doses were administered in solution in a volume of 1.0 mL/kg of body weight. All test compounds, as the free bases, were first dissolved in a small amount of 95% ethanol and were then diluted to the desired concentrations with an isotonic solution of 1% ascorbate in 0.72% sodium chloride solution. All injections were administered intraperitoneally 30 min before the start of discrimination sessions.

Discrimination Training. To avoid positional preference, half of the animals were trained to press LSD-L and SAL-R, while the other half were trained vice versa. Rats were trained on an FR32 schedule with 15-min maintenance sessions. Responding rates were found to be 1450 ± 600 per 15-min session among different animals. No significant difference in responding rate was seen between the training dose of LSD and saline ($p > 0.05$, grouped Student's t-test). The complete training procedure has been published in a previous article.[18]

Stimulus Generalization. Those rats that had successfully acquired 85% correct responding on the appropriate lever during the 6-week training period were included in the stimulus generalization testing procedure. Testing sessions were run on Wednesdays and Saturdays only. Training sessions were held the rest of the week with Sundays off. On test days, the animal was placed in the operant chamber 30 min after injection. Test sessions lasted until the rat emitted 32 responses on either lever or until 5 min had passed, whichever came first. If the rat did not emit 32 responses on either lever within 5 min, he was scored as disrupted and was not included in the calculations. No reinforcement was given during test sessions. In order to receive a test drug, the animals were required to satisfy the 85% correct lever response criterion on each of the two preceding training sessions. Also following the procedure of Colpaert et al.,[19] test data were discarded and the test condition later retested if the test session was followed by failure to meet the 85% criterion in either of the two subsequent training sessions. This procedure was employed to increase the reliability of the individual test data. It has been reported[19] that incorrect lever selections in trained rats typically occur in bursts of one to three sessions. This correction procedure assists in avoiding the contamination of test data that may occur during such bursts. If the animal was not included in the testing procedure on a given day, the session was used for training.

Several preliminary experiments to determine appropriate dosages for new compounds were carried out; these data were discarded. Dosages for each of the test compounds were based on these initial experiments. The drug treatments in this study, including LSD and the vehicle for the ergoline solutions (control), were randomized over the entire experimental period.

Data Analysis. Animals were scored as drug positive if they selected the LSD-appropriate lever (i.e., if they emitted 32 responses on the drug lever before emitting 40 total responses). If generalization occurred (greater than 80% drug appropriate responding), these quantal data were analyzed by the method of Litchfield and Wilcoxon[20] to determine an ED_{50}. Parallelism was tested, and potency ratios were determined from a 3 point × 3 point parallel line bioassay for quantal data.[21]

Acknowledgment. We thank Dr. Federico Arcamone and Farmitalia Carlo Erba for the gift of lysergic acid monohydrate. Major support for this work was provided by PHS Grant DA-02189 from the National Institute on Drug Abuse. The NMR studies were performed at PUBMRL, which is supported by NIH Grant RR01077. Instrumentation for the drug discrimination studies was partially funded by Biomedical Research Support Grant 2-S07-RR05586-15. We also thank Robert Oberlender for excellent technical assistance provided in connection with the discrimination training.

Registry No. 1, 50-37-3; 2, 65527-62-0; 3, 65527-63-1; 4, 96930-86-8; 5, 96930-87-9; 6, 65527-61-9; 7, 96930-88-0; 8, 35779-43-2; 9, 35779-41-0.

(18) Oberlender, R.; Kothari, P.; Nichols, D. E.; Zabik, J. *J. Med. Chem.* **1984**, *27*, 788.
(19) Colpaert, F. C.; Niemegeers, C. J. E.; Janssen, P. A. J. *J. Pharmacol. Exp. Ther.* **1982**, *221*, 206.
(20) Litchfield, J. T., Jr.; Wilcoxon, F. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99.
(21) Finney, D. J. "Probit Analysis", 3rd ed.; Cambridge University Press: London, 1971.

Synthesis and Antiallergic Activities of 1,3-Oxazolo[4,5-*h*]quinolines

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A series of new 1,3-oxazolo[4,5-*h*]quinolines has been prepared. These compounds were tested as inhibitors of antigen-induced release of histamine (AIR) in vitro from rat peritoneal mast cells (RMC) and as inhibitors of IgE-mediated passive cutaneous anaphylaxis in the rat (PCA). After several modifications of the original lead, the most potent compound of the series was determined to be 5-chloro-1,3-oxazolo[4,5-*h*]quinoline-2-carboxylic acid methyl ester (**4a**). It has an IC_{50} of 0.3 μM in the RMC assay and an ED_{50} (intraperitoneal) of 0.1 mg/kg in the PCA test, which is 10 times and 60 times more potent than disodium cromoglycate (DSCG), respectively. Of greater importance, it is orally active (ED_{50} = 0.5 mg/kg) as an inhibitor of the PCA test.

