Structure-Activity Relationships in a Series of Novel 3,4-Dihydro-4-oxopyrimido[4,5-*b*]quinoline-2-carboxylic Acid Antiallergy Agents[1]

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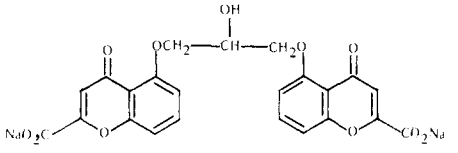
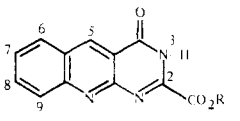
A series of more than 50 new 3,4-dihydro-4-oxopyrimido[4,5-*b*]quinoline-2-carboxylic acid derivatives and related compounds with substituent variations at the 2, 3, and 5–9 positions was prepared and evaluated for antiallergy activity using the rat PCA assay. These compounds were obtained by the condensation of the appropriately substituted 2-aminoquinoline-3-carboxamides with dialkyl oxalates, followed by further chemical transformations. More than two-thirds of the compounds prepared exhibited intravenous activity ranging from 1 to 400 times disodium cromoglycate (DSCG). Structure-activity data suggest that the presence of a carboxylic acid moiety at the 2 position affords optimal potency and that esters are preferred for good oral absorption. Best oral activity, with ED_{50} values ranging from 0.3 to 3.0 mg/kg, was displayed by ethyl esters with methoxy and/or ethoxy groups at the 7 and 8 positions.

The development of 3,4-dihydro-4-oxopyrimido[4,5-*b*]-quinoline-2-carboxylic acid, **8a**, a new prototype with an-



DSCG



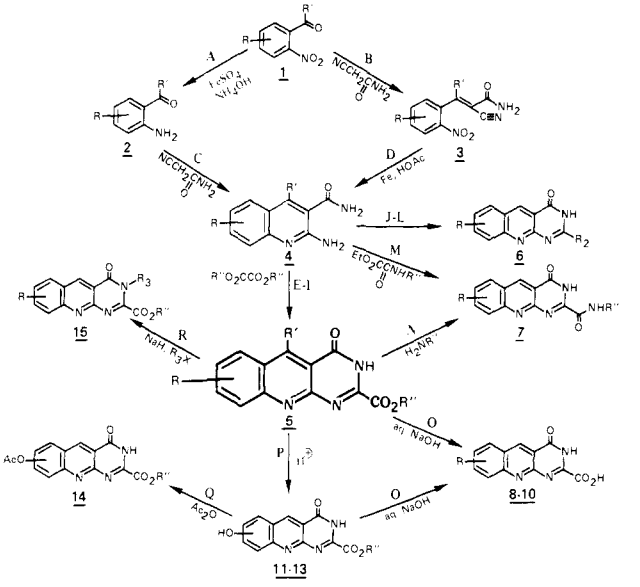
5a R = Et
8a R = H

tiallergy activity of the disodium cromoglycate (DSCG) type, has been previously described.[2] This compound was approximately ten times more potent than DSCG[3] against reagin-mediated anaphylactic responses in the rat passive cutaneous anaphylaxis (PCA) procedure. Furthermore, the corresponding ethyl ester, **5a**, was orally active at doses comparable to those of several other recently reported antiallergy agents.[2]

We now report our efforts to maximize antiallergy activity in these novel pyrimido[4,5-*b*]quinolines, several of which demonstrate intravenous potency from 100 to 400 times DSCG. Unlike DSCG, which is inactive orally, a number of these compounds possess potent oral activity.

Chemistry. The 3,4-dihydro-4-oxopyrimido[4,5-*b*]-quinoline-2-carboxylic acids and related compounds (Tables I–V) were synthesized as illustrated in Scheme I. The 2-aminoquinoline-3-carboxamides (**4**), which served as key intermediates, were prepared from the appropriately substituted *o*-nitrobenzaldehydes (**1**) or *o*-aminobenzaldehydes (**2**). Knoevenagel condensation of the *o*-nitroaldehydes (**1**) with cyanoacetamide occurred readily and provided the benzylidene derivatives, **3**, in good yield. Nucleophilic aromatic substitution (**3c**, R = Cl) with sodium alkylthiolates furnished the alkylthio-substituted compounds (**3i** and **3k**). The benzylidene derivatives were reduced with iron and acetic acid to afford the 2-aminoquinoline-3-carboxamides (**4**) in high yield.[4] Alternately,

Scheme I. Synthesis of Substituted 3,4-Dihydro-4-oxopyrimido[4,5-*b*]quinoline-2-carboxylic Acid Derivatives and Related Compounds



base-catalyzed condensation of *o*-aminobenzaldehydes (**2**) with cyanoacetamide yielded the 2-aminoquinoline-3-carboxamides (**4**) directly.[5]

The synthesis of the pyrimido[4,5-*b*]quinoline-2-carboxylic acid esters (**5**) is similar to that of the preparation of 2-substituted pyrimido[4,5-*b*]quinolin-3(4*H*)-ones (**6**) reported by Taylor and Kalenda,[6] who employed acetic anhydride to effect pyrimidine ring formation. Heating the 2-aminoquinoline-3-carboxamides (**4**) with dialkyl oxalates or alkyl oxamates gave moderate yields of the corresponding 3,4-dihydro-4-oxopyrimido[4,5-*b*]quinoline-2-carboxylic acid esters (**5**) and amides (**7**) (Tables I–V). Higher yields of **5** were usually obtained when the ring closure was effected at room temperature in the presence of excess sodium alkoxide.[7] Evidence of ring closure was readily evident by examination of the NMR spectrum. For example, the proton at position 4 of the 6,7-dialkoxy-2-aminoquinoline-3-carboxamides (**4m–z**) has a chemical shift of about δ 8.95 in deuteriotrifluoroacetic acid, whereas this proton in the ethyl 7,8-dialkoxy-3,4-dihydro-4-oxopyrimido[4,5-*b*]quinoline-2-carboxylates (**5m–z**) has a chemical shift about 1 ppm lower field, at δ 9.67–10.33.

(1) Presented in part at the "Symposium on Drugs Affecting the Respiratory System" at the 175th National Meeting of the American Chemical Society, Anaheim, Calif., Mar 13–17, 1978, see abstract MEDI-2.
(2) For paper 1 in this series, see T. H. Althuis, P. F. Moore, and H.-J. Hess, *J. Med. Chem.*, **22**, 44 (1979).
(3) J. S. G. Cox, J. E. Beach, A. M. J. N. Blair, A. J. Clark, J. King, T. B. Lee, D. E. E. Loveday, G. F. Moss, T. S. C. Orr, Jean T. Ritchie, and P. Sheard, *Adv. Drug Res.*, **5**, 115 (1970).
(4) E. Campaigne and G. Randau, *J. Heterocycl. Chem.*, **8**, 111 (1971).
(5) E. C. Taylor and N. W. Kalenda, *J. Org. Chem.*, **18**, 1755 (1953).
(6) E. C. Taylor and N. W. Kalenda, *J. Am. Chem. Soc.*, **78**, 5108 (1956).
(7) S. Nakanishi and S. S. Massett, *Org. Prep. Proced. Int.*, **12**, in press (1980).

