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Antiallergy Activity of Substituted 11-Oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxylic Acids

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A series of substituted 11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxylic acids were prepared and evaluated as antiallergy agents. Several analogues were orally active. 2-Methyl-11-oxo-11*H*-pyrido[2,1-*b*]quinoazoline-8-carboxylic acid (**6**) was superior to cromolyn sodium and doxantrazole orally and intravenously in the rat PCA test and a rat allergic bronchospasm model.

A new orally active antiallergy agent was recently reported having the structure 11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxylic acid.[1] A series of substituted 11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxylic acids have been prepared and compared with two clinically effective antiallergy agents, cromolyn sodium and doxantrazole. While cromolyn sodium is ineffective orally, it is useful insufflated as a powder. Doxantrazole has been reported to be an orally active antiallergy agent in humans.[2]

The reversible narrowing of bronchial airways and accompanying edema in bronchial mucosa observed in asthma may be caused by a specific allergic response or by a nonspecific irritant. β-Adrenergic bronchodilators, anticholinergics, theophylline, or steroids are useful for symptomatic relief. An alternate approach is the use of an antiallergy agent, such as cromolyn sodium, which appears to act by preventing the release of histamine and other possible allergic mediators resulting from antibody–antigen interactions.[3]

We report the synthesis and effects of substitution on the antiallergy activity of a series of oxopyrido[2,1-*b*]quinazoline-8-carboxylic acids.

Chemistry. The 11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxylic acids were prepared in one step from 6-chloronicotinic acid and the appropriately substituted anthranilic acid by refluxing the mixture in glacial acetic acid[4] (method A) or ethanol containing hydrochloric acid (method B). Compounds **8** and **18** were prepared from their corresponding methoxy analogues **10** and **16** utilizing a pyridine hydrochloride fusion method (method C).[5]

The alkoxy derivatives **11**–**15** were prepared from **4** by alkylation with an alkyl halide and potassium carbonate (method D).

Discussion

The substituted pyrido[2,1-*b*]quinazolinones were evaluated for their antiallergy activity by their ability to inhibit passive cutaneous anaphylaxis (PCA) in rats as described in the Experimental Section.[6] Those agents which showed an inhibition greater than 50% at 0.5 mg/kg iv were studied further for their ID_{50} and oral efficacy. Since the 8-carboxylic acid **1** was more active than the corresponding 6 and 7 isomers,[1] the effects of aromatic substitution upon biological activity were studied in a series of 8-carboxylic acids. The activities are summarized in Table I.

The most potent analogues of the series were **4**–**6**, **13**, and **16**. However, **4**, **13**, and **16** were less active orally compared with **5** and **6**. Replacement of the methoxy group of **5** by ethoxy, hydroxyethoxy, propoxy, butoxy or allyloxy (**11**–**15**) resulted in reduced oral activity. In the rat PCA test, **6** is about 30 times more potent than cromolyn sodium and doxantrazole intravenously (Table I). Orally, **6** is 4–6.7 times better than doxantrazole, while cromolyn sodium is inactive orally. When analogue **6** was compared with cromolyn sodium and doxantrazole in the rat allergic bronchospasm model,[7] **6** was ten times more potent orally than doxantrazole in preventing death in 50% of the animals (ID_{50}) and 4.6 times better in preventing the fall in respiratory flow rate by 40% (ID_{40}). All three agents effectively inhibit the anaphylactic histamine release from rat mast cells[6,9] (Table II).

Analogue **6** is not a histamine antagonist, since it did not inhibit histamine-induced contractions of the guinea pig ileum[8] at concentrations up to 10^{-4} M.

In summary, several 11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxylic acids are orally active antiallergy agents which prevent anaphylactic reactions in both skin and pulmonary tissues of rats. 2-Methyl-11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxylic acid (**6**) appears to be superior to other known antiallergy agents, cromolyn sodium, and doxantrazole.