Since the introduction of disodium cromoglycate (DSCG) for the treatment of asthma and allergy disease,[2] a large number of chemical series have been reported as antiallergic agents.[3] As part of a program to develop new antiallergic agents,[4] we synthesized some 1,3-oxazolo[4,5-*h*]quinolines.

Our interest in the 1,3-oxazolo[4,5-*h*]quinoline ring system evolved from a chemical lead discovered in our selective screening program. The known 8-quinolinyl 2-methoxycarbanilate (**1**)[5] was found active as an inhibitor (IC_{50} = 2.0 μM) of anaphylactically induced histamine

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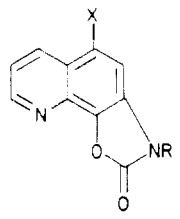
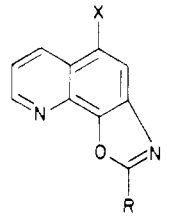
(2) Cox, J. S. G. *Nature (London)* **1967**, *216*, 1328. Cox, J. S. G.; Beach, J. E.; Blair, A. M. J. N.; Clarke, A. J.; King, J.; Lee, T. B.; Loveday, D. E. E.; Moss, G. F.; Orr, T. S. S.; Ritchie, J. T.; Sheard, D. *Adv. Drug Res.* **1970**, *5*, 115. Speight, R. N.; Avery, G. S. *Drugs* **1974**, *7*, 164.
(3) Musser, J. H.; Kreft, A. F.; Lewis, A. J. *Annu. Rep. Med. Chem.* **1984**, *19*, 93 and earlier volumes.

0022-2623/85/1828-1255$01.50/0

Table I. Inhibition of RMC and Rat PCA by Oxazoloquinolines



2



4

			RMC[a]		PCA[b]			
					% inhibn, mg/kg		ED_{50}, mg/kg	
compd[d]	X	R	% inhibn, μM	IC_{50}, μM	ip	po	ip	po
2a	H	$COCH_3$	<30 (50)[e]				10	4 [2][c]
2b	H	H				0 (25)		
2c	Cl	H		18 (50)		14 (125)		
2d	Cl	$COCH_3$				15 (100)		
2e	Cl	$CO_2C_2H_5$	*f*			43 (100)		
2f	Cl	COC_6H_4-2-OCH_3	16 (50)			34 (125)		
2g	Cl	$COCH{=}CH_6H_5$[g]	18 (50)			0 (25)		
2h	Cl	$CON(CH_3)_2$	0 (50)					
2i	Cl	CH_3				51 (125)		102
4a	Cl	CO_2CH_3		0.3[e]	100 (10)		0.1	0.5 [4]
4b	Cl	$CO_2C_2H_5$		0.1	59 (10)			
4c	Cl	$CO_2^-Na^+$		0.3	51 (10)			
4d	Cl	$CO_2^-H_3N^+C(CH_2OH)_3$			47 (10)			
4e	Cl	$CH_2CO_2C_2H_5$	<30 (100)		9 (10)			
4f	Cl	H	<30 (100)			28 (10)		
4g	Cl	CH_3		33	15 (10)			
4h	Cl	C_6H_5	<30 (30)		3 (10)			
4i	Cl	CCl_3		0.03	28 (10)			
DSCG				3.0			6.0	

[a] For all compounds, inhibition of AIR was concentration dependent, and inhibition at <10 μM was significant at the 0.05 level. [b] Unless noted otherwise, results represent a single trial and are statistically significant using the Student's *t* test ($p \leq 0.05$). [c] Total number of trials shown in brackets. [d] All compounds had elemental analysis within ±0.4 of theoretical values except **2c** and **4e**. Anal. ($C_{10}H_5ClN_2O_2$) C, N; H: calcd, 2.28; found, 2.82. Anal. ($C_{14}H_{11}ClN_2O_3$) H, N; C: calcd, 57.84; found, 56.95. [e] Histamine release was assayed by a radioenzymatic method. [f] Stimulation of AIR. [g] Trans.

release from rat mast cells (RMC) but was inactive as an inhibitor of IgE-mediated passive cutaneous anaphylaxis in the rat (PCA). With the goal of preparing compounds active in vivo, we decided to synthesize compounds based on **1** but with the quinolinyl C-7 carbon bonded to the carbanilate nitrogen (**2**). Although some PCA activity is observed with compounds of general structure **2**, none were of sufficient activity to warrant further development.

