butyrylcholinesterase from horse serum with a specific activity of 2.5 mmoles of ACh hydrolyzed/ml per min were used. The acid produced during hydrolysis of the esters was titrated with standardized 0.001 *N* NaOH at 37.5° under N_2 with a Radiometer titrator, type TTTld, titrigraph type SBR2c, and a syringe buret SBU1a. The reaction medium consisted of 0.03 *M* NaCl and 0.02 *M* $MgCl_2$. The ester substrate (0.8 ml) was placed in the reaction vessel and the pH and vol were adjusted to 7.4 and 0.9 ml, respectively, with NaOH. Spontaneous hydrolysis was measured for 5 min, whereupon 0.1 ml of the enzyme soln was added. The final concns of the enzyme were 4.64×10^{-6} g/l. and 2.5×10^{-3} g/l. for AChE and butyrylcholinesterase, respectively. After measuring enzymatic hydrolysis for 5 min, ACh (0.1 ml) was added and the inhibition of ACh hydrolysis was measured. The final concns of ACh were 10^{-3} *M* for AChE and 10^{-2} for butyrylcholinesterase. Appropriate ACh control rates were measured separately.

Phenothiazines as Local Anesthetics

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The possibility that *N*-(acylamino)phenothiazines and iminodibenzyls reported herein, might possess local anesthetic properties similar to those of corresponding phenothiazines[1] led us to undertake their synthesis and pharmacological evaluation as local anesthetics. These compounds, referred to in Chart I, were prepared using the procedure[2] reported for the synthesis of related phenothiazines. Thus, appropriately substituted phenothiazines or iminodibenzyls when treated with haloacylchlorides afforded the corresponding *N*-haloacyl derivative. The latter when condensed with *t*-$BuNH_2$ or *N*-β-hydroxyethylpiperazine gave the respective 10-*N*-aminoacylphenothiazine or *N*-aminoacyliminodi-

TABLE I

RELATIVE CORNEAL ANESTHETIC ACTIVITY OF THE COMPOUNDS[a]

Compd	Minimum effective concn (%)[b]	Median effective concn (%)[c] (ME_{50})	Relative potency (intensity)
A	0.2	0.12	0.46
B	0.2	0.11	0.50
C	0.1	0.06	0.92
D	0.1	0.05	1.10
E	0.1	0.10	0.55
F	0.1	0.09	0.61
G	0.5	0.355	0.155
H	0.5	0.50	0.11
I	0.2	0.16	0.34
J	0.2	0.18	0.31
K	0.5	0.50	0.11
Lidocaine[e]	0.075	0.055	1.00

[a] Three guinea pigs of either sex were used for each concn of test compd. [b] Different concn (%) were used and thereby the minimal effective concn (%) that produced complete loss of blink reflex in all 3 animals was detd. Opposite eye of each animal served as control. The onset of anesthesia for such concn of each compd (A–K) was 0.7, 0.8, 1.3, 3.5, 4.2, 4.2, 4, 4.3, 3.2, 3.5, 4.5 and 3.5 min, while duration of activity lasted for 39, 42, 10, 33, 15, 12, 10, 15, 17, 15, 10, and 7 min, respectively. [c] Average per cent loss of corneal reflex was noted for different concn of each drug. On plotting the log concn against its per cent response, a median effective concn (ME_{50}) which caused 50% loss of blink reflex was calcd. [d] Obtd by dividing ME_{50} of lidocaine by ME_{50} of test compound. [e] Standard drug for comparison.

TABLE II

RELATIVE RATING (PROCAINE) INDICES FOR INFILTRATION ANESTHESIA OF THE COMPOUNDS[a]

Compd	Relative potency[b] Intensity	Relative potency[b] Duration	Relative toxicity[c]	Relative rating[b] Intensity	Relative rating[b] Duration
A	10	10	0.56	17.92	17.92
B	10	10	0.66	15.60	15.60
C	5	2	0.56	8.96	3.58
D	10	2	0.498	20.10	4.10
E	5	2.5	0.56	8.96	4.48
F	10	10	0.498	20.10	20.10
G	2	2	0.33	6.02	6.02
H	2	1	0.29	6.70	3.35
I	2	2	0.29	6.82	6.82
J	5	2	0.33	15.01	6.03
K	1	1	0.29	3.41	
Procaine[d]	1	1	1.00	1.00	1.00

[a] Guinea pigs of either sex were used. Six tests were performed. for each concn. [b] The relative potency (regarding intensity) is a ratio of the concns, behaving alike, of the standard drug for comparison and of the test compd. Similarly relative potency (regarding duration) is a ratio of the concn of the standard drug and that of the test compd, producing anesthesia for nearly the same duration. Hence the relative rating (regarding intensity) would be obtd by dividing relative potency (regarding intensity) by relative toxicity; while relative rating (regarding duration) would be a ratio of relative potency (regarding duration) and the relative toxicity exhibited by the test compound. For details refer to Hamilton, *et al.*[3b] [c] Refer to Table IV for relative toxicity. [d] Standard drug for comparison.

