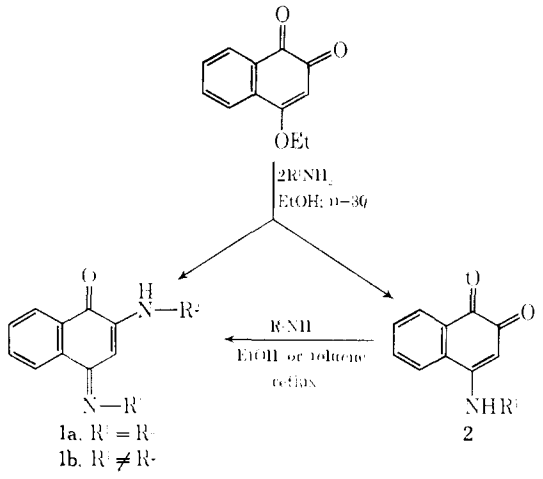
tailed several facile syntheses. We now wish to report the preparation and results of an evaluation of the series in Table I.

The synthetic routes used here are outlined in Scheme I. For the compounds **1a** the route from 4-ethoxy-1,2-naphthoquinone[6] was employed, which gives in addition the 4-alkylamino-1,2-naphthoquinone **2**.[7] For synthesis of **1b** the starting point was the appropriate 4-alkyl- or arylamino-1,2-naphthoquinone **2**. With this latter reaction limitations are imposed by the thermal instability of some compounds.

SCHEME I



The new compounds in Table I have been evaluated against *Plasmodium berghei* infected mice, *P. gallinaceum* infected chicks and against the sexual phase of *P. gallinaceum* in mosquitoes (*Aedes aegypti*).[8] None of the compounds evaluated showed any activity in chicks or mosquitoes, but unmistakable activity was found in mice. Compound **15** increased the mean survival time of infected mice 5.4 days at 640 mg/kg, 2.7 days at 160 mg/kg, and 1.7 days at 40 mg/kg with no toxicity; **22** increased the mean survival time an average of 3.2 days at 640 mg/kg, also with no toxicity. Quinones bearing hydrophilic groups (**13**, **28**, **29**) showed toxicity. The other quinone imines were essentially inactive and no clear structure–activity relationships emerged within this series except for the possibly coincidental fact that both **15** and **22** contain a cycloalkylalkyl chain reminiscent of that found in the side chain of the most active 2-hydroxy-1,4-naphthoquinones.[4]

Experimental Section

All melting points were obtained with a Fisher–Johns apparatus and are uncorrected. Ir (KBr pellets), uv, and nmr spectra were as expected for assigned structures. Nmr spectra were also discussed in our previous report.[5] All compounds were analyzed for C, H, N and were within ±0.4% of the theoretical values unless otherwise indicated. Analyses were carried out by Galbraith Laboratories, Knoxville, Tenn., or Dr. S. N. Nagy, Massachusetts Institute of Technology, Cambridge, Mass.

2-Amino-1,4-naphthoquinone Imines. The series **1a** was prepared by method A described in detail in the Experimental Section of our recent paper.[5] The series **1b** was prepared by method E of the same paper. All the compounds of Table I were recrystallized from EtOH except for quinones **26** and **27** where petroleum ether (60–90°) was used. The amines used here were commercial materials or prepared as described previously.[5]

Substituted *N*-Phenylanthranilic Acid Hydrazides as Potential Antimalarial and Antimicrobial Agents[1]

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In view of the current interest in antifolics as antimalarials[2] and because of the role of *p*-aminobenzoic acid (PAB) in folic acid synthesis,[3] we have prepared several of the title compounds, congeners of the *ortho* isomer, anthranilic acid, of PAB, for antimalarial evaluation. Since anthranilic acid is reported to be a metabolite of tryptophan for certain microorganisms,[4] (*e.g.*, *Escherichia coli*[4]) and in view of the numerous reports of antimicrobial activity of anthranilic acid derivatives[5] the title compounds were evaluated for microbial inhibition. The substituted anthranilic acids required as starting materials for these compounds were available from a previous investigation of substituted dibenz[*b*,*f*]azepines as potential antimalarials.[6] The anthranilic acids were esterified and subsequently treated with hydrazine to give the desired hydrazides as described in the Experimental Section.