Experimental Section

Rat Reaginic Passive Cutaneous Anaphylaxis (PCA). The PCA test[6] involved immunization of rats with 1 mg of ovalbumin intramuscularly and approximately 10^{10} *B. pertussis* organisms as pertussis vaccine, intraperitoneally. Fourteen days later, the rats were bled and serum was prepared. Suitable dilutions of antiserum were injected intradermally at various sites on the back of rats 48 h before an intravenous injection of 1 mg of ovalbumin in 1 mL of physiological saline and 0.25% Evan's Blue. Thirty

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Table I. Inhibition of Rat PCA by Pyrido[2,1-*b*]quinazoline Analogues

compd	R	formula	mp, °C (dec)	recrystn solv	meth	anal.	rat PCA test: % inhibn, 0.5 mg/kg iv	rat PCA test: ID_{50},[a] mg/kg iv	rat PCA test: ID_{50},[a] mg/kg po
1	H	$C_{13}H_8N_2O_3 \cdot HCl$					45	0.5	2.5
2	2-Br	$C_{13}H_7BrN_2O_3$	346-351	pyridine	A	C, H, N, Br	34		1-2
3	2-Cl	$C_{13}H_7ClN_2O_3$	347-348	pyridine	B	C, H, N, Cl	40		
4	2-OH	$C_{13}H_8N_2O_4 \cdot {}^1/_8 H_2O$	375-378	pyridine	A	C, H, N	100	0.05	5.0
5	2-OCH_3	$C_{14}H_{10}N_2O_4$	328-330	pyridine	A	C, H, N	100	0.05	0.5
6	2-CH_3	$C_{14}H_{10}N_2O_3$	328-333	pyridine	B	C, H, N	100	0.05	0.75
7	3-Cl	$C_{13}H_7ClN_2O_3$	330-336	EtOH	A	C, H, N, Cl			5-10
8	3-OH	$C_{13}H_8N_2O_4$	374-377	pyridine	C	C, H, N	36		
9	3-CH_3	$C_{14}H_{10}N_2O_3$	307-310	pyridine	A	H, N;[b] C	54[c]		
10	3-OCH_3	$C_{14}H_{10}N_2O_4$	310-316	pyridine	B[d]	C, H, N	100[e]		>2
11	2-OC_2H_5	$C_{15}H_{12}N_2O_4$	293-296	EtOH	D	C, H, N			>1[f]
12	2-OCH_2CH_2OH	$C_{15}H_{12}N_2O_5 \cdot {}^1/_3 H_2O$	269-274	2-PrOH	D	C, H, N			>1[f]
13	2-OC_3H_7	$C_{16}H_{14}N_2O_4$	280-286	MeOH	D	C, H, N	100		2
14	2-OC_4H_9	$C_{17}H_{16}N_2O_4$	258-260	1-PrOH	D	C, H, N	88		>2
15	2-$OCH_2CH=CH_2$	$C_{16}H_{12}N_2O_4$	262-264	2-PrOH	D	C, H, N			>1[f]
16	2,3-$(OCH_3)_2$	$C_{15}H_{12}N_2O_5 \cdot 0.25H_2O$	348-353	pyridine	A[g]	C, H, N	100	0.05	10
17	2,3-$(CH_3)_2$	$C_{15}H_{12}N_2O_3$	361-364	pyridine	A[h]	C, H, N	25		2
18	2,3-$(OH)_2$	$C_{13}H_8N_2O_3 \cdot 0.25H_2O$	383-393	pyridine	C	C, H, N			>1[f]
19	2,3-$(OCH_2O)_2$	$C_{14}H_8N_2O_5$	353-361	pyridine	A[i]	C, H, N	30		
cromolyn sodium								1-2	*j*
doxantrazole								1.5	5

[a] Dose required to achieve 50% inhibition in PCA test. [b] Calcd: H, 3.97; N, 11.02. Found: H, 4.54; N, 10.56. [c] Tested at 10 mg/kg ip. [d] 4-Methoxyanthranilic acid was prepared according to ref 11. [e] Tested at 1 mg/kg iv. [f] Inactive at 1 mg/kg po. [g] 4,5-Dimethoxyanthranilic acid was prepared according to ref 12. [h] 4,5-Dimethylanthranilic acid was prepared according to ref 13. [i] 4,5-Methylenedioxyanthranilic acid was prepared according to ref 14. [j] Inactive.

