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Comparison of Pharmacologic Activity in a Series of Benzimidazole Compounds. (29406)

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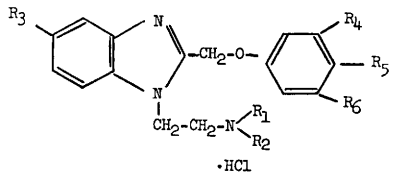
Hunger and associates(1) reported on the synthesis of a series of 1-(2-dialkylaminoethyl)-2-benzyl-5-nitro-benzimidazoles. These compounds were subsequently described as a new class of potent analgetics(2). Following these reports, several others appeared describing the synthesis and analgetic activity of a number of analogs in which various substitutions on the 2-benzyl benzimidazole nucleus were made. The structure-activity relationships of these compounds were reviewed by Beckett and Casy(3). The purpose of this study was to explore the structure-activity relationships of some 1-(2-dialkylaminoethyl)-2-phenoxymethyl benzimidazoles produced by varying substitutions on the benzimidazole and phenyl rings. In addition, the effects of alteration of the alkyl groups on the nitrogen of the ethyl amino side chain of certain derivatives were compared.

Methods. Analgetic activity as well as acute toxicity was determined in male, Swiss-Webster mice using a modification of the hot plate method of Woolfe and MacDonald(4). The heat source was a 250 watt infrared lamp (General Electric) to which 18 volts were applied from a variable transformer. One mouse at a time was placed on the surface of the bulb and the time recorded until the animal jumped off. The sum of 4 consecutive trials was recorded as the reaction time. Reaction times were recorded 3 times for each mouse approximately 20 minutes apart before a drug was administered. Any animal showing a reaction time greater than 6 seconds on the third trial was discarded. The reaction times of the animals were then determined at 30, 60 and 90 minutes following subcutaneous administration of drugs. A 60 second cut off time was utilized for mice which did not jump off the bulb. Since no significant difference could be demonstrated between reaction times determined 30 and 60 minutes following administration of any of the drugs tested, it was decided to use a function of the 30 minute post drug reaction time as the response metameter. Since animals which displayed reaction times greater than 6 seconds on the third trial before drug administration were discarded, the number of animals in dose groups were frequently unequal, ranging from 6 to 10 animals per group. For this reason the unsymmetrical bioassay described by Finney (5) was used to compare statistically the analgetic activity of the analogs to a dose-response regression derived from responses to 2, 4 and 8 mg/kg of morphine sulfate. This statistical method provides for the assessment of potency relative to a standard. A value of 100 has been assigned to the potency of morphine sulfate in this bioassay, hence the relative potency (R) calculated for each analog is in terms of % of morphine sulfate activity.

Acute toxicity, as reflected by lethality within 24 hours following injection of drugs was conducted in Swiss-Webster mice. Intraperitoneal LD-50 values were obtained by determining the effect of 3 dose levels yielding between 10 and 90% effect. At least 10 mice were utilized in determining each point. The computations for fitting the probit-log dose regression line determining the LD-50 and 95% confidence intervals were done ac-

TABLE I. Relative Analgetic Potency and Toxicity of the Analogs Studied.



Compound No.	R_1	R_2	R_3	R_4	R_5	R_6	LD-50, mg/kg (95% C.I.)	R* (95% C.I.)
1	C_2H_5	C_2H_5	H	CH_3	H	CH_3	148 (125–175)	1.9 (1.7–2.0)
2	C_2H_5	C_2H_5	H	H	H	H	149 (106–209)	3.4 (3.3–3.5)
3	C_2H_5	C_2H_5	H	H	CH_3O	H	127 (116–140)	24.2 (23.5–24.8)
4	C_2H_5	C_2H_5	CH_3	H	C_2H_5O	H	107 (82–139)	97.5 (81.3–116.9)
5	C_2H_5	C_2H_5	H	H	C_2H_5O	H	56 (45–72)	52.2 (51.3–53.2)
6	CH_3	CH_3	H	H	C_2H_5O	H	106 (96–117)	5.4 (5.3–5.6)
7	C_2H_5	C_2H_5	NO_2	H	C_2H_5O	H	27 (18–40)	515.2 (490.9–540.8)

* Assigning a standard value of 100 to morphine sulfate, the relative potencies of the analogs are expressed as % of the standard.

cording to the method of Litchfield and Wilcoxon(6).