A publication reporting a quantitative structure–activity correlation for three seemingly distinct classes of molecules that act as inhibitors of mediator release in the PCA[6] prompted us to make one further modification. By incorporating a C-2 carboxylate group into our 1,3-oxazolo[4,5-*h*]quinoline system, the system was endowed with all the essential elements of the reported moiety **3**.[7] Thus, 5-chloro-1,3-oxazolo[4,5-*h*]quinoline-2-carboxylic acid methyl ester (**4a**) was prepared and tested in the RMC assay and PCA test. In rats, compound **4a** is the most potent, orally active inhibitor of IgE-mediated PCA yet discovered by our Research and Development Division. Currently, it is under clinical investigation primarily for the prophylactic treatment of asthma.[8]

Chemistry. Previous references to the oxazolo[4,5-*h*]-quinoline heterocyclic system are limited in scope. For example, 2,6-dimethyl-8-oxo-9*H*-oxazolo[4,5-*h*]quinoline was synthesized as proof of structure for an intermediate used in the total synthesis of the antibiotics nybomycin and deoxynybomycin.[9] In addition, a series of 2-aryloxazolo[4,5-*h*]quinoline-5-arylidines was prepared and shown to be active as bactericidal agents.[10] However, as far as we were able to determine, the oxazolo[4,5-*h*]-quinolines **2** and **4** are new.

The oxazolo[4,5-*h*]quinolines herein described were prepared from the 7-amino-8-hydroxyquinolines **5** (Scheme I). Intermediate **5** was synthesized from 5-chloro-8-hydroxyquinoline by a two-step process, first nitration and then either catalytic reduction with palladium on carbon to give **5a** (X = H) or chemical reduction with sodium dithionite to give **5b** (X = Cl). Compounds **2b** (X = R = H) or **2c** (X = Cl, R = H) was prepared by treating a

(4) For the first in the series see: Musser, J. H.; Brown, R. E.; Loev, B.; Bailey, K.; Jones, H.; Kahen, R.; Huang F.; Khandwala, A.; Leibowitz, M.; Sonnino-Goldman, P.; Donigi-Ruzza, D. *J. Med. Chem.* **1984**, *27*, 121.

(5) Karsein, K. S.; Karabinos, J. V. *U.S. Specification* 3 946 016, 1976. Nuesslein, L.; Muftic, M. South African Specification 67 06 898, 1968. Aries, R. French Specification 1 510 067, 1966.

(6) Cheny, B. V.; Wright, J. B.; Hall, C. M.; Johnson, H. G. *J. Med. Chem.* **1978**, *21*, 936.

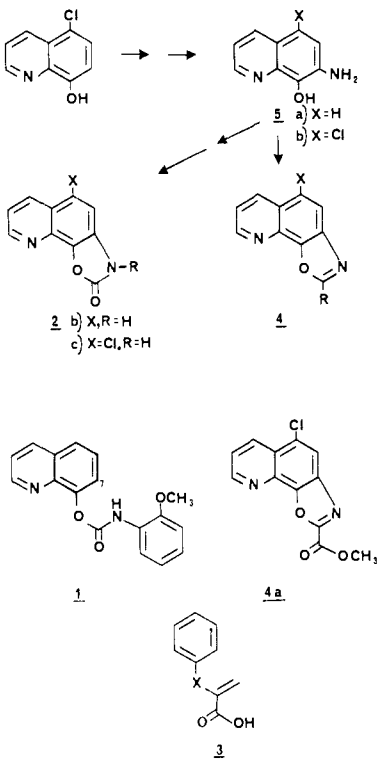
(7) Although the introduction of a methyl carboxylate group to the 2-position of **4** does not, strictly speaking, give the carboxylic acid group of the reported moiety **3**, one must consider that our data are based on oral or intraperitoneal dosing, whereas the data of ref 6 are based on intravenous administration of drug. We have shown that serum readily hydrolyzes **4a** to the free carboxylic acid (details of the metabolic profile for **4a** will be published elsewhere). It is reasonable that the carboxylic acid of **4a** is the probable active species; therefore, the analogy is consistent.

(8) Szabadi, R. R.; Shoupe, T. S.; Berger, B.; Caruso, F. S.; Vukovich, R.; Neiss, E. *Abstr. Annu. Meeting Am. Soc. Clin. Pharm. Ther.* **1984**, *85*, 279–A44.

(9) Forbis, R. M.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1970**, *92*, 6995; **1973**, *95*, 5003.

(10) Osman, A. M.; Khalil, Z. H.; Yanni, A. S. *J. Appl. Chem. Biotech.* **1977**, *27*, 33.

