dihydrochloride (**45a**; 5.2 g, 70%), mp 233–233.5 °C dec. Anal. ($C_{13}H_{22}N_2O\cdot 2HCl$) C, H, N. Iodination with ICl in dilute HCl provided **45** (4.9 g): NMR δ 1.2 (9 H, s), 3.3 (4 H, br s), 4.5 (2 H, br s), 7.6 (2 H, br s).

1-[5-(1,1-Dimethylethyl)-2-hydroxy-3-iodophenyl]methanesulfamic Acid (46). A solution of 5-(1,1-dimethylethyl)-2-hydroxybenzaldehyde[17] (3.56 g, 20 mmol) and ammonium sulfamate (2.28 g, 20 mmol) in anhydrous MeOH (100 mL) was stirred and heated at reflux for 1.5 h. The resulting clear, yellow solution was cooled to 5 °C, treated with $NaBH_4$ (0.76 g, 20 mmol) added portionwise over 5 min, and stirred at 20 °C for 20 h. Evaporation of the solvent left a residue, which was triturated with H_2O (150 mL). The resulting mixture was cooled to 0 °C, cautiously acidified with concentrated HCl, and filtered to give 2.3 g (44.3%) of 1-[5-(1,1-dimethylethyl)-2-hydroxyphenyl]methanesulfamic acid (**46a**), mp 260–262 °C dec. Crystallization from Et_2O–THF afforded an analytical sample as colorless crystals, mp 264–265 °C dec. Anal. ($C_{11}H_{17}NO_4S$) C, H, N.

Iodination with ICl in H_2O–THF (5:1, v/v, 90 mL) provided **46** (1.9 g): NMR δ 1.2 (9 H, s), 4.3 (2 H, s), 4.10 (H, d, J = 2 Hz), 4.33 (H, d, J = 2 Hz).

***N,N,N*-Trimethyl-1-[5-(1,1-dimethylethyl)-2-hydroxy-3-iodophenyl]methanaminium Iodide (48).** Methyl iodide (5 mL) was added to a solution of **46** (3.3 g, 10 mmol) in DMF (5 mL). After standing at 20 °C for 16 h, the cloudy solution was evaporated, and the gummy residue was crystallized to give **48** (3.4 g): NMR δ 1.2 (9 H, s), 3.1 (9 H, s), 4.8 (2 H, s), 4.25 (H, d, J = 2 Hz), 4.50 (H, d, J = 2 Hz).

Ethyl [2-(Aminomethyl)-4-(1,1-dimethylethyl)-6-iodophenoxy]acetate Hydrochloride (49). This compound was prepared similarly to **20**, starting with 4-(1,1-dimethylethyl)-2-iodophenol[3] (13.8 g, 50 mmol). The crude amide was collected, air-dried, and hydrolyzed in EtOH–concentrated HCl (4:1, v/v, 250 mL) heated at reflux for 3 h. The residue obtained by evaporation was triturated with Et_2O and crystallized to provide **49** as colorless needles (3.5 g).

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Supplementary Material Available: Intravenous dog diuretic data providing the milliequivalent per minute values for Na^+, K^+ and Cl^-, along with urine volume and creatinine clearance vs. controls and time of maximum effect (2 pages). Ordering information is given on any current masthead page.

Notes

Alkaline Phosphatase Inhibition by a Series of Pyrido[2,1-*b*]quinazolines: A Possible Relationship with Cromolyn-like Antiallergy Activity

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Several known antiallergic agents, including cromolyn sodium and a series of pyrido[2,1-*b*]quinazolines, inhibit human alkaline phosphatase (ALP), a membranal enzyme associated with calcium uptake in certain tissues. A comparison of ALP and rat passive cutaneous anaphylaxis (PCA) inhibition indicates that PCA inhibition may be associated with drug–ALP interaction, since ALP inhibition potency parallels PCA inhibitory activity. The unpredictability of the PCA test toward clinical efficacy could in part be related to the uncompetitive nature of these inhibitors. The results also suggest that alkaline phosphatase may be a component of membranal calcium channels.