The compounds listed in Table I were screened for antimalarial activity against *Plasmodium berghei* in mice by the method of Rane, *et al.*,[7] by the Walter Reed Army Institute of Research. The compounds were also tested against *P. gallinaceum* in mosquitoes.[8] Only slight activity is observed in the mouse screen. The longest increase in survival time noted, 1.3 days at a dose of 320 mg/kg, occurred when the animals were treated with **4**. Supression of oocysts as well as some toxicity was observed for most compounds in the mosquito screen. We are indebted to Drs. D. P.

(1) We acknowledge the U. S. Army Medical Research and Development Command under Contract No. DADA17-68-C-8035 for partial support of this work. This is Contribution No. 740 from the Army Research Program on Malaria.

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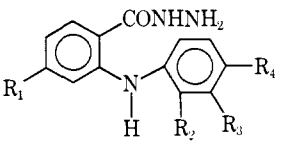
(7) Antiprotozoal Quinones. II. F. J. Bullock, J. F. Tweedie, D. D. McRitchie, and M. A. Tucker, *J. Med. Chem.*, **13**, 97 (1970).

(8) Evaluation was carried out by the methods detailed in our previous paper, Antiprotozoal Quinones. III. F. J. Bullock, and J. F. Tweedie, *J. Med. Chem.*, **13**, 261 (1970).

TABLE I



Compound[a]	R_1	R_2	R_3	R_4	% yield	Mp, °C	Formula[b]
1	H	H	H	H	25	119–120[c]	$C_{13}H_{13}N_3O$
2	Cl	H	H	H	50[d]	160–162	$C_{13}H_{12}ClN_3O$
3	Cl	H	Cl	H	35[e]	140–141	$C_{13}H_{11}Cl_2N_3O$
4	Cl	H	H	F	40	187–188	$C_{13}H_{11}ClFN_3O$
5	H	H	H	Br	60	168–169	$C_{13}H_{12}BrN_3O$
6	H	H	H	OCH_3	50	129–130	$C_{14}H_{15}N_3O_2$
7	H	H	H	Cl	40	161–162	$C_{13}H_{12}ClN_3O$
8	Cl	H	H	Cl	30[e]	185–186.5	$C_{13}H_{11}Cl_2N_3O$
9	H	H	H	F	45	135–137	$C_{13}H_{12}FN_3O$
10	H	Cl	H	Cl	35	191.5–193.5	$C_{13}H_{11}Cl_2N_3O$
11	Cl	Cl	H	Cl	24	229–230	$C_{13}H_{10}Cl_3N_3O$

[a] Unless otherwise noted the recrystallization solvent is EtOH. [b] All compounds analyzed satisfactorily for C, H, N. [c] Lit. mp 121°, see A. Albert, "The Acridines," 2nd ed, St. Martin's Press, New York, N. Y., 1966, p 90. [d] Recrystallization from MeCN. [e] Recrystallization from MeOH.

Jacobus, T. R. Sweeney, and E. A. Steck of the Walter Reed Army Institute of Research for these results.

The title compounds were tested against six microorganisms which included *Bacillus subtilis, Staphylococcus aureus, E. coli, Serratia marcescens, Neisseria catarrhalis,* and the yeast-like fungus *Candida albicans.* The *in vitro* tests employed were the paper disk–agar diffusion method[9] and the method of turbidity measurement of growth in a nutrient broth.[10] The details of these methods are described in the Experimental Section. As can be seen from Table II, these compounds inhibit the growth of the representative Gram-negative coccus, Gram-positive rod and coccus, and the one fungus tested. They are ineffective, at all concentrations tested, against the Gram-negative rods, *E. coli* and *S. marcescens.* It is interesting that none of the anthranilic acid hydrazides tested inhibit the growth of *E. coli.* A similar observation was made when anthranilate esters were tested against this microorganism.[5] In contrast, simple substituted anthranilic acids are potent inhibitors of the growth of *E. coli.*[11]

TABLE II

ANTIMICROBIAL RESULTS[a]