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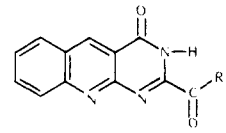
Table I. Antiallergic 3,4-Dihydro-4-oxopyrimido[4,5-*b*]quinoline-2-carboxylic Acid Derivatives



compd	R	synth proc	yield, %	mp, °C	solvent	formula	anal.	rat PCA ED_{50}, mg/kg iv	rat PCA ED_{50}, mg/kg po
DSCG								0.8	(300)[a]
5a	OEt	E	45	247–248 dec	$CHCl_3$	$C_{14}H_{11}N_3O_3$	C, H, N	0.1	3
8a	OH	O	73	345–347 dec	aq H^+	$C_{12}H_7N_3O_3 \cdot 0.5H_2O$	C, H, N[b]	0.2	25
5ab	O-*n*-Bu	E, I	36	218–220	$CHCl_3$	$C_{16}H_{15}N_3O_3$	H[c]	0.06	30
7a	NH_2	M	28	320	MeOH	$C_{12}H_8N_4O_2$	C, H	1.3	(60)[a]
7ab	NHEt	N	54	263–264	EtOH	$C_{14}H_{12}N_4O_2$	C, H, N	(3)[a]	nt[d]

[a] Numbers in parentheses indicate highest dose at which the compound was tested and found inactive. [b] Analyzed as the disodium salt. [c] C: calcd, 64.71; found, 64.12. [d] Not tested.

The pyrimido[4,5-*b*]quinoline-2-carboxylic acid esters (**5**) are also useful synthetic intermediates. They can be transesterified using basic or acidic catalysts. These esters are also sufficiently reactive to undergo aminolysis at slightly elevated temperatures to furnish amides (**7**). In addition, they undergo rapid hydrolysis in dilute aqueous sodium hydroxide at room temperature to give the respective acids (**8–10**). Upon heating, the acids decarboxylate to give **6** (R_2 = H).[8] Ester hydrolysis occurs much more slowly under acidic conditions. Compounds **5** are weak bases and as such are readily soluble in strong nonaqueous acids but sparingly soluble in aqueous mineral acids and weak organic acids. The acidic proton at position 3 renders these compounds soluble in dilute sodium hydroxide. Formation of this sodium salt with sodium hydride in DMF, followed by reaction with alkyl halides, provided the 3-substituted compounds (**15**). Debenzylation of the esters **5h**, **5x**, **5y**, and **5z** to hydroxy compounds **11–13** was conveniently effected in trifluoroacetic acid.[9,10] Acetylation of **11–13** by standard techniques gave **14**.

Structure–Activity Relationships. The intravenous and oral activities displayed by 3,4-dihydro-4-oxopyrimido[4,5-*b*]quinolines **5–15** against reagin (IgE) induced PCA reactions in the rat are shown in Tables I–V. Compounds in these tables are numbered according to the structural types shown in Scheme I. Different letters following the numbers designate different substitution patterns with the same letters always representing the same substitution patterns.

The carbethoxy function at position 2 is an important structural feature for antiallergy activity.[2] As can be seen from Table I, both the free carboxylic acid **8a** and the butyl ester **5ab**, although about as potent as **5a** on intravenous administration, are 8 to 10 times less potent orally. This, together with a further decrease in intravenous and oral potency displayed by carboxamides **7a** and **7ab**, suggests that the carbethoxy moiety is an optimal 2 substituent for oral activity.

The effect on activity of substitution in the quinoline ring is illustrated in Table II. Introduction of chloro or fluoro substituents in positions 6, 7, or 8 (**5b–e**) or a phenyl group in position 5 (**5f**) led to equivalent or slightly reduced intravenous potency compared to **5a** but at least a tenfold decrease in oral activity. A threefold increase in intravenous potency occurred with the 7-methoxy substituent (**5g**). Activity decreased slightly with 7-(methylthio) (**5i**) and 7-(methylsulfinyl) (**5j**) substituents but considerably more with the larger benzyloxy (**5h**) and phenylthio (**5k**) substituents. Although the 8-methoxy analogue (**5l**) was less than one-twentieth as potent as **5g**, a dramatic increase in intravenous potency, 100 times that of DSCG, resulted with the 7,8-dimethoxy analogue, **5m**. More importantly, **5m** elicited potent oral activity (ED_{50} = 1.0 mg/kg). Other dimethoxy (**5n–p**) and trimethoxy (**5q**) substituted derivatives were less potent than **5a**. The decrease in PCA activity demonstrated by the 9-substituted pyrimido[4,5-*b*]quinoline suggests that hydrogen bonding between the nitrogen at position 10 and a receptor may be important and that such interactions are blocked in the presence of 9 substituents. Similar reasoning may explain the 25-fold increase in potency of 3,4-dihydro-4-oxopyrimido[4,5-*b*]quinoline-2-carboxylic acid (**8a**) over the previously reported 3,4-dihydro-4-oxobenzo[*g*]-quinazoline-2-carboxylic acid (ED_{50} = 5 mg/kg),[2] which lacks a comparably situated nitrogen.

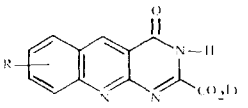
Further studies demonstrated that activity is sensitive to the size of the alkoxy substituents in the 7 and 8 positions (Table III). In contrast to the 7,8-(methylenedioxy) (**5r**) and 7,8-(ethylenedioxy) (**5s**) analogues, which are 70–140 times less potent than **5m**, the 7-methoxy-8-ethoxy and 7-ethoxy-8-methoxy isomers, **5t** and **5u**, are as, or more, potent than **5m** intravenously. Moreover, **5u** displayed marked oral activity (ED_{50} = 0.3 mg/kg). Activity diminished in the presence of larger substituents, such as 7,8-diethoxy (**5v**), and progressively decreased to that of DSCG with butoxy and benzyloxy substitution (**5w** to **5z**).

While the 7-hydroxy (**11**) and 7-methoxy-8-hydroxy analogues (**10**) are somewhat less potent (Table IV) than their corresponding O-methylated derivatives, **5g** and **5m**, the 7-hydroxy-8-methoxy derivative (**13a**) is seven times more potent than **5m** (400 times DSCG), suggesting that the activities of **5m**, **5t**, and **5u** may be due to metabolic dealkylation to **13a**. Dialkoxy-substituted nitrogen heterocycles are known to be metabolically dealkylated, and, in at least one case, this occurs preferentially on the methoxy group para to the nitrogen in the adjacent ring.[10] The hydroxy-substituted pyrimido[4,5-*b*]quinolines (Table IV), however, are generally less active orally than their respective O-alkyl counterparts.

The effect upon PCA activity of substitution at positions 2 and 3 of the heterocyclic nucleus was studied while retaining the activity-enhancing 7,8-dimethoxy substitution pattern. As can be seen in Table V, the carboxylic acid **8m** and esters **5m**, **5ma**, and **5mb** displayed similar activity intravenously, presumably due to hydrolysis of the esters to **8m**. Esters, however, displayed significant oral activity, with the ethyl ester **5m** apparently being optimal. The suggestion that the activity of the esters can be attributed

(8) B. R. Baker and P. I. Almaula, *J. Org. Chem.*, **27**, 4672 (1962).
(9) J. P. Marsh and L. Goodman, *J. Org. Chem.*, **30**, 2491 (1965).
(10) T. H. Althuis and H.-J. Hess, *J. Med. Chem.*, **20**, 146 (1977).