Scheme I



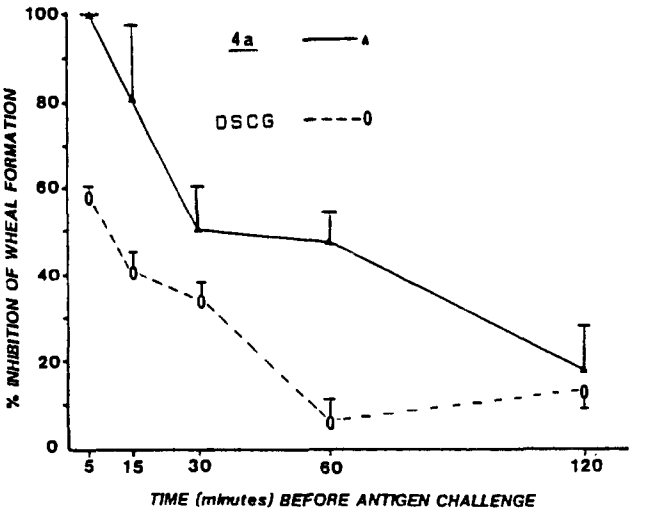


Figure 1. Effect of varying the time of drug treatment on the inhibition of passive cutaneous anaphylaxis by ip administration of **4a** (1.0 mg/kg) and DSCG (6.0 mg/kg). The values shown represent the mean percent inhibition ± SD for each group.

suspension of **5a** or **5b**, respectively, in methylene chloride with phosgene followed by the addition of triethylamine. Reaction of **2c** or **2b** at N-3 with the appropriate acylating agent gave compounds **2d–h** or **2a**, respectively, and N-3 alkylation of **2c** with methyl iodide gave compound **2i** (Table I). Treatment of chloro intermediate **5b** with the appropriate ortho ester or methoxy imidate gave compounds **4a**,**b**,**e–i**. Hydrolysis of **4a** in dilute base followed by treatment with 1 equiv of sodium hydroxide or tris(hydroxymethyl)aminomethane gave **4c** or **4d**, respectively.

Compound **4a** was originally prepared by the ring contraction of 3,6-dichloro-2-oxooxazinyl[5,6-*h*]quinoline with methanol.[11] However, this approach proved unsatisfactory when attempted on a larger scale. Other methods such as the oxidation of the 2-methyl followed by esterification,[12] methanolysis of the 2-cyano,[13] and cleavage of the aryl–silicon bond of the 2-trimethylsilyl derivative[14] of **4** were also unsatisfactory. We reasoned that these low-yield, multistep sequences could be avoided and the desired transformation could be effected in one step if the ortho ester of ethyl hydrogen oxalate was available. A review of the literature showed that although methyl trimethoxyacetate was known,[15] no references to its use in the synthesis of methyl benzoxazole-2-carboxylates could be found. Nevertheless, the expected reaction does in fact proceed as planned, giving **4a** from chloro intermediate **5b** in good yield.[16] Compound **4a** has been prepared in kilogram scale with use of methyl trimethoxyacetate.

Biological Results and Discussion

The results obtained for oxazoloquinolines **2** and **4** in the RMC and PCA assays are listed in Table I. In general, oxazoloquinolines **4** displayed greater activity than compounds of general structure **2** as inhibitors of AIR in vitro from RMC. The best compound of the first series is **2d** and is acylated on nitrogen. Unfortunately, all of the compounds of general structure **2** were less active than DSCG. In contrast, several compounds in the second series were potent inhibitors of AIR. The most potent, **4i**, with an IC_{50} value of 0.03 μM, was 100 times more potent than DSCG as an inhibitor of AIR. It is interesting to note that this compound shows only minor activity in vivo. Other compounds more potent than DSCG in vitro were **4a–c**.

Two oxazoloquinolines (**2a**, **4a**) in Table I showed good oral activity as inhibitors of the IgE-mediated passive cutaneous anaphylaxis (PCA) reaction in the rat. Although compound **2a** is orally active, it is inactive in vitro. This observation may indicate a mechanism of action other than mediator release inhibition for **2a**. Metabolism of **2a** to a mediator release inhibitor in vivo is less likely since **2d** shows only minor activity in vivo and one might expect both to undergo similar metabolism. Compound **4a** has an ED_{50} of 0.5 mg/kg when given orally. When tested intraperitoneally, it is approximately 60 times as potent as DSCG (dose–response curve not shown; see Figure 1 for a single-dose study). Three additional compounds (**4b–d**) are active when injected intraperitoneally.