During the past several years, many potential antiallergy series have been reported in the literature. Except for cromolyn sodium, none have been proven efficacious enough in clinical trials to warrant introduction onto the market.[1] A major problem with the discovery of better antiallergy agents has been the apparent lack of a pharmacological model which can predict efficacy in humans. Also, a poor understanding as to the mechanism of cromolyn sodium action has contributed to this problem.

Calcium ions appear to play a major role in allergic mediator release. Several mediator release inhibitors, including bufrolin, cromolyn sodium, doxantrazole, and lodoxamide, were reported to block antigen-induced calcium ion uptake and subsequent histamine release.[2-4]

Recently, a series of structurally diverse mediator release inhibitors were reported to inhibit human leukocyte alkaline phosphatase (ALP), a membranal enzyme associated with calcium ion uptake in certain tissues.[5] We wish to report that this same series has also been evaluated against human placental alkaline phosphatase. In addition, a series of pyrido[2,1-*b*]quinazolines possessing antiallergy activity was also evaluated against human placental alkaline phosphatase. Activities as inhibitors of the enzyme and rat PCA, an allergy model, were compared for a possible relationship.

Chemistry. The synthesis of the 11-oxo-11*H*-pyrido[2,1-*b*]quinazolinecarboxylic acids in Table II has previously been reported.[6] The synthesis of the tetrazole analogues was accomplished in the following manner. Equal quantities of the substituted 2-aminobenzoic acid and 6-chloronicotinamide were reacted in refluxing ethanol

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(4) H. G. Johnson, C. A. Van Hout, and J. B. Wright, *Int. Arch. Allergy Appl. Immunol.*, **56**, 416 (1978).
(5) C. F. Schwender, *Biochem. Pharmacol.*, **30**, 217 (1981).
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Table I. Inhibition of Leukocyte vs. Placental Human Alkaline Phosphatase

compd	I_{50},[a] mM leukocyte ALP[b]	I_{50},[a] mM placenta ALP
cromolyn sodium	9.68 ± 0.52[c]	>16[d]
bricafen	2.88 ± 0.67	7.60 ± 0.80
M& B 22948[e]	1.75 ± 0.75	6.25 ± 0.25
doxantrazole	2.00 ± 0.43	5.42 ± 0.02
bufrolin	0.97 ± 0.03	2.22 ± 0.03
8	0.34 ± 0.02	0.28 ± 0.01

[a] Concentration required for 50% inhibition when substrate, *p*-nitrophenyl phosphate, is present at 10 mM. [b] Leukocyte ALP I_{50} cited in ref 5. [c] Mean ± SEM. [d] Ten percent inhibition at 16 mM. [e] 2-(2-Propoxyphenyl)-8-azapurin-6-one.

containing concentrated hydrochloric acid (method A). The carboxamido-substituted 11-oxo-11(*H*)-pyrido[2,1-*b*]quinazoline intermediate obtained was dehydrated to the nitrile using *p*-toluenesulfonyl chloride in a pyridine–DMF mixture (method B). The desired tetrazole product was obtained by reacting the nitrile with sodium azide and ammonium chloride in DMF.

Results and Discussion

The relative potencies of a series of known mediator release inhibitors and PCA-active agents were compared against human leukocyte and placental ALP (Table I). The potency order was essentially the same with each enzyme. The most potent compound was **8**, while cromolyn sodium was the least active. It was previously noted[5] that members of this series of inhibitors, while structurally diverse, were all uncompetitive inhibitors of leukocyte ALP with K_i values falling within a range of 5×10^{-5} to 7×10^{-6} M. As uncompetitive inhibitors, their inhibition was characteristically not reduced by increasing substrate concentration from 0.2 to 10 mM. Since the placental enzyme inhibition was also insensitive to those concentration changes, uncompetitive inhibition is probably occurring with that enzyme as well.