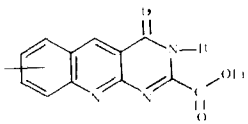
Table II. Antiallergic Ethyl 5-, 6-, 7-, 8-, or 9-Substituted 3,4-Dihydro-4-oxopyrimido[4,5-*b*]quinoline-2-carboxylates



no.	R	synth proc	yield, %	mp, °C	solvent	formula	anal.	rat PCA ED_{50}, mg/kg iv	rat PCA ED_{50}, mg/kg po
5a	H							0.1	3
5b	6-Cl	E	9	278-279 dec	CH_2Cl_2-EtOH (9:1)	$C_{14}H_{10}ClN_3O_3$	H, N[b]	0.2	30
5c	7-Cl	F	37	260.5 dec	HOAc	$C_{14}H_{10}ClN_3O_3 \cdot 0.5MeOH$	H, N[c]	0.4	30
5d	7-F	E	71	272 dec		$C_{14}H_{10}FN_3O_3$	C, H, N	0.3	(30)[a]
5e	8-F	E	29	248-251	*i*-PrOH	$C_{14}H_{10}FN_3O_3$	C, H, N	0.3	30
5f	5-Ph	E	32	225 dec	EtOAc	$C_{20}H_{15}N_3O_3$	C, H, N	0.3	(10)[a]
5g	7-MeO	E	10	263-264 dec	$CHCl_3$	$C_{15}H_{13}N_3O_4$	C, H, N	0.03	20
5h	7-$PhCH_2O$	E	32	256-257 dec	$CHCl_3$	$C_{21}H_{17}N_3O_4$	C, H, N	0.9	(10)[a]
5i	7-MeS	E	11	240-242	$CHCl_3$	$C_{15}H_{13}N_3O_3S$	H, N[d]	0.1	8
5j	7-MeS(→O)-	G	56	257-259 dec	EtOH	$C_{15}H_{13}N_3O_4S$	C, H, N	0.2	(30)[a]
5k	7-*p*-MeO-Ph-S-	E	11	248-251	$CHCl_3$	$C_{21}H_{17}N_3O_4S$	H, N[e]	(3)[a]	(30)[a]
5l	8-MeO	E	9	263-264 dec	$CHCl_3$	$C_{15}H_{13}N_3O_4$	C, H, N	0.8	10
5m	7,8-$(MeO)_2$	E	15	272-273 dec	$CHCl_3$	$C_{16}H_{15}N_3O_5$	C, H, N	0.007	1.0
5n	8,9-$(MeO)_2$	E	35	233-234 dec	$CHCl_3$	$C_{16}H_{15}N_3O_5$	H, N[f]	0.2	(60)[a]
5o	7,9-$(MeO)_2$	E	35	256-257 dec	$CHCl_3$	$C_{16}H_{15}N_3O_5 \cdot H_2O$	C, N[g]	0.5	30
5p	6,9-$(MeO)_2$	E	24	254-255 dec	$CHCl_3$	$C_{16}H_{15}N_3O_5$	C, H, N	0.6	(30)[a]
5q	7,8,9-$(MeO)_3$	Ea	31	251 dec	DMF	$C_{17}H_{17}N_3O_6$	H, N[h]	0.8	(10)[a]

[a] Numbers in parentheses indicate highest dose at which the compound was tested and found inactive. [b] C: calcd, 55.50; found, 55.03. [c] C: calcd, 53.98; found, 53.49. [d] C: calcd, 57.20; found, 56.72. [e] C: calcd, 61.90; found, 61.42. [f] C: calcd, 58.36; found, 57.57. [g] H: calcd, 4.93; found, 4.31. [h] C: calcd, 56.82; found, 56.25. [i] DEO = diethyl oxalate.

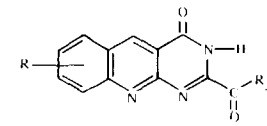
Table III. Antiallergic Ethyl 7,8-Dialkoxy-3,4-dihydro-4-oxopyrimido[4,5-*b*]quinoline-2-carboxylates



no.	R	synth proc	yield, %	mp, °C	solvent	formula	anal.	rat PCA ED_{50}, mg/kg iv	rat PCA ED_{50}, mg/kg po
5m	7,8-$(MeO)_2$	E						0.007	1.0
5r	7,8-OCH_2O	F	20	267 dec	EtOH-CH_2Cl_2	$C_{15}H_{11}N_3O_5$	C, H, N	0.5	(30)[a]
5s	7,8-OCH_2CH_2O	Ea	11	253 dec	DMF	$C_{16}H_{13}N_3O_5$	H[c]	1.0	nt[b]
5t	7-MeO, 8-EtO	E	16	264-265 dec	$CHCl_3$	$C_{17}H_{17}N_3O_5$	C, H, N	0.007	2.0
5u	7-EtO, 8-MeO	E	15	252-253 dec	$CHCl_3$	$C_{17}H_{17}N_3O_5 \cdot 0.5H_2O$	C, H, N	0.002	0.3
5v	7,8-$(EtO)_2$	E	19	243-244 dec	$CHCl_3$	$C_{18}H_{19}N_3O_5$	C, H, N	0.01	1.0
5w	7-EtOH, 8-*n*-BuO	E	16	201-202 dec	PhH	$C_{20}H_{23}N_3O_5$	C, H	0.3	5.0
5x	7-$PhCH_2O$, 8-MeO	E	25	274-275	$CHCl_3$	$C_{22}H_{19}N_3O_5$	C, H	(3)[a]	(10)[a]
5y	7-MeO, 8-$PhCH_2O$	E	8	254-255 dec	$CHCl_3$	$C_{22}H_{19}N_3O_5 \cdot H_2O$	C, H	0.8	(30)[a]
5z	7-EtO, 8-$PhCH_2O$	E	41	241-242 dec	PhH	$C_{23}H_{21}N_3O_5$	H, N[d]	0.9	(30)[a]

[a] Numbers in parentheses indicate highest dose at which the compound was tested and found to be inactive. [b] Not tested. [c] C: calcd, 58.71; found, 58.25. N: calcd, 12.84; found, 12.36. [d] C: calcd, 65.86; found, 65.01.

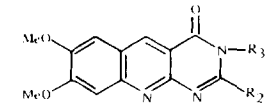
Table IV. Antiallergic 7- or 8-Hydroxy-3,4-dihydro-4-oxopyrimido[4,5-*b*]quinoline-2-carboxylic Acids and Esters



no.	R	R_2	synth proc.	yield, %	mp, °C	solvent	formula	anal.	rat PCA ED_{50}, mg/kg iv	rat PCA ED_{50}, mg/kg po
5g	7-MeO	OEt							0.03	20
9	7-HO	OH	O	95	340 dec	aq H^+	$C_{12}H_7N_3O_4 \cdot H_2O$	C, H	0.01	(10)[a,c]
11	7-HO	OEt	P	74	274-275 dec	TFAA-Et_2O	$C_{14}H_{11}N_3O_4 \cdot 0.5CF_3CO_2H$	C, H, N	0.7	10[d]
5m	7,8-$(MeO)_2$	OEt							0.007	1.0[d]
12	7-MeO, 8-OH	OEt	P	6	265-266 dec	$CHCl_3$	$C_{15}H_{13}N_3O_5 \cdot H_2O$	C, H	0.01-1.0	(3)[a,c]
10	7-HO, 8-MeO	OH	O	57	281 dec	aq H^+	$C_{13}H_9N_3O_5 \cdot CF_3CO_2H \cdot 0.5H_2O$	C, H	<0.01	(3)[a,c]
13a	7-HO, 8-MeO	OEt	P	72	279 dec	TFAA-Et_2O	$C_{15}H_{13}N_3O_5 \cdot CF_3CO_2H \cdot 0.5H_2O$	C, H, N	0.001	(10)[a,c]
14a	7-AcO, 8-MeO	OEt	Q	58	215-217 dec	PhH	$C_{17}H_{15}N_3O_6 \cdot C_7H_8O_3S$	H[e]	0.007	10[d]
13b	7-HO, 8-MeO	O-*n*-Bu	P	80	240-241 dec	TFAA-Et_2O	$C_{17}H_{17}N_3O_5 \cdot CF_3CO_2H$	C, N, H	0.008	10[d]

[a] Numbers in parentheses indicate highest dose at which the compound was tested and found to be inactive. [b] Not tested. [c] Highly active at the same dose if administered in solution of pH ≥10. [d] pH dependent, more active when administered in solution of pH ≥10. [e] C: calcd, 54.44; found, 53.74.