The observation that ester **4a** is more potent than salt **4c** in the PCA although the two compounds have similar potency in the RMC deserves comment. We found that in an acidic medium **4a** is relatively stable; however, in base or in serum **4a** is readily hydrolyzed to the corresponding carboxylic acid. Specifically, **4a** has the following solution half-lives at 37 °C: in 0.1 N HCl, 8.1 h; in 0.1 N NaOH, 1.3 min; and in human serum diluted 1:10 with pH 7.0 phosphate buffer, 11.5 min. Therefore, it may be inferred that ester **4a** is absorbed intact in the acidic environment of the stomach and is hydrolyzed to the carboxylic acid in the blood stream, whereas **4c** is poorly absorbed, thus

(11) This reaction is a modification of the ring contraction method developed by Dickore et al.: Dickore, K.; Sasse, K.; Bode, K. D. *Liebigs Ann. Chem.* **1970**, *733*, 70.

(12) Skruap, S.; Moser, M. *Ber.* **1922**, *55B*, 1080.

(13) Moller, H. *Liebigs Ann. Chem.* **1970**, *749*, 1.

(14) Pinkerton, F. H.; Thames, S. F. *J. Heterocycl. Chem.* **1972**, *9*, 67.

(15) Anshutz, R.; Stiepel, J. *Liebigs Ann. Chem.* **1899**, *306*, 16. For the analogous ethyl triethoxyacetate, see: Anschutz, R. *Ibid.* **1889**, *254*, 1.

(16) For further examples of the reaction of alkyl trialkoxyacetate with other 1,4 binucleophiles, see: Musser, J. H.; Hudec, T. T.; Bailey, K. *Synth. Commun.* **1984**, *14*, 947.

providing lower serum concentrations of drug.

Compound **4a** has been investigated in detail and was found to have an activity profile similar to that of DSCG. As an inhibitor of AIR in vitro from RMC, **4a** exhibited tachyphylaxis and was cross-tachyphylactic to DSCG. Secondary studies in the rat PCA demonstrated that **4a** when given at 1 mg/kg has an intraperitoneal time course of activity similar to that of DSCG given at 6 mg/kg (Figure 1); inhibition of PCA was greatest when given 5–15 min prior to antigen challenge, and inhibitory activity diminished rapidly as the interval between administration of the compound and antigen challenge was lengthened. These results are to be the subject of future papers. Taken together they suggest that **4a** possesses DSCG-like properties.

We have described the preparation of a new series of oxazoloquinolines that evolved from a quinoline carbanilate lead. After several modifications of the original lead, the most potent compound of the series was determined to be **4a**. It has significant antiallergic activity apparently with a mechanism of action similar to that of DSCG. Currently, it is under clinical investigation primarily for the treatment of asthma.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Spectra were recorded for all compounds and were consistent with assigned structures. NMR spectra were recorded on a Varian EM-390 spectrometer at 90 MHz. IR spectra were recorded on a Perkin-Elmer Model 298 spectrophotometer. All compounds, unless otherwise indicated, had elemental analyses within ±0.4% of theoretical values.

5-Chloro-7-nitro-8-hydroxyquinoline. To a solution of 5-chloro-8-hydroxyquinoline (90.0 g, 0.5 mol) in sulfuric acid (500 mL) at 0 °C was added 90% nitric acid (0.6 mol) at such a rate that the temperature did not exceed 2 °C. The clear solution was stirred for 1 h at 0 °C and then allowed to slowly warm to room temperature. The mixture was poured into ice (2 L) and stirred overnight. The resulting yellow precipitate was filtered, washed with water, and dried. The yellow cake was crystallized from methyl ethyl ketone, giving 85.0 g (76% yield) of a solid, mp 192–194 °C.

7-Amino-8-hydroxyquinoline (5a). A suspension of 5-chloro-7-nitro-8-hydroxyquinoline (19.8 g) and 5% Pd/C (1 g) in methanol (500 mL) was subjected to hydrogen (50 psi) while the mixture was shaken overnight. The reaction mixture was filtered through Celite and the solvent was removed in vacuo to give 13.9 g of a solid (99% yield), mp 200 °C dec.

5-Chloro-7-amino-8-hydroxyquinoline (5b). To a suspension of 5-chloro-7-nitro-8-hydroxyquinoline (85.0 g, 0.38 mol) in a 1:1 mixture of methanol and water (2.5 L) was added sodium dithionite (340 g, 2.0 mol). The reaction, which is slightly exothermic, was stirred overnight under nitrogen. The resulting yellow solid was filtered, washed with water, and crystallized from ethanol, giving 53 g (70% yield) of a solid, mp 162–164 °C.