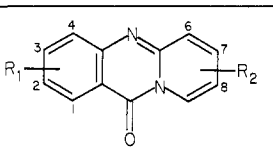
While inhibition of ALP by known antiallergic agents is interesting, we felt that an expanded study within a series of antiallergic pyrido[2,1-*b*]quinazolines might further support the hypothesis of ALP involvement in the process of allergic mediator release. The inhibitory effects of the series was compared for ALP and rat PCA tests (Table II).

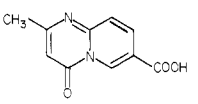
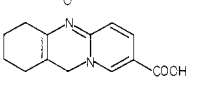
Generally, the enzyme and PCA inhibitions were in agreement. The least active enzyme inhibitors were weak or inactive in rat PCA. The most potent PCA inhibitors were also the better inhibitors of the enzyme.

The exact potency order of the series was not identical for ALP and rat PCA inhibition (Table II). PCA active compounds did inhibit the enzyme, whereas not all ALP inhibitors (**1** and **2**) demonstrated PCA activity. These differences may be a function of the limited PCA testing data available. Then too, differences may be related to species or drug availability when comparing human placental enzyme against rat skin.

Due to the limited PCA data available, only a rough structure–activity relationship can be constructed for the series applicable to both test models. A flat polycyclic nucleus, such as in **6**, **13**, and **14**, is preferred over analogues **18** and **19**. The 7- and 8-COOH isomers, **6** and **14**, were the most active isomers within the series. Generally, no significant activity differences were observed between the

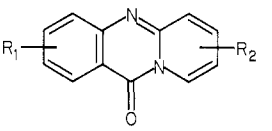
Table II. Comparison of ALP and Rat PCA Inhibition by Pyrido[2,1-*b*]quinazolines and Analogues



no.	R_1	R_2	ALP I_{50},[a] mM	Rat PCA ED_{50},[c] mg/kg iv
1	H	6-COOH	0.80 ± 0.04[b]	inactive[d]
2	2,3-(CH=CH)$_2$	6-COOH	0.71 ± 0.01	inactive[d]
3	3-OCH$_3$	8-(5-tetrazolyl)	0.62 ± 0.11	0.5
4	2,3-(OCH$_3$)$_2$	8-COOH	0.41 ± 0.02	0.05
5	2,3-(OCH$_3$)$_2$	8-(5-tetrazolyl)	0.34 ± 0.01	(100)[d]
6	H	8-COOH	0.35 ± 0.01	0.5
7	H	8-(5-tetrazolyl)	0.28 ± 0.01	(68)[d]
8	2-OH	8-COOH	0.28 ± 0.01	0.05
9	2,3-(CH=CH)$_2$	8-(5-tetrazolyl)	0.23 ± 0.02	(100)[d]
10	2,3-(OH)$_2$	8-COOH	0.22 ± 0.01	>1[e]
11	2,3-(OCH$_2$O)	8-(5-tetrazolyl)	0.23 ± 0.01	(24)[d]
12	2-CH$_3$	8-(5-tetrazolyl)	0.21 ± 0.04	(100)[d]
13	2,3-(CH=CH)$_2$	8-COOH	0.18 ± 0.01	0.1
14	H	7-COOH	0.17 ± 0.01	(26)[d]
15	2-CH$_3$	8-COOH	0.16 ± 0.02	0.05
16	2-OCH$_3$	8-(5-tetrazolyl)	0.14 ± 0.02	0.01
17	2-OCH$_3$	8-COOH	0.10 ± 0.01	0.05
18			>10	inactive[d]
19			>10	inactive[d]
20	doxantrazole		5.42 ± 0.02	1.5

[a] Concentration of inhibitor required for 50% of human placenta alkaline phosphatase. The concentration of substrate, *p*-nitrophenyl phosphate, was 10 mM. [b] Mean ± SEM. [c] Dose required for 50% inhibition of rat passive cutaneous anaphylaxis allergy model. [d] Percent inhibition at 0.5 mg/kg iv. [e] Inactive orally at 1 mg/kg.