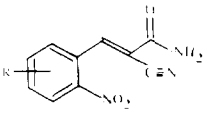
Table V. Antiallergic 2- and/or 3-Substituted 7,8-Dimethoxy-3,4-dihydro-4-oxopyrimido[4,5-*b*]quinolines



no.	R_3	R_2	synth proc.	yield, %	mp, °C	solvent	formula	anal.	rat PCA ED_{50}, mg/kg iv	rat PCA ED_{50}, mg/kg po
5m	H	CO_2Et							0.007	1.0
8m	H	CO_2Na	O	54	281-283 dec	TFAA-Et_2O	$C_{14}H_{11}N_3O_5 \cdot CF_3CO_2H \cdot H_2O$	C, H, N	0.005	(10)[a]
5ma	H	$CO_2CH_2CH_2OH$	E, H	54	252	DMF	$C_{16}H_{15}N_3O_6$	C, H, N	<0.03	10
5mb	H	CO_2-*n*-Bu	E	15	263-264 dec	$CHCl_3$	$C_{18}H_{19}N_3O_5$	C, H, N	0.003	10
7m	H	$C(=O)NH_2$	N	95	310 dec	MeOH/$CHCl_3$	$C_{14}H_{12}N_4O_4 \cdot 0.5CHCl_3$	*f*	3	(30)[a]
7ma	H	$C(=O)NHCH_3$	N	88	334 dec	$CHCl_3$-EtOAc	$C_{15}H_{14}N_4O_4 \cdot 0.5CHCl_3 \cdot 0.5H_2O$	C, H, N	(3)[a]	nt[b]
7mb	H	C(=O)NHEt	N	18	237-238 dec	MeOH	$C_{16}H_{16}N_4O_4 \cdot HCl \cdot 2H_2O$	*e*	(3)[a]	nt[b]
7mc	H	C(=O)NHOH	N	55	337 dec	EtOH	$C_{14}H_{12}N_4O_5 \cdot 3HCl$	H, N	<3	(60)[a]
7md	H	$C(=O)NHCH_2CO_2Et$	N	90	281-282 dec	$CHCl_3$	$C_{18}H_{18}N_4O_6 \cdot 2HCl$	C, N	6	nt[b]
6m	H	H		63	311-312	DMF	$C_{13}H_{11}N_3O_3$	C, H	(3)[a]	nt[b]
6ma	H	CH_3	J		301 dec	EtOH/$CHCl_3$	$C_{15}H_{15}N_3O_3 \cdot 0.33CHCl_3$	C, H[g]	0.4	1.0
6mb	H	CH_2CH_3	J	39	293 dec	EtOH	$C_{15}H_{15}N_3O_3$	*h*	3	(60)[a]
6mc	H	CF_3	J	25	>310	DMF	$C_{14}H_{10}F_3N_3O_3$	C, H, N	>10[d]	nt[b]
6md	H	$C(=O)CH_3$	K		300 dec	EtOH/$CHCl_3$	$C_{15}H_{13}N_3O_4$	*h*	2	(10)[a]
6me	H	Ph	L	27	>310	DMF	$C_{19}H_{15}N_3O_3$	C, H, N	(3)[a]	nt[b]
6mf	H	OH	*c*		359-360 dec		$C_{13}H_{11}N_3O_4 \cdot HOAc$		(3)[a]	nt[b]
15ma	CH_3	CO_2Et	R	61	212 dec	EtOH	$C_{17}H_{17}N_3O_5$	C, H, N	0.8	(30)[a]
15mb	CH_2CO_2Et	CO_2Et	R	43	198-199 dec	EtOH-$CHCl_3$	$C_{20}H_{21}N_3O_7$	C, H, N	(3)[a]	nt[b]
15mc	$(CH_2)_3$-CO_2Et	CO_2Et	R	29	144-146	PhH	$C_{22}H_{25}N_3O_7$	C, H, N	0.1	(10)[a]

[a] Numbers in parentheses indicate highest dose at which the compound was tested and found to be inactive. [b] Not tested. [c] W. Borsche, M. Wagner-Roemmich, and J. Barthenheier, *Justus Liebigs Ann. Chem.*, 550, 160 (1942). [d] Tested ip. [e] C: calcd, 47.95; found, 48.41. H: calcd, 5.28; found, 4.79; N: calcd, 13.98; found, 13.52. [f] C: calcd, 48.38; found, 49.97. H: calcd, 3.50; found, 3.91. N: calcd, 15.57; found, 16.25. [g] N: calcd, 13.51; found, 13.95. TLC [CH_2Cl_2/EtOH (9:1)] one spot, R_f 0.26. [h] See synthetic procedure under the Experimental Section.

Table VI. Physical Properties of α-Cyano-β-(2-nitrophenyl)acrylamides



no.	R	yield, %	mp, °C	solvent	formula	anal.
3c	5-Cl	58	187-189	MeOH	$C_{10}H_6ClN_3O_3$	C, H, N
3d	5-F	65	162-166	EtOH	$C_{10}H_6FN_3O_3$	*a*
3e	4-F	83	177-178	CH_2Cl_2/*i*-Pr_2O	$C_{10}H_6FN_3O_3$	C, H, N
3g	5-MeO	86	207-208 dec	MeOH	$C_{11}H_9N_3O_4$	*a*
3h	5-$PhCH_2O$	48	124-126	MeOH	$C_{17}H_{13}N_3O_4$	*a*
3i	5-MeS	88	230-231	DMF·H_2O	$C_{11}H_9N_3O_3S$	*a*
3k	5-*p*-MeOPhS	53	179-182	DMF·H_2O	$C_{17}H_{13}N_3O_4S$	*a*
3l	4-MeO	95	151-152 dec	EtOH	$C_{11}H_9N_3O_4$	*a*
3m	4,5-$(MeO)_2$	93	265-266	MeOH	$C_{12}H_{11}N_3O_5$	C, H, N
3n	3,4-$(MeO)_2$	64	194-195	MeOH	$C_{12}H_{11}N_3O_5$	*a*
3o	3,5-$(MeO)_2$	20[b]	231-232 dec	MeOH	$C_{12}H_{11}N_3O_5$	*a*
3p	3,6-$(MeO)_2$	83	247-248 dec	MeOH	$C_{12}H_{11}N_3O_5$	*a*
3q	3,4,5-$(MeO)_3$	74	244.5 dec	MeCN	$C_{13}H_{13}N_3O_6$	C, H, N
3s	4,5-OCH_2CH_2O	98	301.5 dec	MeOH	$C_{12}H_9N_3O_5$	C, H, N
3t	4-EtO, 5-MeO	62	242-243 dec	MeOH	$C_{13}H_{13}N_3O_5$	*a*
3u	4-MeO, 5-EtO	85	244-245 dec	MeOH	$C_{13}H_{13}N_3O_5$	*a*
3v	4,5-$(EtO)_2$	79	191-193 dec	MeOH	$C_{14}H_{15}N_3O_5$	*a*
3w	4-BuO, 5-EtO	54		MeOH	$C_{16}H_{19}N_3O_5$	*a*
3x	4-MeO, 5-BzO	46	157-158	EtOH	$C_{18}H_{15}N_3O_5$	*a*
3y	4-BzO, 5-MeO	52	182-183	MeOH	$C_{18}H_{15}N_3O_5$	*a*
3z	4-BzO, 5-EtO	73	167-168	MeOH	$C_{19}H_{17}N_3O_5$	*a*

[a] Not analyzed; TLC indicated sample was sufficiently pure to use in the next step of the synthesis. [b] Refluxed for 18 h.

to the free acid is supported by the fact that carboxamides, which are much more resistant to hydrolysis, were only weakly active (**7a**, **7m**, **7mc**, and **7md**) or inactive (**7ab**, **7ma** and **7mb**). Of interest, the 2-methyl analogue (**6ma**) exhibited oral activity similar to that of the ethyl ester, **5m**, although **6ma** was much less potent intravenously. Comparison of the 2-hydrogen (**6m**), 2-hydroxy (**6mf**), 2-(trifluoromethyl) (**6mc**), and 2-phenyl (**6me**) analogues, all of which were inactive, with **6ma** suggests that the activity of **6ma** may be due to metabolic oxidation to acid **8m**.[11] Consistent with this hypothesis is the fact that the 2-ethyl (**6mb**) and 2-acetyl (**6md**) derivatives were considerably less active intravenously and lacked oral activity.