2-Oxo-3*H*-oxazolo[4,5-*h*]quinoline (2b). A suspension of **5a** (3.2 g, 20.0 mmol) in methylene chloride (50 mL) was treated with 25 mL of 1.6 M phosgene gas in methylene chloride. Triethylamine (12 mL) was slowly added and the reaction was stirred overnight. The reaction mixture was partially concentrated and water was added. After treatment of the resulting mixture with aqueous ammonium hydroxide, a precipitate formed, which was filtered and dried to give 3.4 g (91% yield) of a solid, mp >300 °C. In a similar manner starting with **5b**, compound **2c** was prepared (89% yield), mp 300 °C (Table I).

3-Acetyl-2-oxo-3*H*-oxazolo[4,5-*h*]quinoline (2a). A suspension of **2b** (1.86 g, 10.0 mmol), acetic anhydride (50 mL), and sulfuric acid (0.5 mL) was refluxed overnight. The reaction mixture was filtered and the filtrate concentrated. Methylene chloride was added and the resulting solution was washed with water (three times) and brine, dried ($MgSO_4$), and concentrated to a solid. The solid was crystallized from ethanol to give 1.82 g (80% yield) of product, mp 192–193 °C. In a like manner as above, with use of acetic anhydride and sulfuric acid, compound **2d** was prepared (74% yield), mp 210–212 °C (Table I).

3-Carbethoxy-5-chloro-2-oxo-3*H*-oxazolo[4,5-*h*]quinoline (2e). To a solution of **2c** (3.0, 13.6 mmol) in THF with 1 equiv of triethylamine was added a solution of ethyl chloroformate (1.5 g, 13.6 mmol) in THF. After stirring overnight at room temperature, the mixture was filtered through Celite and silica gel and the solvent was removed in vacuo. The solid was crystallized from ethanol, giving 1.7 g (42% yield) of product, mp 199–201 °C. In a like manner as above, with use of **2c** and 2-methoxybenzoyl chloride, cinnamoyl chloride, and *N,N*-dimethylcarbamyl chloride, respectively, the following compounds were prepared: **2f** (59% yield), mp 199–201 °C; **2g** (51% yield), mp 233–238 °C; **2h** (46% yield), mp 222–225 °C.

5-Chloro-3-methyl-2-oxo-3*H*-oxazolo[4,5-*h*]quinoline (2i). To a solution of **2c** (3.0 g, 13.6 mmol), degreased sodium hydride (1.0 g, 50% in oil, 20.8 mmol), and DMF (30 mL) at 60 °C was slowly added (45 min) methyl iodide (11.6 g, 81.6 mmol). The reaction was stirred for 1 h. Water was added and the mixture was extracted with chloroform (three times). The organic extracts were combined and concentrated. The remaining solid was crystallized from ethanol to give 1.4 g (45% yield) of product, mp 219–221 °C.

5-Chloro-1,3-oxazolo[4,5-*h*]quinoline-2-carboxylic Acid Methyl Ester (4a). A mixture of **5b** (6.0 g, 30.8 mmol) and methyl trimethoxyacetate[17] (16.3 g, 123.2 mmol) was heated at 100 °C overnight. The reaction was cooled in an ice bath and a precipitate formed, which was filtered and dissolved in acetone (250 mL). The resulting solution was treated with charcoal at reflux, filtered through a pad of Celite and silica gel, and partially concentrated. Crystals formed, which were filtered and dried to give 4.1 g (51% yield) of product, mp 217–218 °C. In a like manner as above, with use of **5b** and ethyl triethoxyacetate,[17] ethyl 3,3,3-triethoxypropanoate, ethyl orthoformate, ethyl orthoacetate, ethyl benzimidate hydrochloride, and methyl (trichloro)acetimidate, respectively, the following compounds were prepared: **4b** (61% yield), mp 150–152 °C; **4e** (35% yield), mp 118–121 °C; **4f** (68% yield), mp 217–218 °C; **4g** (65% yield), mp 148–152 °C; **4h** (53% yield), mp 195–196 °C; and **4i** (29% yield), mp 167–168 °C (Table I).

5-Chloro-1,3-oxazolo[4,5-*h*]quinoline-2-carboxylic Acid Sodium Salt (4c). A suspension of **4b** (1.5 g, 5.4 mmol) in water (100 mL) was treated with 16.3 mL of 1 N NaOH. After stirring for 10 min, the mixture was washed with chloroform. The aqueous solution was treated with saturated ammonium chloride, causing a precipitate to form. The precipitate was filtered and dried, giving the ammonium salt, mp 212–216 °C. The ammonium salt was suspended in water (50 mL), treated with 1 equiv of sodium hydroxide, and lyophilized to give 0.9 g (61% yield) of product, mp 200 °C dec. In a like manner with use of tris(hydroxymethyl)aminomethane, compound **4d** (70% yield) was prepared, mp 90 °C dec (Table I).