TABLE III

RELATIVE PLEXUS ANESTHETIC ACTIVITY OF THE COMPOUNDS

Compd	Time (min) for a 0.5% soln of the drug to cause anesthesia[a]	Relative potency[b]
A	6.16	0.81
B	8.00	0.625
C	5.00	1.00
D	3.60	1.40
E	6.33	0.79
F	6.00	0.835
G	10.00	0.50
H	11.30	0.44
I	5.66	0.88
J	11.60	0.43
K	11.00	0.45
Cocaine[c]	5.00	1.00

[a] For each concn of every compd 3 frogs were used. Criteria for anesthesia was the abolishment of the stimulatory reaction due to 0.2 *N* HCl. [b] Refer to footnote *a* of Table II. [c] Standard drug for comparison.

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(1) (a) R. Dahlbom and T. Ekstrand, *Acta Chem. Scand.*, **5**, 102 (1951); (b) K. Hirose and Y. Ogawa, *Shionogi Kenkyusho Nempo*, **7**, 517 (1957); *Chem. Abstr.*, **52**, 9419 (1958); (c) Japanese patent 7222 (1959); *Chem. Abstr.*, **54**, 16470 (1960).

(2) (a) British patent, 622,903 (1951); *Chem. Abstr.*, **46**, 11250 (1952). (b) British patent, 740,932 (1955); *Chem. Abstr.*, **51**, 500 (1957). (c) Belgian patent, 586,033 (1960); *Chem. Abstr.*, **54**, 18561d (1960).

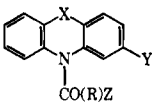
TABLE IV
ACUTE INTRAVENOUS LD_{50} IN ALBINO MICE

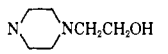
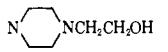
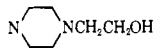
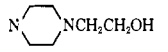
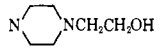
Compd	LD_{50} mg/kg[a]	Relative toxicity
A	89	0.56
B	75	0.66
C	89	0.56
D	100	0.498
E	89	0.56
F	100	0.498
G	150	0.33
H	166.3	0.29
I	169.8	0.29
J	150.0	0.33
K	169.8	0.29
Procaine[b]	49.8	1.00

[a] Experiments were conducted at room temp (28 ± 1°). Besides following Karber's method,[6] the log of each dose was plotted against its percentage mortality and from this graph a dose causing 50% mortality was calcd. Results reported here are the average values obtd by these two methods. [b] Standard drug for comparison.

benzyl (A–K) which were isolated as hydrochlorides. The ability of these compounds to induce surface,[3]

CHART I



	X	Y	R	Z
A	S	H	CH_2CH_2	$NHC(CH_3)_3$
B	S	Cl	CH_2CH_2	$NHC(CH_3)_3$
C	S	H	CH_2	$NHC(CH_3)_3$
D	S	Cl	CH_2	$NHC(CH_3)_3$
E	S	Cl	$CH(CH_3)$	$NHC(CH_3)_3$
F	CH_2CH_2	H	CH_2	$NHC(CH_3)_3$
G	S	H	CH_2CH_2	
H	S	Cl	CH_2CH_2	
I	S	H	CH_2	
J	S	Cl	CH_2	
K	CH_2CH_2	H	CH_2	

A–F as hydrochlorides; G–K as dihydrochlorides.