Substitution at the 3 position caused a dramatic decrease in activity, which is exemplified by the 3-methyl (**15ma**) and 3-(carboethoxyalkyl) (**15mb** and **15mc**) analogues, both of which were at least 15 times less potent than **5m** intravenously and were devoid of oral activity.

Detailed pharmacological characterization of **5a** and **5m** confirms that these compounds have a DSCG-like mechanism of action. For example, doses of **5m** as high as 1.0 mg/kg iv or 60 mg/kg po did not antagonize the changes in vascular permeability induced by intradermal injections of histamine or serotonin. Compound **5m** inhibited plasma histamine increases induced by antigen challenge in rats passively sensitized with homologous antisera. At 0.1 mg/kg iv, **5m** produced 92% inhibition of histamine release, while DSCG displayed a similar degree of inhibition at a dose of 3 mg/kg. The bronchoconstrictive effect of a histamine aerosol in conscious guinea pigs was unaltered by **5m** at a dose of 30 mg/kg iv. This indicates that the blockade of the reagin-induced PCA reaction by **5m** is due to inhibition of mediator release.

In summary, synthetic variations in a series of 3,4-dihydro-4-oxopyrimido[4,5-*b*]quinoline-2-carboxylic acid esters led to compounds displaying intravenous activity 400 times greater than DSCG. The most potent compounds were those bearing 7-ethoxy-8-methoxy (**5u**) and 7-hydroxy-8-methoxy (**13a**) substitution patterns. Ethyl esters **5m** and **5t–v** exhibit optimal oral activity, and several of these compounds are comparable in potency to other recently reported orally active agents. Although highly potent intravenously, hydroxy-substituted analogues display weak oral activity. The 2-methyl derivative (**6ma**) also demonstrates potent oral activity, suggesting that the 2-methyl substituent may be oxidized in vivo to a 2-carboxylic acid. Carboxylic acids are only moderately active orally but are as potent as their corresponding ethyl esters when administered intravenously. Pharmacological studies confirm that 3,4-dihydro-4-oxopyrimido[4,5-*b*]-quinoline-2-carboxylic acid derivatives inhibit the release of the mediators of anaphylaxis.

Experimental Procedures

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Microanalyses were performed by the Analytical Department of Pfizer, Inc. Where analyses are indicated only by symbols of the elements, analytical values were within ±0.4% of theoretical values. NMR and/or mass spectra were obtained on all compounds and were consistent with structures and assignments. NMR spectra were recorded on either a Varian A-60 or T-60 spectrometer, and the mass spectra were obtained on a Hitachi Perkin-Elmer RMU-6E spectrometer. Representative procedures for the preparation of compounds **2–15** (Scheme I) are illustrated below. Capital letters designate the step of the synthetic procedure as given in Scheme I and in Tables I–V. TLC was performed on silica gel.

Procedure A. 2-Aminobenzaldehyde (**2a**), 2-amino-6-chlorobenzaldehyde (**2b**), and 2-amino-4,5-(methylenedioxy)-benzaldehyde (**2m**) were prepared by reduction of the respective 2-nitrobenzaldehydes (**1**) using literature procedures.[12] The *o*-nitrobenzaldehydes were prepared by conventional routes.[13-15]

Procedure B. Preparation of α-Cyano-β-(2-nitro-4,5-di-

(11) H. Novack, G. Schorre, and R. Struller, *Arzneim.-Forsch.*, 16, 407 (1966).

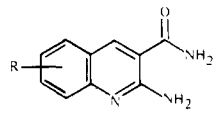
(12) E. I. Smith and J. W. Opie in "Organic Syntheses", Collect. Vol. III, E. C. Horning, Ed., Wiley, New York, 1955, p 56.

(13) K. W. Merz and J. Fink, *Arch. Pharm. (Weinheim)*, **289**, 347 (1956).

(14) M. Julia, P. Manoury, and M. E. Voillaume, *Bull. Soc. Chim. Fr.*, 1417 (1965).

(15) C. A. Fetscher in "Organic Syntheses", Collect. Vol. IV, N. Rabjohn, Ed., Wiley, New York, 1963, p 735.

Table VII. Physical Properties of 2-Aminoquinoline-3-carboxamides



no.	R	synth proc	yield, %	mp, °C	solvent	formula	anal.
4a	H	C	84	240–242	EtOH	$C_{10}H_9N_3O$	*a*
4b	5-Cl	C	77	270–273 dec	EtOH	$C_{10}H_8ClN_3O$	C, H, N
4c	6-Cl	D	56	249–250	aq NaOH	$C_{10}H_8ClN_3O$	C, H, N
4d	6-F	D	90	232–236	EtOAc	$C_{10}H_8FN_3O$	H, N[b]
4e	7-F	D	57	267–268	*i*-PrOH	$C_{10}H_8FN_3O$	H, N[c]
4f	4-Ph	*h*		248–250[d]	EtOAc	$C_{16}H_{13}N_3O$	*a*
4g	6-MeO	D	80	231–232 dec	aq NaOH, MeOH	$C_{11}H_{11}N_3O_2$	C, H, N
4h	6-$PhCH_2O$	D	94	238–239	aq NaOH	$C_{17}H_{15}N_3O_2$	*e*
4i	6-MeS	D	56	248–250	aq NaOH	$C_{11}H_{11}N_3OS$	*e*
4k	6-*p*-MePhS-	D	80	236–239	aq NaOH	$C_{17}H_{15}N_3O_2S$	C, H, N
4l	7-MeO	D	82	281–282 dec	aq NaOH	$C_{11}H_{11}N_3O_2$	C, H, N
4m	6,7-$(MeO)_2$	D	84	274–275	aq NaOH	$C_{12}H_{13}N_3O_3$	C, H, N
4n	7,8-$(MeO)_2$	D	50	218–219	aq NaOH	$C_{12}H_{13}N_3O_3$	*e*
4o	6,8-$(MeO)_2$	D	52	284–286 dec	aq NaOH	$C_{12}H_{13}N_3O_3$	*e*
4p	5,8-$(MeO)_2$	D	79	263–264 dec	aq NaOH	$C_{12}H_{15}N_3O_3$	C, H, N
4q	6,7,8-$(MeO)_3$	D	83	240–241.5 dec	$PhNO_2$	$C_{13}H_{17}N_3O_4$	C, H[f]
4r	6,7-OCH_2O	C	89	308 dec	HOAc	$C_{11}H_9N_3O_3 \cdot 2CH_3CO_2H$	H, N[g]
4s	6,7-OCH_2CH_2O	D	27	269.5 dec	DMF–*i*-PrOH	$C_{12}H_{11}N_3O_3$	C, H, N
4t	6-MeO, 7-EtO	D	84	234–235 dec	aq NaOH	$C_{13}H_{15}N_3O_3$	C, H, N
4u	6-EtO, 7-MeO	D	87	263–264 dec	aq NaOH	$C_{13}H_{15}N_3O_3$	C, H, N
4v	6,7-$(EtO)_2$	D	91	243–244 dec	aq NaOH	$C_{14}H_{17}N_3O_3$	*e*
4w	6-EtO, 7-BuO	D	85	225	aq NaOH	$C_{16}H_{21}N_3O_3$	*e*
4y	6-MeO, 7-$PhCH_2O$	D	95	282–283 dec	aq NaOH	$C_{18}H_{17}N_3O_3$	*e*
4z	6-EtO, 7-$PhCH_2O$	D	73	279–280 dec	$CHCl_3$/MeOH	$C_{19}H_{19}N_3O_3$	C, H, N