Rat Mast Cell (RMC). The RMC test was conducted as described in ref 4. Rat peritoneal cells obtained by lavage contained 5–10% mast cells. Since the intrinsic fluorescence of some oxazoloquinolines interfered with the automated fluorometric analysis, a radioenzymatic method (HARM) was employed to quantitate the levels of histamine.[18] Samples containing histamine were incubated with [^{14}C]methyl-*S*-adenosylmethionine and an enzyme, histamine-*N*-methyltransferase, prepared from rat kidneys. A trace of tritiated histamine was an internal standard. The product of the enzymatic reaction, 1-[methyl-^{14}C]histamine, was extracted into chloroform and quantitated by scintillation counting. The assay was linear in the range between 0 and 22 ng of histamine.

Passive Cutaneous Anaphylaxis in the Rat (PCA). The effect of compounds on IgE-mediated cutaneous wheal formation in the rat was determined by the method described in ref 4. A comparative time-course study was carried out for one of the oxazoloquinolines (**4a**) and DSCG. These studies used the same general PCA protocol, except that 1.0 mg/kg of compound 4a or

(17) Methyl trimethoxyacetate and ethyl triethoxyacetate were made by a modification of a procedure reported by Jones: Jones, R. G. *J. Am. Chem. Soc.* **1951**, *73*, 5168.

(18) Shaft, R. E.; Breaver, M. A. *Anal. Biochem.* **1979**, *94*, 425.

6.0 mg/kg of DSCG was given intraperitoneally at intervals ranging from 5 to 120 min prior to challenge.

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Registry No. **2a**, 97000-05-0; **2b**, 85064-08-0; **2c**, 97000-06-1; **2d**, 97000-07-2; **2e**, 97000-08-3; **2f**, 97000-09-4; **2g**, 97000-10-7; **2h**, 97000-11-8; **2j**, 97000-12-9; **4a**, 86048-40-0; **4b**, 88362-76-9; **4c**, 88362-77-0; **4c** (ammonium salt), 97000-19-6; **4d**, 97000-14-1; **4e**, 88362-75-8; **4f**, 97000-15-2; **4g**, 97000-16-3; **4h**, 97000-17-4; **4i**, 97000-18-5; **5a**, 18472-06-5; **5b**, 18471-93-7; $ClCO_2C_2H_5$, 541-41-3; *trans*-$ClCOCH{=}CHC_6H_5$, 17082-09-6; $ClCON(CH_3)_2$, 79-44-7; $(CH_3O)_3CCO_2CH_3$, 18370-95-1; $(C_2H_5O)_3CCO_2C_2H_5$, 57267-03-5; $(C_2H_5O)_3CCH_2CO_2C_2H_5$, 32650-62-7; $(C_2H_5O)_3CH$, 122-51-0; $(C_2H_5O)_3CCH_3$, 78-39-7; $C_6H_5C{=}NH(OC_2H_5)\cdot HCl$, 5333-86-8; $Cl_3CC{=}NH(OCH_3)$, 2533-69-9; 5-chloro-8-hydroxyquinoline, 130-16-5; 5-chloro-7-nitro-8-hydroxyquinoline, 18472-03-2.

Anticonvulsant Activity of Some 4-Aminobenzanilides

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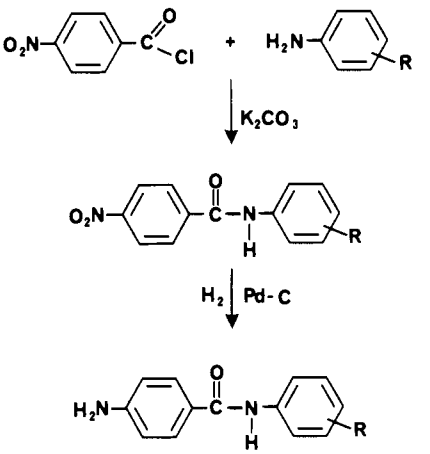
A series of 4-aminobenzanilides derived from ring-alkylated anilines were prepared and evaluated for anticonvulsant activity. These benzanilides were prepared in the course of studies designed to determine the relationship between the benzamide structure and anticonvulsant effects. The compounds were tested in mice against seizures induced by electroshock and metrazole (pentylenetetrazole) and in the rotorod assay for neurologic deficit. All of the 4-aminobenzanilides showed activity at doses of 300 mg/kg against maximal electroshock seizures (MES). The 4-aminobenzanilide derived from 2,6-dimethylaniline (**8**) was the most potent anti-MES compound with an ED_{50} of 2.60 mg/kg and a protective index of 5.77 ($PI = TD_{50}/ED_{50}$). The activity profile for **8** compares quite favorably with that for phenobarbital and phenytoin in the same assays.

