile—LD_{50} Determination. White male mice of the Carworth Farm strain CF-1 weighing 18–22 g were used in these determinations. The test compound, regardless of solubility, was suspended in a 0.15% aqueous methylcellulose solution. Intraperitoneal injections were administered in logarithmic progression and sequentially. The dose levels employed routinely were 10, 30, 100, and 300 mg/kg, using four male mice at each dose level. Since this test was conducted in a sequential manner, the first dose administered was at 300 mg/kg. The mice were injected at this dose level and observed for gross changes produced by the drug, such as behavioral, neurological, autonomic, and toxic effects.

The number of animals alive at the end of 48 h was recorded and the LD_{50} value estimated according to the method of Litchfield and Wilcoxon.[9] If none were dead after 48 h at 300 mg/kg, the LD_{50} value was estimated to be greater than 300 mg/kg and no further dosing was required. If numerous deaths occurred at 300 mg/kg, the compound was dosed at 100 mg/kg and the LD_{50} value estimated from the number of deaths at that dose.

Dose–Response SHR Assay. The procedures for the dose–response SHR assay were identical to the primary level procedure, except rats were dosed at 100, 30, 10, and 3 mg/kg. Five rats were used for each dose, and heart rate and blood pressure were recorded.

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Synthesis of 2,3,4,4a,5,9b-Hexahydro-1*H*-pyrido[4,3-*b*]indole Derivatives and Their Central Nervous System Activities

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The synthesis and some pharmacological effects of *cis*- and *trans*-2-substituted 2,3,4,4a,5,9b-hexahydro-1*H*-pyrido[4,3-*b*]indole derivatives are described. In these derivatives, the substituents of the 2, 5 and 8 position, together with the relative configuration of the 4a and 9b position, influenced the potency of the central nervous system activities. A *cis*-2-[3-(*p*-fluorobenzoyl)propyl] analogue (**5k**) of carbidine (1) possessed not only thymoleptic-like biological activity but had more potent neuroleptic activity than the parent drug.

It has been reported that carbidine (*cis*-2,8-dimethyl-2,3,4,4a,5,9b-hexahydro-1*H*-pyrido[4,3-*b*]indole dihydrochloride, 1) is an effective antipsychotic agent with thymoleptic properties.[1,2] In animal studies, 1 not only depresses the central nervous system (CNS) but also enhances the stereotyped behavior induced by methamphetamine,[1-3] a property which is characteristic of thymoleptics.[2,4] However, the CNS-depressing activities of 1 were found to be extremely weak when tested on the basis of criteria for the typical neuroleptics, as will be shown. So, an attempt was made to prepare novel hexahydro-1*H*-pyrido[4,3-*b*]indole derivatives which were as potent as the existing neuroleptics in CNS-depressing activities but which, like 1, still possessed the ability to potentiate the methamphetamine-induced stereotypy. It was found that *cis*-2-[3-(*p*-fluorobenzoyl)propyl]-8-methyl-2,3,4,4a,5,9b-hexahydro-1*H*-pyrido[4,3-*b*]indole (**5k**) possessed such pharmaceutical properties.

Chemistry. Hydrogenolysis of 2-benzyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indoles **2** on palladium/carbon gave the debenzylated compounds **3**, which were catalytically hydrogenated in dilute hydrochloric acid on platinum dioxide to afford (4a,9b-*cis*)-2,3,4,4a,5,9b-hexahydro derivatives **4** (Scheme I). The alkylation of **4** afforded the 2-substituted products **5**. The alkylation or acylation of the butyrophenone derivative **5k** gave the 5-substituted derivatives **6**.

Reaction of 2-benzyl-8-methyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (**2c**) with BH_3–THF, followed by acid hydrolysis and subsequent neutralization, gave the *trans*-2,3,4,4a,5,9b-hexahydro derivative **7b**. The same treatment of the 8-fluoro derivative **2b**, however, gave the mixture of trans (**7a**) and cis compounds. This mixture was treated with acetic anhydride for separation to give the 5-acetyl derivatives **8b** and 8a in a ratio of 6:1. Compound **8b** was hydrolyzed to **7a**, while 8a gave **4b** by the same hydrolysis followed by catalytic hydrogenolysis. Hydrogenolysis of **7** gave the debenzylated compounds **9**, which were converted into the 2-substituted derivatives **10a–e** and the 2,5-disubstituted ketones **11** in similar procedures mentioned above.

Compounds **4c** and **9b** were benzoylated and then treated with formaline, followed by catalytic hydrogenation, to give the 2-benzoyl-5-methyl derivatives **13**, which were quarternized with methyl iodide to give the 5,5-dimethyl derivatives **14a** and **14b**, respectively (Scheme II). In the NMR spectra, the difference in chemical shifts of the methyl group owing to the anisotropic shielding of the benzene ring[5] is 10 Hz for **14a** and 40 Hz for **14b**, which suggest the former to be the cis form and the latter to be the trans form, as was expected from the reduction procedures.[6]

Pharmacological Results and Discussion. The CNS-depressing property of hexahydropyrido[4,3-*b*]indole derivatives obtained in this study was examined by their effect on locomotor, muscle relaxating, and cataleptic activities in mice; the results are shown in Table I. It seems that the CNS-depressing property of these derivatives is affected by the substituent in the 2, 5, and 8 positions. Among the cis compounds (**5a–h** and **5k**) with a methyl group in the 8 position similar to 1, the compounds (**5g**, **5h**, and **5k**) with the bulky substituent group, such as 3-(2-chlorophenothiazin-10-yl)propyl, 4,4-bis(*p*-fluorophenyl)butyl, and 3-(*p*-fluorobenzoyl)propyl, in the

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