Results and discussion. In preparing various plots of the analgetic data it was found that utilizing the reciprocal of the 30 minute post drug reaction time and the $\log_{10}$ dose, the curves appeared to assume the best degree of linearity and parallelism. For this reason, these metameters were used in calculation of relative analgetic potencies.

The analogs studied are presented in Table I. LD-50 values, analgetic potency in relation to morphine sulfate and their 95% confidence intervals are also stated. The least potent compound of the series is compound 1. When its structure is compared to that of compound 2, it would appear that replacement of the methyl groups with hydrogen at R_4 and R_6 enhances analgetic potency without significantly affecting toxicity. In comparing compound 2 with compounds 3 and 4, it appears that alkoxylation at R_5 favors analgetic potency. An increase in toxicity accompanies the increase in analgetic potency produced by these substitutions. Methylation of the benzimidazole ring at position R_3 approximately doubles the analgetic potency while reducing the toxicity to about half (compounds 4 and 5). On the other hand, substitution at R_3 with a nitro group (compound 7) increases toxicity by 2-fold while increasing analgetic potency to over 5 times that of morphine. One of the effects of nitration at R_3 would be to decrease the electron density about the nitrogens in the benzimidazole ring. This may alter lipid solubility and possibly favor higher levels of the compound in the central nervous system. The benzyl analog of this compound was found by Eddy(7) to be almost 1500 times as potent as morphine. Replacement of the ethyl groups by methyl groups at R_1 and R_2 cause a sharp decrease in analgetic potency as can be seen with compound 6. A decrease in toxicity appears to accompany this substitution. A decrease in lipid solubility might also be expected to accompany this change.

In the determination of acute toxicity in mice, it was observed that compounds 4, 5 and 7 produced the "Straub Tail Effect." These analogs are also the 3 most potent compounds of the series.

Summary. A series of seven 1-(2-dialkylamino-ethyl)-2-phenoxymethyl benzimidazoles featuring alterations in the nucleus and side chain have been studied. Analgetic ac-

tivity in a modified hot plate method and acute toxicity were determined in mice. The data obtained from the analgetic test were statistically analyzed and the relative potency of the analogs in relation to morphine sulfate was determined. The structure-activity relationships of the analogs with respect to relative analgetic potency and acute toxicity have been discussed.

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Electrophoretic and Immunolelectrophoretic Studies of the Serum of Germfree and Conventional Guinea Pigs.* (29407)

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When germfree guinea pigs were kept on a diet of essentially vegetable origin, one year old animals displayed low serum beta globulin levels and gamma globulins were undetectable(1). Using a modification of the same diet Newton and DeWitt showed that, although the caesarian born germfree guinea pig starts life with a gamma globulin level of approximately 7% of total serum protein, at the age of 3 months this level has already declined to values of 2 to 3%(2). Incorporation of bovine milk proteins into the diet led to the appearance of appreciable amounts of gamma globulins and an increase in beta globulin levels of the serum of the mature germfree guinea pig(3).

We present here an analysis of the sera of germfree and conventional guinea pigs of various ages. The results indicate that both microbial and dietary stimulation play an important role in determining the quantitative pattern of the serum globulins.

Materials and methods. Animals. The germfree and conventional guinea pigs used were obtained from the Lobund animal colonies. The first group of 4 germfree and 6 conventional guinea pigs had been fed the L-445 diet(4). Subsequently, a second group of 18 germfree and 13 conventional guinea pigs, of varying ages, were maintained on diet L-462, a formula high in bovine milk protein(5). Animals were bled via cardiac puncture.

Quantitative electrophoresis. Electrophoretic patterns of the sera from the first group were obtained with the Antweiler Micro-free boundary electrophoresis apparatus, using a sodium barbital buffer of pH 8.6 and the ionic strength of 0.12(1). Analysis of sera from the second group was made on cellulose acetate paper with the Shandon Universal Electrophoresis apparatus, and a sodium barbital buffer of pH 8.6 and ionic strength of 0.06. Cellulose strips were stained with Amido Black 10B(6) and analyzed in a light reflecting densitometer (Chromoscan by Joyce Loeb & Co. Ltd.).

Antisera. Three different anti-guinea pig-rabbit immune sera were produced using 3 different lots of conventional guinea pig serum. Each serum lot consisted of the sera from 2 different animals. Rabbits were subjected to the following schedule: 40 mg guinea pig serum protein, 0.25 ml of Freund's

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