plasma are summarized in Table II.

Plasma Electrophoresis. Polyacrylamide gel electrophoresis of plasma samples was performed according to the method of Narayan et al.[12] as previously described.[13] The amount of radioactivity associated with each lipoprotein class was determined by sectioning the gels and counting each section in a γ-counter. The radioactivity associated with each lipoprotein band was expressed as a percentage of the total radioactivity applied to the gel.

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Preliminary Studies of Mesoionic 3-(Substituted-aryl)-ψ-oxatriazoles as Potential Antihypertensive Agents

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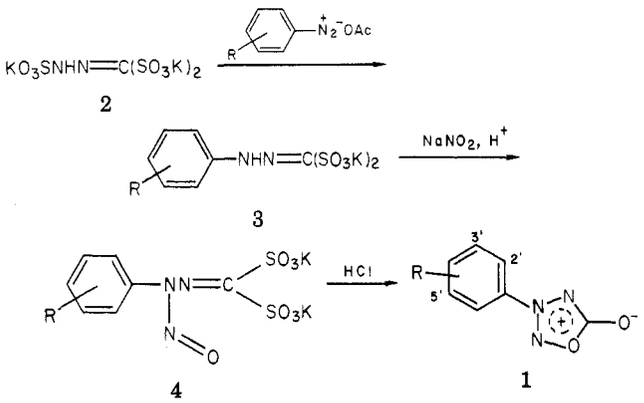
Several mesoionic 3-(substituted-aryl)-ψ-oxatriazole derivatives were prepared and evaluated as potential antihypertensive agents. 3-(4-Methylphenyl)-ψ-oxatriazole was found to produce a significant hypotensive effect in rats, which was characterized by a rapid (2–3 min) onset of action. Maximal effects were achieved within 30 min, and a substantial decrease in mean arterial blood pressure was recorded even 5 h after administration.

Mesionic compounds, as a class, have received considerable attention from a chemical and/or physicochemical standpoint; however, their pharmacological potential, for the most part, remains relatively unexplored. Several years ago, it was discovered that 3-alkyl derivatives of the mesoionic ψ-oxatriazole (i.e., oxatriazolium-5-olate, **1**) possess a moderate degree of hypotensive activity characterized by a rapid onset and a long duration of action as measured in the anesthetized dog.[1,2] The results of these studies were supported and extended by Thomas and co-workers.[3] Because optimal activity appears to be associated with the increased lipophilic nature of the 3-alkyl substituent (e.g., *t*-Bu > Et > Me), it was of interest to explore several 3-aryl derivatives. We now report the synthesis and the results of a preliminary evaluation of a series of 3-aryl-ψ-oxatriazoles as potential hypotensive agents.

Chemistry. The synthesis of the mesoionic compounds is shown in Scheme I. Tripotassium sulfohydrazonomethanedisulfonate (**2**) was prepared in four steps according to the method of von Pechmann and Manck.[4] Compound **2** was allowed to react with the diazonium acetates of the appropriately substituted anilines to yield the corresponding arylhydrazonomethanedisulfonate salts, **3**, which were further nitrosated to afford **4**. The nitroso derivatives **4** were not isolated and characterized but were cyclized by stirring in acid at room temperature to afford the desired mesoionic products **1**. All mesoionic products displayed a negative Liebermann nitroso test.

Scheme I



Results and Discussion

Compounds **1a–d** were initially prepared and evaluated; the 3-(4-methylphenyl) derivative **1b** was studied in greater detail than the other compounds. When administered to rats, **1b**, in doses of 1–90 mg/kg, produced a dramatic dose-dependent decrease in blood pressure; maximal effects were achieved in less than 1 h, and Table II shows the effects of several doses of **1b** recorded during the first hour after administration. Doses of **1b** greater than 10 mg/kg produced a decrease in blood pressure that was of long duration (i.e., after 5 h, blood pressure was still at least 20% below control values), and percent decreases in both systolic and diastolic pressures were essentially equal (data not shown). A typical time course for **1b** (at 15 mg/kg) is shown in Figure 1.