TABLE V
N-(AMINOACYL)PHENOTHIAZINES OR IMINODIBENZYLS

Product[a]	Recrystn solvent[b]	Mp, °C[c]	Yield	Formula[d]	Uv spectral data[g] in H_2O or EtOH[e] λ_{max}, mμ	log ε	in H_2O[f] log ε
A	EH	198	65	$C_{19}H_{23}ClN_2OS$	255	3.982	3.876
					310	3.212	3.090
B	EH	192	68	$C_{19}H_{22}Cl_2N_2OS$	255	3.921	3.800
					310	3.036	2.890
C	EH	215 dec	70	$C_{18}H_{21}ClN_2OS$	258	3.965	3.800
					310	3.019	2.750
D	EH	230 dec	65	$C_{18}H_{20}Cl_2N_2OS$	260	4.006	3.880
					312	2.980	2.880
E	EE	183–190	70	$C_{19}H_{22}Cl_2N_2OS$	260	4.005	3.860
					313	3.075	2.895
F	EE	229 dec	70	$C_{20}H_{25}ClN_2O$	258	3.537	3.390
					310	3.230	3.080
G	EH	198–200	65	$C_{21}H_{27}Cl_2N_3O_2S$	260	4.835	4.728
					312	3.357	3.200
H	EH	204–205	72	$C_{21}H_{26}Cl_3N_3O_2S$	260	3.945	3.860
					310	3.088	2.980
I	EH	218–219	62	$C_{20}H_{25}Cl_2N_3O_2S$	260	3.875	3.680
					312	2.946	2.780
J	EH	230	62	$C_{20}H_{24}Cl_3N_3O_2S$	261	3.940	3.780
					312	3.076	2.900
K	EH	250–251 dec	72	$C_{22}H_{29}Cl_2N_3O_2$	260	3.859	3.675
					312	3.039	2.840

[a] All compds had ir and uv spectra compatible with their assigned structure. [b] EH, EtOH–hexane (1:1); EE, EtOH–Et_2O. [c] Melting points are uncorr and were taken in open capillary tubes. [d] Anal: C, H, N. [e] Initially within 1 hr. [f] After 8 hr. [g] Of hydrochlorides.

infiltration,[4] and conduction anesthesia[4] was evaluated and compared with lidocaine, procaine, and cocaine, respectively. Their local tissue irritancy[5] as well as acute systemic toxicity[6] were also determined. From these results their relative toxicities and relative potencies as well as rating (regarding intensity and duration) have been found (Tables I–IV).

The following correlations between local anesthetic activity and chemical structure were drawn.

(i) The compounds having a *tert*-butylamino group (A–F) were in general more potent than those possessing a β-hydroxyethylpiperazino moiety (G–K). These compounds, therefore, might possess the balance which,

(3) The experiments were designed to yield the following indices of activity: (i) minimal anesthetic concentration, onset of anesthesia, and duration of activity with various concentrations;[a,b] (ii) median effective concentration.[c] (a) A. D. Hirschfelder, *Physiol. Rev.*, **12**, 190 (1932); (b) H. S. Hamilton, B. A. Westfall, and J. R. W. Fergusson, *J. Pharmacol. Exp. Ther.*, **94**, 299 (1948); (c) M. R. A. Chance and H. Lobstein, *ibid.*, **82**, 203 (1944).

(4) E. Bülbring and I. Wajda, *ibid.*, **85**, 78 (1945).

(5) Three methods were employed *viz.*, mucous membrane irritation by the rabbit eye test; intradermal irritation by the trypan blue test in the rabbit,[a] and the subcutaneous irritation by the rabbit ear test.[b] (a) J. O. Hoppe, E. G. Alexander, and L. C. Miller, *J. Amer. Pharm. Ass.*, **39**, 147 (1950); (b) S. Wiedling, *Acta. Pharmacol. Toxicol.*, **45**, 351 (1948).

(6) G. Karber, *Arch. Exp. Pathol. Pharmacol.*, **162**, 480 (1931); quoted by R. A. Turner, "Screening Methods in Pharmacology," Academic Press, London, 1965, p 63.

as Quevauviller[7] has pointed out, must exist between the lipophilic and hydrophilic portion of the molecule for it to exhibit potent local anesthetic activity. Hydrophilicity in the case of G–K, might be dominating and lead to decreased activity. Interestingly, these compounds were more soluble at pH 7.4 than A–F[8] which supports this postulation.

(ii) In the acyl-substituted portion the compds having one CH_2 group were more potent than those having two CH_2 groups (A, B, G, and H); but the duration of activity of the latter class of compounds was definitely greater. Obviously, the rate of hydrolysis of these two classes of compounds might be governing their duration of action.

A marked decrease in the initial activity was observed after keeping the aqueous solutions of these compounds for 8 hr at room temperature. Hence, it became of interest to study their uv absorption pattern in distd H_2O. Initially the uv spectra of all these compounds in EtOH or H_2O exhibited a strong band between 255 and 260 mμ and a wide band around 310 mμ. Although the gross features of their uv spectra in aqueous solution after 8 hr remained unchanged, the band around 255–260 mμ now appeared as only an inflection while the broad band around 310 mμ disappeared. The uv spectral data of their aqueous solution, initially as well as after 8 hr, are presented in Table V. They suggest instability in aq solution and support our postulate that the rate of hydrolysis probably governs the duration of activity. However, the possibility of the sensitivity of these compounds toward enzymatic hydrolysis *in vivo* cannot be overruled. Finally, keeping in mind relative potency and toxicity, most of these compounds might offer a wide spectrum of useful local anesthetic activity.