Table II. Rat Experimental Allergic Bronchoconstriction

compd	PCA: ID_{50},[a] iv	PCA: ID_{100},[b] iv	PCA: ID_{50},[a] po	mast cell anaphyl hist rel: IC_{50},[c] μM	RAM,[f] bronchospasm: ID_{40}[d]	RAM,[f] death: ID_{50}[e]
6	0.05	2.5	0.75	0.94	9.3	12.5
doxantrazole	1.5	10	5	0.48	43	125
cromolyn sodium	1-2			6.5		

[a] Dose (mg/kg) required to achieve 50% inhibition in PCA test. [b] Dose (mg/kg) required to achieve 100% inhibition in PCA test. [c] Concentration required for 50% inhibition. [d] Inhibition of 40% reduction in respiratory flow rate, in mg/kg po. [e] Dose preventing death in 50% of animals, in mg/kg po. [f] Rat allergy model.

minutes later, the animals were killed in ether, the dorsal skin was reflected, and the mean orthogonal diameter was measured. For oral dosing, the drugs were suspended in 1% gum tragacanth in physiological saline and given 10–15 min before intravenous antigen challenge. For intravenous dosing, the compounds were dissolved in the saline/ovalbumin/Evan's Blue solution and given with the antigen. If necessary, the compounds were first dissolved in a slight molar excess of sodium bicarbonate and then diluted into the antigen solution. Groups of five animals were used for all dose levels and control groups.

To quantitate the PCA test, the mean diameter of each spot was graphed as a function of the relative antiserum concentration. The line, fitted by the least-squares equation, was extrapolated to the value at "zero" antiserum concentration (base value). The following equation was then used to calculate the percent inhibition:

$$\% \text{ inhibition} = \left[1 - \left(\frac{\text{diameter of drug} - \text{base value}}{\text{diameter of control} - \text{base value}}\right)\right] \times 100$$

The statistical significance of the results was determined by Student's *t* test ($p \leq 0.05$). An inhibition of 15% was significant.

Rat Experimental Allergic Bronchoconstriction. Rats were immunized, im, with 1 mg of ovalbumin adsorbed on alumina (Amphojel) in the nuchal region three times on alternate days.[7] On days 8, 9, or 10 when IgE-type antibodies were present, the animals were anesthetized with sodium pentobarbital, 60 mg/kg ip, cannulated through the trachea, and connected to a Fleisch no. 0000 pneumotachograph and a Statham pressure transducer. The animals were allowed to breathe spontaneously, and the respiratory flow rate was monitored before and after intravenous (jugular) injection with 1 mg of ovalbumin. Drugs were given orally 15 and 30 min before antigen challenge. The response to antigen was measured by both the fall in respiratory flow rate and the percentage of animals surviving bronchoconstriction for 10 min after challenge.

In Vitro Mast Cell Studies. The effect of drugs on anaphylactic histamine release from passively sensitized rat mast cells was determined.[6,9] In outline, this involved isolation of mast cells from the rat peritoneal cavity and sensitizing them with the same serum as used for PCA. The cells were then washed and resuspended in Hank's balanced salt solution with ovalbumin (20 mcg/mL) and drug. Following a 15-min incubation at 37 °C, the

supernatant solution was assayed for histamine release by a fluorometric method.[10]

Chemistry. Melting points were taken in open capillary tubes on a Mel-Temp apparatus and are uncorrected. Each analytical sample was homogeneous by TLC and had IR, UV, and NMR spectra compatible with its structure. Combustion analysis for C, H, and N and Cl or Br gave results within 0.4% of theory. The procedures for the preparation of the reported compounds are listed as methods A–D and may be considered as general methods of preparation. The reported yields for the products obtained were not maximized.

Method A. 2-Hydroxy-11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxylic Acid (4). A refluxing mixture of 10.0 g (65.5 mmol) of 5-hydroxyanthranilic acid, 10.2 g (65.5 mmol) of 6-chloronicotinic acid, and 100 mL of glacial HOAc[4] was allowed to react for 15 h. The mixture was cooled and the resultant green suspension was filtered to give 9.50 g (55.2%) of crude **4**, mp 363–370 °C dec. The powder was recrystallized from pyridine, giving 4.20 g (24.4%) of analytically pure **4**, mp 375–378 °C dec.