[a] Known compound. [b] C: calcd, 50.88; found, 49.90.. [c] C: calcd, 58.53; found, 58.01. [d] Lit. mp 248–251. [e] Analogue not analyzed. [f] N: calcd, 15.16; found, 15.57. [g] C: calcd, 51.28; found, 50.68. [h] Reference 4.

methoxyphenyl)acrylamide (3m). Piperidine (2.1 g, 23.7 mmol) and 2-cyanoacetamide (22.0 g, 262 mmol) were added to a slurry of 6-nitroveratraldehyde (50.0 g, 237 mmol) in methanol (500 mL). The mixture was heated to reflux for 2 h and then cooled in an ice bath and filtered. The bright-yellow filter cake was washed with cold *i*-PrOH (300 mL) and then air-dried to yield 60.1 g (93%) of **3m**: mp 265–266 °C. Anal. ($C_{12}H_{11}N_3O_5$) C, H, N. Compound **3f** was prepared from *o*-nitroacetophenone. TLC indicated these compounds were sufficiently pure for use in the next step. Except for **3i** and **3k**, the compounds in Table VI were prepared as described above.

Preparation of α-Cyano-β-[2-nitro-5-(methylthio)-phenyl]acrylamide (3i). A solution of the sodium salt of MeSH (2.78 g, 39.8 mmol) in DMF (50 mL) was prepared by bubbling MeSH into a mixture of NaH (1.67 g of 57% NaH) in DMF (50 mL). The mixture was cooled by means of an ice bath until the reaction was complete. The NaSMe solution was then added dropwise to a mixture of α-cyano-β-(2-nitro-5-chlorophenyl)-acrylamide, **3c** (10 g, 39.8 mmol), in DMF (34 mL) cooled in an ice bath. After stirring for 1 h, the mixture was removed from the ice bath, stirred for an additional 2 h, and then poured into water (600 mL). The resulting mixture was thoroughly stirred and Et_2O (30 mL) was added. The resulting precipitate was filtered, washed with Et_2O, and dried to yield 8.4 g of **3i**, mp 227–229 °C. Compound **3k** was prepared in a similar manner, using the sodium salt of *p*-methoxyphenyl mercaptan.

Procedure C. Preparation of 6,7-(Methylenedioxy)-2-aminoquinoline-3-carboxamide (4r). A mixture of 4,5-(methylenedioxy)-2-aminobenzaldehyde (5.0 g, 0.03 mol) and 2-cyanoacetamide (2.50 g, 0.03 mol) was added to NaOMe (1.90 g, 0.03 mol) in MeOH (50 mL). The mixture was heated to reflux for 15 min and then cooled in an ice bath. The resulting bright-yellow solid was filtered and recrystallized from acetic acid to give 6.18 g (89.3%) of the desired product: mp 308 °C dec; MS M^+ 231. Compounds **4a** and **4b** (Table VII) were prepared in an analogous manner.

Procedure D. Preparation of 6,7-Dimethoxy-2-aminoquinoline-3-carboxamide (4m). Over a period of 0.5 h, iron powder (65.2 g, 1.22 mol) was added to a slurry of **3m** (75.0 g, 0.27 mol) in a 50% solution of HOAc–DMF (750 mL) at 75 °C. When addition of the iron powder was complete, the mixture was heated to 90 °C for 4 h and then filtered while hot. The filter cake was washed with hot HOAc (150 mL). The dark red filtrate was added to 1 N HCl (1500 mL), the pink precipitate was recovered by filtration and dissolved in water, and the aqueous solution was made alkaline with 10% NaOH. The solid was filtered, washed with cold *i*-PrOH, and dried to give 57.1 g (83%) of **4m** as yellow crystals, mp 274–275 °C. Anal. ($C_{12}H_{15}N_3O_3$) C, H, N. The physical properties of other compounds of structure 4 prepared by this method and procedure C are reported in Table VII.

Procedure E. Preparation of Ethyl 7,8-Dimethoxy-3,4-dihydro-4-oxopyrimido[4,5-*b*]quinoline-2-carboxylate (5m). To a round-bottom flask equipped with a stirrer, reflux condenser, and Dean–Stark apparatus and containing a mixture of diethyl oxalate (50 mL) and xylene (80 mL) at reflux was added 6,7-dimethoxy-2-aminoquinoline-3-carboxamide (**4m**; 3.0 g, 12 mmol). Xylene, H_2O, and EtOH were distilled over a 4-h period. When all the xylene was removed, the reaction mixture was cooled to about 100 °C and then slowly poured into $CHCl_3$ (300 mL). The $CHCl_3$ solution was cooled and the brown precipitate which formed was removed by filtration. The filtrate was decolorized with charcoal, concentrated, and chilled to give a crystalline solid. This was dissolved in hot chloroform, and the solution was treated with charcoal, filtered, and concentrated to give 0.59 g (15%) of pale yellow crystals, mp 272–273 °C dec. Anal. ($C_{16}H_{15}N_3O_5$) H, N; C: calcd, 58.41; found, 57.91. Alternatively, this procedure (E) is performed with a catalytic amount of acid (designated as procedure Ea in Tables II and III).

Procedure F. Preparation of Ethyl 6,7-(Methylenedioxy)-3,4-dihydro-4-oxopyrimido[4,5-*b*]quinoline-2-carboxylate (5r). A mixture of **4r** (1.50 g, 6.5 mmol), NaOMe (0.05 g, 1.25 mmol), and diethyl oxalate (150 mL) was heated to 150 °C for 3.5 h. The mixture was then cooled to room temperature, and the precipitated product was recovered by filtration and purified by chromatography on silica gel using $CHCl_3$–MeOH (99:1) as eluant. Evaporation of the eluant and recrystallization of the residue from ethanol yielded 0.414 g (20.4%) of product **5r**, mp 267 °C dec. Anal. ($C_{15}H_{11}N_3O_5$) C, H, N.

Procedure G. Preparation of Ethyl 7-(Methylsulfinyl)-3,4-dihydro-4-oxopyrimido[4,5-*b*]quinoline-2-

carboxylate (5j). A solution of 5i (315 mg, 1.0 mmol) in TFAA (2 mL) was heated to 55 °C. H_2O_2 (113 mg of 30% H_2O_2, 1 mmol) was added and the solution was stirred for 10 min. After the solution was cooled to room temperature, absolute ethanol (6 mL) was added, and the resulting yellow precipitate was filtered, washed with Et_2O, and air-dried. Recrystallization from absolute ethanol gave 185 mg (65%) of product 5j, mp 257–259 °C. Anal. ($C_{15}H_{13}N_3O_4S$) C, H, N.