Recent studies in this laboratory[1] have demonstrated significant anticonvulsant potential for the 4-aminobenzamides of some simple dialkyl- and arylalkylamines. Structurally, some of the simplest compounds possessing anticonvulsant properties are the carboxylic acids and their amides.[2] Valproic acid is perhaps the best known example of this class of compounds.[3] The amide of valproic acid has been shown[4] to be as effective as the acid at half the dose. Various reports,[5,6] have described the anticonvulsant effects of substituted cinnamamides. Balsamo et al.[7,8] have described the anticonvulsant and other CNS effects of the *E* and *Z* isomers of some *N*-alkylcinnamamides. Cinromide, 3-bromo-*N*-ethylcinnamamide, has been evaluated as a broad-spectrum anticonvulsant and has a reported anti-MES ED_{50} of 60 mg/kg when administered ip in mice.[6] Several derivatives of 3-phenyl-2-piperidinone have been shown to possess anti-MES and anti-scMet activity in animal models.[9] Amides of substituted benzoic acids[2] including some aminohalobenzamides[10] have also been reported to possess anticonvulsant activity. The local anesthetic amide lidocaine has been shown to suppress the electroencephalogenic manifestations of epileptic seizures in cats.[11]

Scheme I



The unique behavioral profile produced in animals by substituted benzamide neuroleptics such as metoclopramide has generated a considerable amount of interest in recent years.[12] The benzamide neuroleptics are useful in the treatment of schizophrenia and appear to exert their neuroleptic action selectively at a subpopulation of the D-2 type dopamine receptors.[13] These antidopaminergic benzamides differ in some clinical and pharmacological respects from other neuroleptic drugs. In a comparative study with classical neuroleptics these benzamides were generally 1000 times more potent in stimulating rat prolactin secretion than would have been predicted from their potencies in displacing [^{3}H]spiperone from bovine anterior pituitary membranes.[14] The simple 4-aminobenzanilides reported in this paper represent a continuation of our studies on the relationship between benzamide structure

(1) Clark, C. R.; Wells, M. J. M.; Sansom, R. T.; Norris, G. N.; Dockens, R. C.; Ravis, W. R. *J. Med. Chem.* **1984**, *27*, 779.
(2) Murray, W. J.; Kier, L. B. *Med. Chem. (Academic Press)* **1977**, *15*, 591.
(3) Meunier, G.; Carrey, G.; Mennier, Y.; Eymard, P.; Aimard, M. *Therapie* **1963**, *18*, 435.
(4) Carrey, G.; Boucherle, A.; Lebreton, S.; Benoit-Guyod, J. L.; Boitard, M. *Therapie* **1964**, *19*, 917.
(5) British Patent 663 903; *Chem. Abstr.* 46:6336.
(6) Perchalski, R. J.; Karas, B. J.; Wilder, B. J. In "Antiepileptic Drugs", 2nd ed.; Woodbury, D. M. Penry, J. K., Pippenger, C. E., Eds.; Raven Press: New York, 1982; Chapter 72, p 817.
(7) Balsomo, A.; Basili, P. L.; Crotti, P.; Mocchia, B.; Macchia, F.; Cuttica, A.; Passerini, N. *J. Med. Chem.* **1977**, *20*, 48.
(8) Balsomo, A.; Crotti, P.; Lapucci, A.; Mocchia, B.; Mocchia, F.; Cuttica, A.; Passerini, N. *J. Med. Chem.* **1981**, *24*, 525.
(9) Brouillette, W. J.; Grunewald, G. L. *J. Med. Chem.* **1984**, *27*, 202.
(10) Horrom, B. W.; Lynes, T. E. *J. Med. Chem.* **1963**, *6*, 528.
(11) Bernard, C. G.; Bohm, E. *Experientia* **1954**, *10*, 474.
(12) Blaney, F. E.; Clark, M. S. G.; Gardner, O. V.; Hadley, M. S.; Middleton, D.; White, T. J. *J. Med. Chem.* **1983**, *26*, 1747.
(13) Jenner, P.; Marsden, C. D. *Acta Psychiatr. Scand., Suppl.* **1984**, *69*, 109.
(14) Melzer, H. Y.; Simonovic, M.; So, R. *Life Sci.* **1983**, *32*, 2877.

0022-2623/85/1828-1259$01.50/0