Table III



no.	R_1	R_2	mp, °C dec	formula	anal.	method[c]
3	3-OCH_3	8-(5-tetrazolyl)	302-304	$C_{14}H_{10}N_6O_2$	C, H, N	C
3a	3-OCH_3	8-CN	273-275	$C_{14}H_9N_3O_2$	C, H, N	B
3b	3-OCH_3	8-$CONH_2$	313-318	$C_{14}H_{13}N_3O_3 \cdot 0.5H_2O$	H; C, N[a]	A
5	2,3-$(OCH_3)_2$	8-(5-tetrazolyl)	300-303	$C_{15}H_{12}N_6O_3$	C, H; N[b]	C
5a	2,3-$(OCH_3)_2$	8-CN	309-312	$C_{15}H_{11}N_3O_3 \cdot 0.5H_2O$	C, H, N	B
5b	2,3-$(OCH_3)_2$	8-$CONH_2$	313-318	$C_{15}H_{13}N_3O_4 \cdot 0.5H_2O$	C, H, N	A
7	H	8-(5-tetrazolyl)	293-296	$C_{13}H_8N_6O \cdot 0.25C_2H_6O$	C, H, N	C[d]
7a	H	8-CN	249-251	$C_{13}H_7N_3O \cdot 0.25H_2O$	C, H, N	B
7b	H	8-$CONH_2$	338-344	$C_{13}H_9N_3O_2$	C, H, N	A
9	2,3-(CH=CH)$_2$	8-(5-tetrazolyl)	295-302	$C_{17}H_{10}N_6O$	C, H, N	C
9a	2,3-(CH=CH)$_2$	8-CN	354-356	$C_{17}H_9N_3O$	C, H, N	B
9b	2,3-(CH=CH)$_2$	8-$CONH_2$	242-248	$C_{17}H_{11}N_3O_2$	C, H, N	A
11	2,3-(OCH_2O)	8-(5-tetrazolyl)	310-313	$C_{14}H_8N_6O_3$	C, H, N	C
11a	2,3-(OCH_2O)	8-CN	319-323	$C_{14}H_7N_3O_3$	C, H, N	B
11b	2,3-(OCH_2O)	8-$CONH_2$	368-373	$C_{14}H_9N_3O_4$	C, H, N	A
12	2-CH_3	8-(5-tetrazolyl)	284-286	$C_{14}H_{10}N_6O$	C, H, N	C
12a	2-CH_3	8-CN	307-309	$C_{14}H_9N_3O$	C, H, N	B
12b	2-CH_3	8-$CONH_2$	332-336	$C_{14}H_{11}N_3O_2$	C, H, N	A
16	2-OCH_3	8-(5-tetrazolyl)	302-304	$C_{14}H_{10}N_6O_2$	C, H, N	C
16a	2-OCH_3	8-CN	281-285	$C_{14}H_9N_3O_2$	C, H, N	B
16b	2-OCH_3	8-$CONH_2$	329-332	$C_{14}H_{11}N_3O_3$	C, H, N	A

[a] C: calcd, 61.41; found, 61.92. N: calcd, 15.34; found, 14.88. [b] N: calcd, 25.92. Found: 25.45. [c] All samples recrystallized from pyridine. [d] Recrystallized from EtOH.

carboxylic acids (**4**, **6**, **13**, **15**, and **17**) and the corresponding substituted tetrazole analogues (**5**, **7**, **9**, **12**, and **16**). No clear substituent effect on the aryl binding was apparent for the series where R_1 and R_2 were a variety of electron-withdrawing or electron-donating groups. However, it is not likely that the inhibition of ALP by antiallergic compounds is nonspecific, since a wide potency range of over 100-fold was observed for the compounds tested.