Procedure H. Preparation of 2-Hydroxyethyl 7,8-Dimethoxy-3,4-dihydro-4-oxopyrimido[4,5-*b*]quinoline-2-carboxylate (5ma). Triethylamine (1 mL) was added to a slurry of 5m (500 mg) in ethylene glycol (5 mL), and the mixture was stirred for 6 h. After dilution with H_2O (30 mL), the resulting clear yellow solution was acidified with acetic acid, and the precipitate was filtered and recrystallized from DMF (20 mL) to yield 285 mg (53.5%) of product, mp 252 °C. Anal. ($C_{16}H_{15}N_3O_6$) C, H, N.

Procedure I. Preparation of *n*-Butyl 3,4-Dihydro-4-oxopyrimido[4,5-*b*]quinoline-2-carboxylate (5ab). A 3.0-g portion (1.12 mmol) of 5a was added to *n*-BuOH (350 mL) containing 10 drops of concentrated HCl. The mixture was refluxed for 36 h, cooled, and filtered. The solid was dissolved in hot $CHCl_3$, the insoluble matter was removed by filtration, and the filtrate was concentrated to provide 1.19 g (36% yield) of the *n*-butyl ester 5ab, mp 218–219.5 °C. Anal. ($C_{16}H_{15}N_3O_3$) H; C: calcd, 64.71; found, 64.12.

Procedure J. Preparation of 2-Ethyl-7,8-dimethoxypyrimido[4,5-*b*]quinolin-4(3*H*)-one (6mb). Following the procedures of Taylor and Kalenda,[6] concentrated H_2SO_4 (0.5 mL) was added to a stirred mixture of 4m (500 mg, 2.0 mmol) in propionic anhydride (10 mL) at 60 °C. The reaction mixture was stirred for 1 h and then cooled to room temperature and added to water (25 mL). The aqueous mixture was stirred and made basic with 6 N NaOH, stirred overnight, and then acidified to pH 5.0 with 10% HCl. The yellow precipitate was filtered and recrystallized from EtOH to give 283 mg of 6mb, mp 290–291 °C dec. A second crop of product separated on standing. The combined crops were recrystallized from $CHCl_3$–EtOH (1:1) to yield 225 mg (39%) of product: mp 293 °C; TLC [CH_2Cl_2/EtOH (9:1)] one spot, R_f 0.6; MS *m/e* 285 (parent).

Procedure K. Preparation of 2-Acetyl-7,8-dimethoxypyrimido[4,5-*b*]quinolin-4(3*H*)-one (6md). To a solution of SeO_2 (24.5 mg, 2.2 mmol) in dioxane–water (11 mL of 10:1) was added 2-ethyl-7,8-dimethoxypyrimido[4,5-*b*]quinolin-4(3*H*)-one (6mb; 125 mg, 4.4 mmol). The mixture was heated to reflux for 48 h, after which more SeO_2 (24.5 mg) was added and refluxing continued for an additional 24 h. The mixture was cooled, the Se was filtered off, and the filtrate was concentrated to dryness. The residue was taken up in EtOH–CH_2Cl_2 (1:99) and chromatographed on a column of silica gel using the same solvent as eluant (250 mL), followed by EtOH–CH_2Cl_2 (2:98). Concentration of the second eluate (625 mL) gave a yellow solid (23 mg): mp 300 °C; TLC [CH_2Cl_2/EtOH (9:1)] one spot, R_f 0.55; MS *m/e* 299 (parent); IR 1740 and 1695 cm^{-1} (C=O). Anal. Calcd for $C_{15}H_{13}N_3O_4 \cdot CH_3CH_2OH \cdot H_2O$: C, 56.12; H, 5.82. Found: C, 55.58; H, 5.38.

Procedure L. Preparation of 2-Phenyl-7,8-dimethoxypyrimido[4,5-*b*]quinolin-4(3*H*)-one (6me). A 325-mg (1.4 mmol) portion of 2-amino-3-cyano-6,7-dimethoxyquinoline, prepared by adaptation of procedures of Campaigne and Randau,[4] was allowed to reflux for 5 h after the addition of 475 mg (2.1 mmol) of benzoic anhydride in 10 mL of pyridine. The mixture was cooled, poured into 100 mL of ice–water, filtered, and dried to give 390 mg of a tan solid, mg 229–232 °C. This solid was slurried in 10 mL of EtOH, cooled in an ice bath, saturated with HCl, and then stirred at room temperature for 3 h. Concentrated HCl (5 mL) was added and the reaction mixture heated on a steam bath for 0.5 h. Dilution with H_2O gave a precipitate, which was filtered, slurried with saturated $NaHCO_3$, refiltered, and recrystallized from DMF to yield 95 mg of a pale yellow solid, mp >310 °C. Anal. ($C_{19}H_{15}N_3O_3$) C, H, N.

Procedure M. Preparation of 3,4-Dihydro-4-oxopyrimido[4,5-*b*]quinoline-2-carboxamide (7a). A mixture of 2-aminoquinoline-3-carboxamide (4a) (2.0 g, 1.15 mmol) ethyl oxamate (2.71 g, 23.1 mmol), ethylene glycol (10 mL), and NaOMe (10 mg) was heated at 170 °C for 1 h. Slow addition of ice-cold MeOH (50 mL) to the hot reaction mixture, followed by chilling in an ice bath, precipitated the product, which was filtered, washed with cold methanol, and dried in vacuo to yield 768 mg (28%) of 7a, mp 320 °C.

Procedure N. Preparation of 7,8-Dimethoxy-3,4-dihydro-4-oxopyrimido[4,5-*b*]quinoline-2-carboxamide (7m). Anhydrous NH_3 was bubbled into a mixture of 5m (300 mg, 9.00 mmol) and absolute EtOH (75 mL) for 15 min. The reaction mixture was transferred to a pressure bomb (Monel) and heated overnight in an oil bath at 95 °C. The bomb was then cooled to room temperature, the contents were removed with EtOH and the reaction mixture was filtered to recover 260 mg (95%) of product: mp 310 °C; TLC [CH_2Cl_2/EtOH (9:1)] one spot, R_f 0.25.

Procedure O. Preparation of 7,8-Dimethoxy-3,4-dihydro-4-oxopyrimido[4,5-*b*]quinoline-2-carboxylic Acid (8m). Compound 5m (250 mg, 7.4 mmol) was stirred overnight in 5% aqueous NaOH (37.5 mL) at room temperature. The reaction mixture was acidified with TFAA, and the precipitate was filtered, washed with H_2O, redissolved in TFAA, and crystallized by the addition of Et_2O to yield 180 mg (54%) of 8m as the trifluoroacetate monohydrate, mp 281–283 °C. Anal. ($C_{14}H_{11}O_5N_3 \cdot CF_3COOH \cdot H_2O$): C, H, N; F: calcd, 13.15; found, 13.75.

Procedure P. Preparation of Ethyl 7-Hydroxy-8-methoxy-3,4-dihydro-4-oxopyrimido[4,5-*b*]quinoline-2-carboxylate Trifluoroacetate Hemihydrate (13a). A solution of 5x (250 mg, 0.618 mmol) in TFAA (5 mL) was refluxed for 2.5 h. The reaction mixture was then poured into ether (25 mL), and the resulting bright yellow precipitate was recovered by filtration, washed with ether, and dried to yield 194 mg (72%) of 13a, mp 279 °C. Anal. ($C_{15}H_{13}N_3O_5 \cdot CF_3COOH \cdot 0.5H_2O$) C, H, N.