	Disk method[b] (10 μg)				Dilution method[c] (% Inhibition at 48 μg/ml)		
Compound[d]	*B. subtilis*	*S. aureus*	*C. albicans*	*N. catarrhalis*[e]	*B. subtilis*	*S. aureus*	*C. albicans*
1	+	+	−	+	0	0	P
2	+	+	+	+	0	60	30
3	+	+	−[f]	+	60	90[g]	30
4	+	+	−[f]	+	P	70[h]	P
5	+	+	+	+	P	P	P
6	−[f]	−[f]	−[f]	+	P	P	P
7	+	+	+	+	0	40	20
8	+	+	−[f]	+	90[i]	90[i]	60[i]
10	−[f]	−[f]	−	+	50	50	10
11	+	+	−	+	90[i]	90[k]	10

[a] *E. coli* and *S. marcescens* showed no inhibition at any concentration by either method. [b] Results shown are for 10 μg/disk unless otherwise noted. [c] Per cent inhibition recorded for concentration of 48 μg/ml unless otherwise noted. P indicates test compound precipitated out of broth solution at concentration of 48 μg/ml and no inhibition shown at concentration of 24 μg/ml for these compounds. No inhibition was observed at 2.4 μg/ml for any compound. *N. catarrhalis* was not tested by this method. [d] Compound 9 was not tested. [e] *N. catarrhalis* results are for 1 μg/disk. [f] Inhibition positive for 100 μg/disk. [g] 60% inhibition at concentration of 24 μg/ml. [h] 40% inhibition at concentration of 24 μg/ml. [i] 80% inhibition at concentration of 24 μg/ml. [j] 20% inhibition at concentration of 24 μg/ml. [k] 90% inhibition at concentration of 24 μg/ml.

Experimental Section

All melting points were obtained on a Thomas–Hoover Unimelt and are uncorrected. Satisfactory ir spectra were recorded for all compounds using a Perkin-Elmer Model 337 spectrophotometer. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn. Analytical results for the elements indicated were within ±0.4% of the theoretical values.

***N*-Phenylanthranilic Acid Hydrazides.**—The anthranilic acid precursors were prepared by the Ullman condensation of *o*-chlorobenzoic acids with substituted anilines according to Albert[12a] and the carboxylic acids were esterified directly.[12b] In a typical reaction, 80 g of 4'-chlorodiphenylamine-2-carboxylic acid was dissolved in 400 ml of EtOH saturated with HCl. The mixture was refluxed for 8 hr, poured into 2 l. of H_2O, neutralized with the Na_2CO_3, filtered, and dried. The crude ester (75 g), 80 ml of hydrazine hydrate, and 20 ml of EtOH were refluxed 16 hr. The reaction mixture was cooled, filtered, and washed with hexane. The crude carboxyhydrazide was recrystallized from EtOH: mp 161–162°, yield 34.7 g (50%).

Antimicrobial Tests.[13]—The agar plate diffusion method employed 6-mm sterile paper disks impregnated with the test compounds. Three different tests were performed by depositing 100, 10, and 1 μg of compound, respectively, on disks and placing the disks on Difco Tryptic Soy Agar plates which had been previously inoculated with the test organism. Each plate was incubated at the optimum growth temperature for the individual microorganism. Prior to the final inspection, checks were made after 8–12 hr incubation periods to ascertain that no regrowth had occurred in an inhibition area. A ring of inhibited growth around the disk after a 24-hr incubation was considered to indicate antimicrobial activity provided the control disk showed no inhibition.

For the tube-dilution assays, a solution of test compound in an inert solvent, propylene glycol, was sterilized and then injected

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(13) We thank the Department of Biology, Georgia State University, Atlanta, Ga., for use of their facilities to perform these tests.

into a series of 4-ml portions of sterile BBL Trypticase Soy Broth to give concentrations of 200, 48, 24, and 2.4 μg/ml. All of the compounds at concentrations of 200 μg/ml and some at 48 μg/ml precipitated out of the water-based broth solution, and the tests at these concentrations were not performed. The test solutions were inoculated with the microorganisms and incubated, with shaking, for 24 hr. Turbidity readings of the cultures were taken on a Bausch and Lomb Spectronic 20 spectrophotometer at 660 mμ. Per cent inhibition was determined by using a control consisting of broth, solvent, and microorganism, to give a turbidity reading of 100% growth, or 0% inhibition. The percentage inhibition shown is the average from 2 to 4 separate determinations. In view of the limitations of the turbidity method the per cent inhibition shown in Table II has been rounded off to the nearest 10%.