Phosphate monoester cleavage by alkaline phosphatase involves at least two steps: (1) cleavage of the monoester by the enzyme forming a phosphorylated enzyme intermediate and (2) dephosphorylation of the enzyme to regenerate the free enzyme. Uncompetitive alkaline phosphatase inhibitors probably act by preventing the dephosphorylation of the phosphoenzyme. These inhibitors are more effective at high substrate concentrations in which the dephosphorylation step is rate limiting.[7] Low substrate concentrations would favor the first step. During the course of our investigations reported herein, it was noted that all the antiallergic compounds tested showed reduced or variable inhibitory activity at sub-K_m substrate levels.

A problem in the development of antiallergic agents continues to be the inconsistencies between pharmacologic models and clinical efficacy. This might be due to species or tissue differences between animal models and human allergic responses. The unpredictability of certain laboratory models for human allergies may be in part a function of the uncompetitive inhibition kinetics by which these allergic mediator release inhibitors seem to act. It is well established that uncompetitive inhibitors can inhibit, have no effect, or stimulate an enzymic process, depending upon substrate concentrations. Cromolyn-like agents have been reported to exhibit a complex pharmacologic profile, including inhibition, enhancement, and no effect upon an allergic response.[4,8] Being a membranous enzyme, the microenvironment of alkaline phosphatase might have a more limited availability of substrate, resulting in a complex therapeutic response to uncompetitive inhibitors.

In summary, a relationship exists between alkaline phosphatase and rat PCA activity. Cromolyn-like agents may achieve their PCA inhibitory effects by inhibition of ALP, a possible component of membranal calcium channels. While the relationship between the kinetics of ALP inhibition and the therapeutic action of cromolyn-like agents is unclear, the complex pharmacologic profile and clinical inconsistencies of cromolyn-like agents may be related to uncompetitive ALP inhibition.

Experimental Section

Enzyme Assay. Human placenta alkaline phosphatase (B Grade, Calbiochem-Behring) was followed by measuring *p*-nitrophenolate production at 405 nm, using a Gilford Model 250 spectrophotometer. Each assay was run at 24 °C, and the total volume of 1 mL contained 10 mM *p*-nitrophenyl phosphate, 0.2 M Tris-HCl, pH 9.0, and sufficient enzyme amounts to give an easily measurable initial rate of reaction. Solutions of the substrate were prepared daily in water. The concentration of inhibitor (I) required for 50% inhibition (I_{50}) was determined graphically by plotting percent inhibition vs. I; each assay was run in duplicate. All reported values were determined at least twice using several inhibitor concentrations.

Rat Reaginic Passive Cutaneous Anaphylaxis (PCA). The PCA test involved immunization of rats with 1 mg of ovalbumin intramuscularly and approximately 10^{10} *Bordetella 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% Evans blue. Thirty minutes later, the animals were killed in ether, the dorsal skin was reflected, and the mean orthogonal diameter of the wheal was measured. For intravenous dosing, the compounds were dissolved in the saline/ovalbumin/Evans blue solution and

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(8) M. K. Church, *Drugs Today*, **14**, 281 (1978).

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:

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

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

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 gave results within 0.4% of theory.

The procedures for the preparation of the reported compounds are listed as methods A–C and may be considered as general methods of preparation. The reported yields for the products obtained were not maximized.

Method A. 2-Methoxy-11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxamide (16b). A mixture of 2-amino-5-methoxybenzoic acid (27.0 g, 161 mmol), 6-chloro-3-pyridinecarboxamide (25.0 g, 160 mmol), and ethanol (500 mL) containing 15 mL of concentrated HCl was heated at reflux for 24 h. The mixture was cooled to 0 °C, and the resultant solid precipitate was collected by filtration to give 34.0 g (69.5%) of crude hydrochloride salt, mp 286–294 °C dec. This material was recrystallized twice from pyridine to give the analytical sample: yield 7.20 g (16.7%); mp 329–333 °C dec.