Because the maximal effect of 10 mg/kg of **1b** occurred within 30 min, this dose and time parameter were chosen for single dose studies of the unsubstituted, 4-chloro-, and 3,4-dichlorophenyl derivatives (**1a**, **1c**, and **1d**, respectively) in order to make comparisons with **1b**. Table III shows

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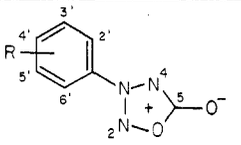
Table I. Properties of 3-(Substituted-aryl)-ψ-oxatriazoles



compd	R	yield, %	mp,[a] °C	emp formula[b]	IR[c] (C=O), cm^{-1}
1a	H	52	83-84[d]	$C_7H_5N_3O_2$	1785
1b	4'-Me	50	96-97	$C_8H_7N_3O_2$	1795
1c	4'-Cl	30	135-136[e]	$C_7H_4ClN_3O_2$	1800
1d	3',4'-Cl_2	35	113-116	$C_7H_3Cl_2N_3O_2$	1805
1e	4'-NO_2	25	164-165[f]	$C_7H_4N_4O_4$	1800
1f	4'-OCH_3	40	135-136	$C_8H_7N_3O_3$	1790
1g	2',6'-Cl_2	18	99-100	$C_7H_3Cl_2N_3O_2$	1795
1h	4'-F	33	122-123	$C_7H_4FN_3O_2$	1795

[a] All compounds were recrystallized from MeOH. [b] Correct elemental analyses (C, H, N) were obtained within 0.4% of theoretical values. [c] Infrared carbonyl stretching band, with CCl_4 as solvent. [d] Literature[5] mp 89 °C. [e] Literature[6] mp 133 °C. [f] Literature[7] mp 166 °C.

Table II. Maximal Hypotensive Response Observed for 3-(4-Methylphenyl)-ψ-oxatriazole (1b) during the First Hour after Administration

dose,[a] mg/kg	N[b]	% decrease in BP[c,e]
0[d]	3	0.0
1	5	13.9 (±3.0)
3	9	23.5 (±6.2)
10	12	28.0 (±8.3)
15	8	37.6 (±7.0)
25	1	48.1
45	1	53.0
90	2	75.3 (±5.4)

[a] Dose administered in less than 1 mL of corn oil by gavage. [b] Number of animals receiving drug. [c] Percent decrease in mean (±SE) arterial pressure (mmHg) from base-line pressure recorded after recovery from anesthesia. [d] Corn oil (1 mL) was administered. [e] Control animals were administered 0.1 mg/kg of clonidine (data not shown), which was found to decrease blood pressure by 13% within the first hour, to a maximum of 15% at 2 h.

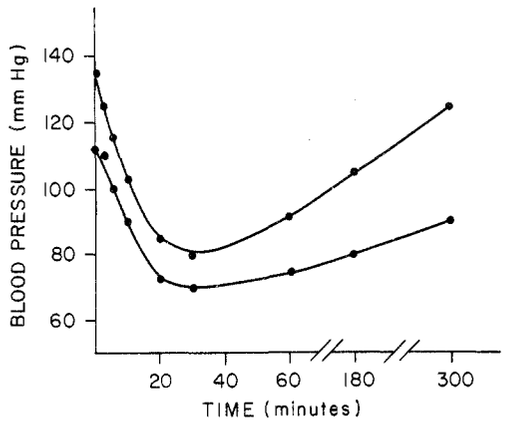


Figure 1. A typical time-course plot for a single 15 mg/kg dose of **1b**. (Upper tracing = systolic pressure; lower tracing = diastolic pressure.)

that there is very little difference in activity amongst **1a–d**, with the 3-(4-chlorophenyl) derivative **1c** being the most active (approximately twice as active as **1b** on a molar basis). Preliminary studies of **1e–g** (Table III) reveal activity roughly comparable to that of **1b**.