Method B. 2-Methyl-11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxylic Acid (6). An ethanolic mixture (80 mL) containing 5.00 g (33.1 mmol) of 5-methylanthranilic acid, 5.20 g (33.1 mmol) of 6-chloronicotinic acid, and 3.0 mL (30 mmol) of hydrochloric acid was heated at reflux for 6 h. The resultant suspension was filtered, and it gave 2.10 g (25.5%) of crude **6**, mp 322–326 °C dec. One recrystallization from pyridine gave the analytical sample: yield 1.05 g (12.3%); mp 328–333 °C dec.

Method C. 3-Hydroxy-11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxylic Acid (8). Over a period of 0.5 h, 2.00 g (7.40 mmol) of **10** was added to 10 g of melted pyridine hydrochloride.[5] The resultant mixture was heated at 220 °C for 2 h. The reaction mixture was poured into ice–H_2O (200 mL) and stirred. Filtration of the resultant precipitate yielded 1.60 g (84.2%) of crude **8**, mp 363–370 °C dec. The solid material, obtained by recrystallization from pyridine, was triturated with EtOH and gave 1.00 g (52.6%) of the analytical **8**, mp 374–379 °C dec.

Method D. 2-Ethoxy-11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxylic Acid (11). DMF (50 mL) containing 3.00 g (11.7 mmol) of **4**, 19.5 g (125 mmol) of ethyl iodide, and 2.76 g (20.0 mmol) of anhydrous K_2CO_3 was heated at 100 °C for 18 h. The resultant mixture was poured into H_2O (500 mL), and the yellow precipitate which formed was collected to give 2.35 g (70.7%) of crude **11**, mp 263–275 °C dec. The impure **11** was crystallized from MeOH to give 0.99 g (29.8%) of the analytical sample, mp 293–296 °C dec.

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Blood Glucose Lowering Sulfonamides with Asymmetric Carbon Atoms. 3.[1] Related N-Substituted Carbamoylbenzoic Acids

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Both enantiomers of 4-*N*-[1-(5-Fluoro-2-methoxyphenyl)ethyl]carbamoylmethylbenzoic acid display hypoglycemic activity. The more potent (*S*) enantiomer is approximately equipotent with the acylaminoethylbenzoic acids of the type HB 699 (Figure 1; Table I). Observations are given that suggest that these benzoic acids act at the same receptor as the hypoglycemic sulfonylureas and sulfonylaminopyrimidines and further that this receptor includes important binding sites.

Antidiabetic sulfonylamino compounds of the so-called second generation, such as glibenclamide (**2**)[2] and gliflumide [(*S*)-**5**][3] (Figure 1), display a hypoglycemic activity at much lower doses than corresponding drugs of the first generation, such as tolbutamide (**1**) and glymidine (**4**). The hypothesis was advanced that tolbutamide and glymidine are linked via their $-SO_2NH-$ group to a binding site A of a "sulfonamide" receptor, whereas, in addition, the newer drugs are bound through their –CONH– or –NHCO– group to a second binding site B and, as a result, attain higher potencies.[1,3,4]

Recently, the hypoglycemic and β-cytotropic activities of acylaminoethylbenzoic acids[5–7] have been reported; HB 699 (**3**), the most intensively investigated compound, exhibited about the same potency as tolbutamide **1**. The structure of **3** corresponds to a part of the glibenclamide molecule (part b in Figure 1). We suspect that compounds of this type act at the same receptor as the sulfonylamino compounds but via a binding site B and not via A, as in the case of tolbutamide, or via A and B, as in the case of glibenclamide.

If this assumption is correct, benzoic acid derivatives **6**, whose structure corresponds to the carbamoylmethylphenyl part b of the sulfonylaminopyrimidine **5**, should also have β-cytotropic activity. Further, compound **5** possesses an asymmetric carbon atom in this part of the molecule and (*S*)-**5** (gliflumide) has been found to be considerably more potent than (*R*)-**5**. If **6** is to occupy the same binding site B as **5**, an analogous stereospecificity of activity ought to be recognizable in the cases of (*S*)-**6** and

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