Procedure Q. Preparation of Ethyl 7-Acetoxy-8-methoxy-3,4-dihydro-4-oxopyrimido[4,5-*b*]quinoline-2-carboxylate *p*-Toluenesulfonate (14a). A mixture of Ac_2O (4 mL), 13a (250 mg, 0.572 mmol), and *p*-TSA (100 mg, 0.572 mmol) was heated at 100 °C for 24 h. The Ac_2O was then stripped from the reaction mixture in vacuo. The solid residue was dissolved in hot $CHCl_3$ and the solution decolorized with activated charcoal. Benzene (4 volumes) was added to the decolorized solution, which was chilled in ice to yield crystals. These were recovered by filtration and air-dried to give 174 mg (58%) of product, mp 215–217 °C. Anal. ($C_{17}H_{15}O_6N_3 \cdot C_7H_8O_3S$) H; C: calcd, 54.43; found, 53.74; N: calcd, 7.98; found, 7.24.

Procedure R. Preparation of Ethyl 3-Methyl-7,8-dimethoxy-3,4-dihydro-4-oxopyrimido[4,5-*b*]quinoline-2-carboxylate (15a). NaH (470 mg of 50% in oil, 11 mmol) was added to a slurry of 5m (3.3 g, 0.01 mol) in DMF (75 mL). The mixture was stirred and heated on a steam bath for 10 min and then at room temperature for 0.5 h. It was cooled in an ice bath and MeI (2.1 g, 15 mmol) was added dropwise over a 0.5-h period. Following completion of the addition, the mixture was stirred for an additional 15 min in an ice bath and then for 30 min at room temperature before pouring into ice–water (200 mL). The resulting solid was filtered, air-dried, and recrystallized from EtOH to give 2.1 g (61.2%) of yellow crystals, mp 211.5 °C dec. Anal. ($C_{17}H_{17}N_3O_5$) C, H, N.

Pharmacological Procedures. Passive Cutaneous Anaphylaxis (PCA). The ability of agents to interfere with PCA reactions was measured in male Charles River CD rats, 170–210 g. Reaginic antisera were prepared according to Mota,[16] using hen egg albumin and *B. pertussis*, or according to Petillo and Smith,[17] using hen egg albumin and $Al(OH)_3$. Forty-eight hours prior to antigen challenge, the reaginic antiserum was injected intradermally into the shaved skin of a normal rat's back; 60 μg of histamine dihydrochloride and 0.5 μg of serotonin–creatinine sulfate were injected id just prior to antigen challenge as controls for antihistaminic, antiserotonin, or unspecific types of blockade. The intravenous challenge of 5 mg of egg albumin and 2.5 mg of Evan's Blue dye was injected immediately after intravenously administered drugs and 5 min after orally administed drugs. Thirty minutes later, animals were asphyxiated using chloroform, and the skin of the back was reversed for observation. A score

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equal to the product of the diameter of the dye site in millimeters and a grade of 0.5, 1, 2, 3, or 4, which was proportional to intensity of dye coloration, were assigned each injection site. The scores of a given injection site were summed for each group (n) of five or seven animals and compared to the control. The difference was expressed as percent inhibition. For compounds with an iv ED_{50} greater than 0.01 mg/kg, n was usually small and the ED_{50} was estimated from a dose-response curve fitted by eye. For compounds of greater potency, n was larger and the ED_{50} was calculated from the least-squares regression line. ED_{50} values differing by a factor of 3 or more are significantly different. The method is easily reproducible in other laboratories, as judged by the ED_{50} value of ~1 mg/kg reported by many for DSCG; we observed an ED_{50} value of 0.8, n = 25.

Rat Plasma Histamine Procedure. Hyperimmune antisera to chicken egg albumin (crystallized five times, Pentex) were prepared according to Orange, Valentine, and Austen[18] and 0.5 mL was injected iv into normal male Charles River CD rats 20 h before challenge. Control animals were injected with normal rat sera 20 h before challenge or were injected with hyperimmune sera but given saline in place of antigen challenge. Animals were deprived of food, but not water, after being passively sensitized. Animals were anesthetized 20 h later with diabutal, 40 mg/kg ip. Drug or saline was injected into the inferior vena cava 1 min prior to challenge with 5 mg of egg albumin. Five minutes after challenge, 3 mL of blood was withdrawn from the inferior vena cava into a syringe containing 0.3 mL of 3% sodium citrate. The histamine concentration of the plasma was determined by the method of Shore, Burkhalter, and Cohn.[19]

Guinea Pig Histamine Aerosol Procedure. Bronchodilator activity was evaluated according to the method of Van Arman, Miller, and O'Malley[20] in conscious female Reed-Willet guinea pigs (200–250 g) fasted overnight. One minute following iv administration of saline or the test drug in saline, each animal was challenged with histamine aerosol as follows: a 0.4% aqueous solution of histamine was placed in a Vaponephrine standard nebulizer (Vaponephrine Co., Edison, N.J.) and sprayed under an air pressure of 6 psi into a closed 8 × 8 × 12 in. transparent plastic container for 1 min. Immediately thereafter, the guinea pig was placed in the container. The respiratory status of the guinea pig after 1 min in the container was scored as follows: 0, normal breathing; 1, slightly deepened breathing; 2, labored breathing; 3, severely labored breathing and ataxia; 4, unconsciousness. The scores for a control group and a test group (eight animals per group) were summed and compared, and the difference was expressed as percent protection.

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Potential Antitumor Agents. 33. Quantitative Structure–Activity Relationships for Mutagenic Activity and Antitumor Activity of Substituted 4′-(9-Acridinylamino)methanesulfonanilide Derivatives

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A series of substituted 4′-(9-acridinylamino)methanesulfonanilide (AMSA) derivatives have been tested for mutagenicity using *Salmonella typhimurium* strain TA 1537 and for antitumor activity against the L1210 leukemia in mice. Two measures of mutagenic activity were determined and quantitative structure–activity relationships (QSAR) developed for them. M_{50}, the percentage of drug-induced mutant colonies observed at the concentration providing 50% inhibition of bacterial growth, is a measure of mutagenic efficiency. The lowest molar drug concentration ($1/C$) needed to induce a fixed proportion of revertants (chosen as 50 per 10^8 bacteria) is a measure of mutagenic effectiveness. The two measures of antitumor activity modeled were ILS_{max} (the percent increase in life span observed for each derivative at its LD_{10} dose), a measure of tumor cell selectivity, and $1/D_{40}$ (the dose of drug to provide an ILS of 40%), a measure of dose potency. These measures of bioactivity were intercompared and modeled in terms of a number of drug physicochemical properties. The results show that drug lipophilic/hydrophilic balance is the dominant factor in determining both mutagenic and antitumor activity, although other factors are involved. The two different types of activity can be readily separated in the AMSA drug series by appropriate choice of substituent and adjustment of overall drug lipophilic/hydrophilic balance.

The current successes of chemotherapy in the treatment of cancer have brought their attendant problems. As more patients are provided with longer periods of remission (in many cases achieving a normal life span), there is an increasing literature[1] describing the onset of drug-induced secondary cancer in patients treated with a number of chemotherapeutic drugs. Since almost all of the antitumor agents currently in use are carcinogenic to some degree,[2] the need to separate antitumor activity and carcinogenicity and provide effective but noncarcinogenic antitumor agents has become one of the major tasks facing the drug designers.

Since animal carcinogenicity testing is too costly to carry out on a routine basis, attention has focused on bacterial mutagenicity tests as suitable systems for quantitative evaluation of carcinogenic risks. There is currently a brisk discussion concerning what relevance results from such tests might have for predicting carcinogenic risks for humans.[3,4] However, these tests do measure the results of events which take place at the molecular level in similar ways in both bacterial and mammalian cells. By estimating the ability of a chemical to be active in the first stage

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