Doses of 1 mg/kg of **1b** produce a decrease in blood pressure equivalent to that observed after administration of 0.1 mg/kg of clonidine. However, whereas blood pressure, after clonidine administration, is back to base-line levels within 3 h, **1b** produces effects that are still evident at 5 h after administration. Thus, it appears that 3-aryl-substituted ψ-oxatriazoles constitute a novel class of moderately active but relatively long-acting hypotensive agents. Additional studies are currently ongoing.

Table III. Hypotensive Responses at 30 min for a Single 10 mg/kg dose of Mesoionic Compounds 1a-g

compd	N[a]	% decrease in BP[b] systolic	% decrease in BP[b] diastolic
1a	4	51.7 (±3.4)	52.3 (±3.2)
1b	12	28.5 (±8.1)	27.5 (±8.8)
1c	2	47.0 (±2.0)	47.9 (±1.5)
1d	2	31.7 (±0.1)	30.9 (±0.6)
1e	1	24.8	23.8
1f	1	22.2	20.0
1g	1	11.5	8.3
clonidine[c]	2	12.0 (±0.0)	13.5 (±0.4)

[a] Number of animals employed. [b] Mean (±SE) percent decrease from base line (i.e., blood pressure of animals receiving 1 mL of corn oil) pressure (mmHg). [c] Clonidine (0.1 mg/kg) was administered in corn oil.

Experimental Section

Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 257 spectrophotometer, and ^1H NMR spectra were determined on a Perkin-Elmer R-24 spectrometer using Me_4Si as an internal standard. Mass spectra were obtained on a Finnigan 4015 GC-MS data system at 70 eV. The structures of all compounds are consistent with their IR, NMR, and mass spectral data. Microanalyses were performed by Atlantic Microlab, Atlanta, GA, and results are within 0.4% of the calculated values.

General Procedure for the Preparation of 3-(Substituted-aryl)-ψ-oxatriazoles (1). The appropriate aniline (1 mol) was diazotized by treatment with concentrated HCl (2.3 mol), followed by the addition of enough water to effect solution; the solution was chilled to 0 °C, and an aqueous solution of KNO_2 (1.1 mol) was added at such a rate as to maintain a temperature of 0–5 °C. At 15 min after the addition was completed, KOAc was added at 0 °C to adjust the solution to pH 5.5. An amount of this cold solution, corresponding to approximately 0.15 mol of the diazonium salt, was added to an aqueous solution of tripotassium sulfohydrazonomethanedisulfonate (0.1 mol) with stirring; pH 5 was maintained by addition of granular KOAc. When all effervescense had ceased, the reaction mixture was filtered, and the filtrate was concentrated under reduced pressure until the formation of a colored precipitate was observed; addition of 95% EtOH further precipitated the product. Yields of the hydrazones **3** varied widely; these salts were used without further purification. Solid $NaNO_2$ (55 mmol) was added to a solution of **3** (50 mmol) in H_2O (145 mL); after stirring for 30 min, the reaction mixture was filtered, and the filtrate was added to 2 N hydrochloric acid (300 mL). After the solution was stirred for

5 h, the precipitated product was collected by filtration and recrystallized from MeOH. Physical and spectral data for the mesoionic compounds 1 are given in Table I.

Pharmacology. Male Sprague–Dawley rats (250–350 g) were anesthetized with sodium pentobarbital (35 mg/kg ip), and a femoral artery was cannulated. The animals were restrained in a supine position, and the excision area was bathed in normal saline for the duration of the experiment. A 10% heparin–saline solution (0.01 mL) was injected into the cannulated artery 30 s before recording blood pressure. Blood pressure was recorded with a Statham transducer interfaced with a Grass Model 7 polygraph. After the animals recovered from anesthesia, blood pressure was recorded continuously for 1 h. Control experiments were carried out and showed that the length of time of restraint, time of day of blood pressure recording, and administration of vehicle alone did not affect blood pressure. Compound **1b** was found by the procedure of Weil[8] to possess an approximate LD_{50} of 250 mg/kg; therefore, in the early studies of hypotensive effects, doses of up to 90 mg/kg were used. Test compounds were dissolved in corn oil (0.7–0.8 mL) and administered by gavage. Blood pressure was recorded continuously for 30 min and then intermitantly for the next several hours. Animals were sacrificed at the conclusion of each experiment.