Method B. 2-Methoxy-11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carbonitrile (16a). A solution of 1.2 L of pyridine, 300 mL of DMF, 7.46 g (39.4 mmol) of *p*-toluenesulfonyl chloride, and 7.45 g (27.6 mmol) of **16b** was heated at 100 °C for 42 h. The mixture was cooled and poured onto 4 L of ice/H_2O and acidified to pH 1 with concentrated HCl. The solid that formed was collected: yield 5.0 g (72.2%); mp 273–280 °C dec. The analytically pure nitrile was obtained in 56.2% yield after one recrystallization from pyridine, mp 281–285 °C dec.

Method C. 2-Methoxy-8-(1*H*-tetrazol-5-yl)-11*H*-pyrido[2,1-*b*]quinazolin-11-one (16). A mixture of 3.00 g (12.0 mmol) of **16a**, NaN_3 (2.22 g, 34.2 mmol), NH_4Cl (1.83 g, 34.2 mmol), and 250 mL of DMF was heated at 115 °C for 20 h. The mixture was cooled, poured onto 1.5 L of ice/H_2O, and acidified with concentrated HCl. The solid that formed was collected: yield 2.93 g (83%); mp 279–299 °C dec. The sample of analytical purity was obtained by recrystallization from pyridine: yield 2.25 g (64%); mp 302–304 °C dec.

Acknowledgment. The authors thank André Salama for his assistance and many stimulating discussions. We also thank Mrs. U. Zeek and Dr. R. C. Greenough for microanalyses and spectra and Dr. D. J. Herzig for the rat PCA determinations.

Isoxazolidine-3,5-diones as Lens Aldose Reductase Inhibitors

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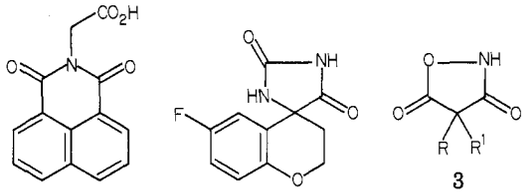
Diabetes is a serious, debilitating disease which has several chronic complications, such as retinopathy, nephropathy, neuropathy, and cataracts.[1] The tissues involved in these complications have been shown to accumulate abnormally high amounts of sorbitol, the polyol which arises from the reduction of D-glucose by the enzyme aldose reductase. The pathogenic potential of sorbitol accumulation has been documented in the lens where elevated concentrations lead to osmatic swelling, resulting in disturbance in the metabolism and membrane transport mechanisms. Experimental models support the hypothesis that this contributes to the increased incidence of cataracts in human diabetes mellitus.[2-4]

The existence of aldose reductase in the aortic intima, brain, pancreas, and renal cortical tubules provides a pathogenic mechanism by which hyperglycemia can alter the metabolism and function of cells in these tissues via sorbitol accumulation. Therefore, an inhibitor of aldose reductase that is nontoxic and of appropriate duration of action in vivo may have value as a therapeutic agent against those diabetic complications in which sorbitol accumulation is thought to play a pathogenic role.[5]

The search for these agents showing activity as aldose reductase inhibitors has produced compounds such as alrestatin (1) and sorbinil (2). While clinical experience with aldose reductase inhibitors is limited, sorbinil remains one of the most promising aldose reductase inhibitors. The structural similarity of isoxazolidine-3,5-diones (3) to the spiro[chroman-4,4′-imidazolidine]-2′,5′-dione (2) has prompted us to explore isoxazolidine-3,5-diones for activity against aldose reductase.



1 2 3

Results and Discussion

Compounds 8–17 were evaluated for activity in the rat lens aldose reductase assay. The percent inhibition of aldose reductase prepared from rat lenses was determined for each of the test compounds at a concentration of 50 μM by the procedure of Hayman and Kinoshita.[6] Compounds which inhibited partially purified aldose reductase

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