(7) A. Quevauviller, *Proc. Pharmacol.*, **7**, 533, 585 (1952).

(8) All these compounds (A–F) were also investigated for their CNS depressant activity. Their solubilities at pH 7.4 were also determined spectrophotometrically because Green[a] has shown that CNS depressat activity of phenothiazines is associated with their low solubility at pH 7.4. The details of this work including some interesting account of structure activity relationship in phenothiazine series has been communicated.[b] (a) A. L. Green, *J. Pharm. Pharmacol.*, **19**, 10 (1967); (b) H. L. Sharma, S. P. Banerjee, V. N. Sharma, and R. L. Mital, *Ann. Soc. Sci. Bruxelles, Ser. 2*, **84**, 55 (1970).

Preparation and Antimalarial Activity of Compounds Related to Dypnone Guanylhydrazone[1]

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Early in the current Walter Reed *Plasmodium berghei* screening program, dypnone guanylhydrazone (**1**) was demonstrated to have interesting activity.[2,3] We wish to report the preparation and testing of a series of compounds designed to probe several of the structural parameters of this lead.

An early variant, the *p,p'*-dichloro compound **7**, showed a striking increase in activity and a decrease in toxicity (Table I). That the chlorodypnone residue alone was not responsible for the activity was shown by the results with the parent ketone and some simple ketone derivatives (**2–6**).

The fact that a high level of activity remained in the desmethyl (chalcone) analog **9** led us to prepare a small series in which the ketonic portion of the molecule was varied. Vinylogs and tris-*p*-chlorophenyl compounds (**10–13**) showed decreased activity. Table II presents a short series of aldehyde guanylhydrazones. The hydrocarbon nuclei used for this series are associated with quite good antimalarial action when other side chains are incorporated. Only marginal effect was noted for **14–16**, however. The results with these series suggest that peak activity resides in the more compact dypnone–chalcone type guanylhydrazones. It should be pointed out that the activity and toxicity of a large series of highly active, substituted benzophenone guanylhydrazones reported by DoAmaral, *et al.*, were strongly influenced by minor variations in ring substitution patterns.[4,5] The most active benzophenone compounds were variously substituted with Br, Cl, F, I, and CF_3 or OCF_3. The work of DoAmaral and French[5,6] also indicates that alkyl substitution of the aminoguanidine moiety is inimical to activity.

An attempt was made to prolong the activity of **7** by the use of the "repository" pamoate salt **8**. The decreased activity of **8**, however, roughly corresponded to the decreased proportion of active drug in the salt when tested in the standard mouse screen.

The guanylhydrazone derivatives were prepared by the same method used to prepare the parent dypnone derivative **1**.[7] It should be noted that aminoguanidine bicarbonate is the only aminoguanidine salt, of several available, that successfully derivatized these ketones in our hands. Other procedures, for the preparation of benzophenone guanylhydrazones, have since been reported.[4]

The 4,4'-dichloro analog **2** was prepared by $POCl_3$-catalyzed self-condensation of 4-chloroacetophenone.[8] The carbonyl derivatives **3–6** were prepared by standard procedures.

The base-catalyzed condensation of 4-chlorobenzaldehyde and 4-chloroacetophenone by a published procedure[9] gave 4,4'-dichlorochalcone,[10] the precursor to **9**.

The tris(*p*-chlorophenyl) ketone **11** was obtained from the reaction of the ylid derived from *p*-chlorophenacyl

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(2) This information was provided through the courtesy of Dr. R. E. Strube of the Walter Reed Army Institute of Research.

(3) Biological investigation performed by Dr. Leo Rane of the University of Miami, Miami, Fla. by a published procedure: T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431 (1967).

(4) J. R. DoAmaral, E. J. Blanz, Jr., and F. A. French, *ibid.*, **12**, 21 (1969).

(5) J. R. DoAmaral, D. A. French, E. J. Blanz, Jr., and F. A. French, 158th National Meeting of the American Chemical Society, New York, N.Y., Sept 1969, MEDI 57.

(6) Personal communication from Dr. F. A. French.

(7) We wish to thank Dr. R. E. Strube of the Walter Reed Army Institute of Research for this procedure.

(8) E. Ziegler and H. Schredt, *Monatsh. Chem.*, **85**, 1191 (1954); *Chem. Abstr.*, **50**, 321 (1956).

(9) E. P. Kohler and H. M. Chadwell, Organic Syntheses, Collect. Vol. I, 2nd ed, Wiley, New York, N. Y., 1946, p 78.

(10) H. O. House, *J. Amer. Chem. Soc.*, **78**, 2298 (1956).