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Ammonium 7*H*-Purin-6-yl 1-Thio-β-D-glucopyranosiduronate, a Latent, Selective Anticancer Agent

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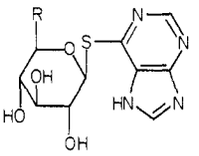
Ammonium 7*H*-purin-6-yl 1-thio-β-D-glucopyranosiduronate (**1**), a good substrate of β-glucuronidase, causes a 22% decrease in the growth of L1210 cells while not affecting the growth of Chinese hamster lung fibroblasts from a nontumor line. 7*H*-Purin-6-yl 1-thio-β-D-glucopyranosiduronamide (**2**), a poor substrate of β-glucuronidase, has no effect on the growth of either cell type.

In 1927, Armand J. Quick wrote[1] that "the significance of glycuronic [sic] acid in the economy of the organism is not fully understood, but it seems rather certain that its biological importance is underestimated," and that "little emphasis has been given to the marked ability of the organism to produce relatively large amounts of glycuronic [sic] acid without any perceptible embarrassment," during his study of the metabolism of borneol in dogs. Today we know that there exists higher than normal levels of the enzyme β-glucuronidase (EC 3.2.1.31) in human cancer tissues[2] and this may be used to advantage in devising prodrugs. Futher, the activity of β-glucuronidase is enhanced when cells become more acidic, since the pH–velocity maximum of the enzyme is between 4 and 5. The acidity of cancer cells, already known to be more acidic than normal, can specifically be increased by glucose.[3,4] Taken together, these findings indicate that glucuronides of known anticancer compounds can selectively deliver these drugs to cancer tissue. This approach is currently being explored in some laboratories[4-8] and here we report the synthesis of ammonium 7*H*-purin-6-yl 1-thio-β-D-glucopyranosiduronate (**1**) and of 7*H*-purin-6-yl 1-thio-β-D-glucopyranosiduronamide (**2**), the hydrolysis of **1** and **2** catalyzed by bovine liver β-glucuronidase, and the activity of **1** vs. **2** against L1210 cells.

Table I. Michaelis Constants for Hydrolysis of 1 and 2 Catalyzed by Bovine Liver β-Glucuronidase[a]

no.	pH	V_{max}[b]	V_{max}/K_m[c]
1	3.38	3.73×10^{-8}	6.59×10^{-4}
1	4.40	3.95×10^{-8}	4.09×10^{-4}
1	4.70	3.92×10^{-8}	2.97×10^{-4}
1	5.58	2.18×10^{-8}	9.51×10^{-5}
2	3.38	7.85×10^{-11}	8.80×10^{-9}
2	4.40	1.24×10^{-10}	1.06×10^{-8}
2	4.70	9.85×10^{-11}	1.06×10^{-8}
2	5.58	8.80×10^{-11}	1.04×10^{-8}

[a] Solvent = 0.1 M potassium acetate buffer; μ = 0.1 M (KCl); t = 30 °C. [b] Millimoles per minute per Fishman unit of enzyme activity. [c] Milliliters per minute per Fishman unit of enzyme activity. Here, 1 Fishman unit (FU) of enzyme activity is that amount of enzyme necessary to produce 1 μg of phenolphthalein from phenolphthalein glucuronide per hour at pH 5 at 30 °C. Spontaneous hydrolysis of 1 and 2 was negligible as determined by stable absorbance values for 1 and 2 in acetate buffer at the assay pH. For 1 at pH 4.7, $V_{max} = 3.92 \times 10^{-7}$ M min^{-1} when enzyme activity = 10 FU/mL; this corresponds to 0.49 absorbance unit/h. For 2, at pH 4.7, $V_{max} = 2 \times 10^{-6}$ M min^{-1} when enzyme activity = 20 278 FU/mL; this corresponds to 2.5 absorbance units/h.



1, R = CO_2NH_4
2, R = $CONH_2$

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