1-Adamantanecarboxylic Acid Amide of 4-Aminoantipyrine

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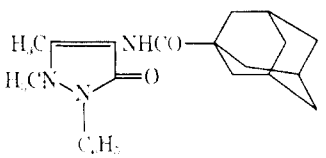
We have prepared 2,3-dimethyl-1-phenyl-4-(1-adamantanecarboxamido)-5-pyrazolone



in order to consider the effect of the highly symmetrical cagelike adamantane molecule and its hydrophobic binding in the form of adamantanecarboxamide of 4-aminoantipyrine, and to test for analgetic and antipyretic activity of this new compound.

Experimental Section

Chemistry.—A mixture of 9.9 g of 1-adamantanecarboxylic acid chloride (0.05 mol) (Aldric Chemical Co., Inc.); 10.5 g of 4-aminoantipyrine (0.05 mol) and 100 ml of dioxane, was kept at room temperature for 3 hr. The reaction mixture was diluted with 200 ml of cold H_2O and the crystalline reaction product was filtered off. It was washed with 5% $NaHCO_3$, and recrystallized from dioxane. The yield was 16.2 g (87%) of white crystals, mp 206–207° (uncorr). *Anal.* ($C_{22}H_{27}N_3O_3$) C, H, N.

Spectroscopic Results.—Ir spectra were recorded on a Perkin-Elmer Model 221 ir spectrophotometer (Nujol) and were as expected: N–H stretching bands near 3320 cm^{-1} and 3420 cm^{-1} (4-aminoantipyrine, starting material) were absent from the spectrum, and a C=O stretching band in the 1650 cm^{-1} region due to the NH–CO group was present. Uv spectrum were characterized by the maximum at 284 mμ (14 mcg/ml) in dioxane using a Beckman spectrophotometer Model D. U. $E_{1cm}^{1\%}$ calculated was 290.

Pharmacology.—The compound was administered in physiological solution containing 6% Tween 80 and its analgetic and antipyretic actions were tested in Swiss mice and rabbits.

Analgetic Activity. Hot Plate Test.[1]—Antipyretic activity was measured by the method of Baker and Coll.[2] Hyperthermia was produced in rabbits by injection of 0.3 ml/kg of TAB-D-ISM vaccine in the marginal vein of the ear.

Mice which received doses of 20 mg/kg i.p. and p.o. of 2,3-dimethyl-1-phenyl-4-(adamantanecarboxamido)-5-pyrazolone showed 30% more heat resistance than mice which received the equivalent dose of 4-aminoantipyrine.

Doses of 19 mg/kg i.p. in rabbits were shown to cause the same antipyretic activity as that produced by an equivalent dose of 4-aminoantipyrine.

In mice the LD_{50} i.p. was 820 mg/kg; *vs.* 280 mg/kg for 4-aminoantipyrine.

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2-(Substituted Amino)quinolizinium Bromides. A New Class of Anthelmintic Agents

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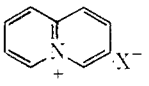
Many different types of compounds have been shown to have varying degrees of anthelmintic activity. Several of these classes, according to Harfenist, have a group bearing a positive charge and a cyclic moiety, which may or may not be separated. Examples of these include naphthamidines, dihydropyridines, pyrrocolines, and stilbazoles.[1] Two additions to veterinary helminthic therapy, tetramisole and pyrantel, contain these two structural features.[2,3]

Emetine dihydrochloride is another example of a compound which possesses these features. This alkaloid, used in the treatment of several helminthic disorders,[4] contains the positively charged quinolizine ring. The investigation reported here was undertaken to determine whether a related but less complicated structure, the completely aromatic quinolizinium ring system (**1**), might retain some of the antiparasitic activity of this alkaloid.



1

In our work a series of 2-(substituted amino)quinolizinium salts was synthesized for screening for prevention of lung invasion by the larvae of *Ascaris suum* in mice.

Chemistry.—The preparation of the 2-(substituted amino)quinolizinium compounds, shown in Table I, proceeded through either of the two intermediates, 2-bromoquinolizinium bromide (**2**) or 2-bromo-6-methylquinolizinium bromide (**3**). These were readily pre-

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