tion and represents the "efficiency of catalysis" of the reaction of a given substrate.[13] Thus, since the intracellular concentrations of deoxynucleoside substrates are far below their K_m values for dThd phosphorylase, those with higher V_{max}^{rel}/K_m values will be more rapidly catabolized and the QSAR described for this parameter should be of predictive utility. Important drugs which are degraded by dThd phosphorylase are also converted to their active form via initial phosphorylation at the 5'-hydroxyl by dThd kinase. It follows that the efficacy of such drugs in a given cell will largely depend on the relative rates of these divergent pathways. Thus, at low substrate concentrations, the fraction of a 5-substituted 2'-deoxyuridine which will be converted to its 5'-phosphate rather than be catabolized by dThd phosphorylase is described by eq 3,

$$\frac{v_{TK}}{v_{TK} + v_{TP}} = \frac{(k_{cat}/K_m)_{TK}[TK]}{(k_{cat}/K_m)_{TK}[TK] + (k_{cat}/K_m)_{TK}[TP]} \quad (3)$$

where v_{TK} and v_{TP} are the velocities of reaction with dThd kinase (TK) and dThd phosphorylase (TP), respectively. If, as described here for dThd phosphorylase, a QSAR could be established for the catalytic efficiencies (k_{cat}/K_m) for phosphorylation of 5-substituted 2'-deoxyuridines by dThd kinase, it should be possible to predict their metabolism in cells once the relative activities of these two enzymes are ascertained. As these activities vary in differing cell lines,[14] it may be possible to predict the sensitivity of a given cell to these analogues. Studies to test this hypothesis are in progress.

Experimental Section

General. dThd phosphorylase from horse liver was purchased from Gipep Co., Ltd. (Paris, France). Using conditions described below, the phosphorolysis of Urd proceeded at less than 2% the rate of dThd; the absence of Urd phosphorylase in this preparation was further verified by lack of inhibition of dThd phosphorolysis by 0.45 mM 5-benzyluridine, a potent inhibitor of Urd phosphorylase.[15] dUrd, dThd, BrdUrd, CldUrd, CF_3dUrd, FdUrd, and IdUrd were obtained from P-L Biochemicals. CHOdUrd[16] and CNdUrd[17,18] were prepared by the cited procedures. 5-Ethyl-, 5-allyl- and 5-propenyl-2'-deoxyuridines were gifts from Dr. Donald Bergstrom. Other materials were of the highest commercial grade available. All nucleosides used had proper UV spectra and were homogeneous as determined by high-performance LC analysis using a Lichrosorb RP18 column (4.6 × 250 mm) and 2% (v/v) CH_3CH/water as eluant; flow rate was 1 mL/min and compounds were detected at 254 and/or 280 nm with an ISCO UA-5 detector. Ultraviolet spectra and kinetic analysis were obtained on a Cary 118 recording spectrophotometer equipped with a repetitive scan accessory.

Enzyme Assays. With substrates of dThd phosphorylase, the wavelength of maximal difference ($\Delta\lambda_{max}$) between each nucleoside and its corresponding base and the magnitude of absorbance change ($\Delta\epsilon_{max}$) were determined as follows: The UV spectrum of a solution of the nucleoside (ca. 0.1 mM) in 0.1 M potassium phosphate (pH 6.0) was obtained and, from reported ϵ_{max} values, the concentration was determined. Identical amounts of the dThd phosphorylase preparation in 1% of the total volume were added to both reference and sample cuvettes to give a final concentration of ca. 0.08 mg/mL. The UV spectrum was scanned repetitively until no further changes were observed. The final spectrum was scanned repetitively until no further changes were observed. The final spectrum of the pyrimidine base was subtracted from the initial spectrum of the nucleoside to obtain the difference spectrum from which $\Delta\lambda_{max}$ and $\Delta\epsilon_{max}$ were obtained. Completion of reaction was verified by high-performance LC analysis using the systems described above.

For initial velocity experiments of *phosphorolysis*, the change in absorbance was continuously monitored at $\Delta\lambda_{max}$ for each nucleoside, and $\Delta\epsilon_{max}$ was used to quantitate the extent of reaction. Reaction mixtures contained variable amounts of nucleoside (60–320 μM) in 0.1 M potassium phosphate (pH 6.0) and limiting enzyme; assays were performed at 25.0 °C. *Pentosyl transfer* (trans-deoxyribosylation) was assayed by monitoring the increase in absorbance at 300 nm which accompanies the formation of IdUrd upon transfer of the deoxyribose moiety from a nucleoside donor which does not absorb at 300 nm (e.g., dThd) to IUra. A typical reaction mixture contained, in 1 mL, 75 mM morpholinoethyl sulfonate (pH 6.2), 0.1 mM IUra, 0.1 mM potassium phosphate, 90 μg of enzyme, and 0.1 to 1 mM of dThd as the deoxyribosyl donor. The validity of this assay was confirmed by high-performance LC separation of reactants and products, which were then quantitated by UV spectroscopy.

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Antiallergy Activity of 10-Oxo-10*H*-pyridazino[6,1-*b*]quinazoline-2-carboxylic Acids

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A series of substituted 10-oxo-10*H*-pyridazino[6,1-*b*]quinazoline-2-carboxylic acids was prepared and evaluated as antiallergy agents. The 8-chloro and unsubstituted analogues were more potent than cromolyn sodium and doxantrazole intravenously in the rat PCA test. None of the analogues possessed significant oral activity.

A new series of 11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxylic acids was recently reported to possess significant oral antiallergy activity. 2-Methyl-11-oxo-pyrido[2,1-*b*]-quinazoline-8-carboxylic acid (11) was 30 times more potent than cromolyn sodium in the rat PCA test intravenously and 4–6.7 times better than doxantrazole orally.[1]

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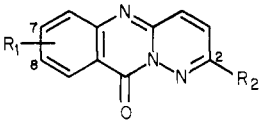
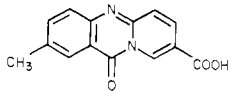
Table I. 10-Oxo-10*H*-pyridazino[6,1-*b*]quinazolines



no.	R_1	R_2	formula	mp, °C	anal.	solvent	method of preparation	rat PCA: % inhibn at 0.5 mg/kg, iv[a,c]
1	H	COOH	$C_{12}H_7N_3O_3$	239-243	C, H, N	2-PrOH	A	76
2	8-OCH_3	COOH	$C_{13}H_9N_3O_4$	241-245	C, H, N	MeOH	A	8
3	8-CH_3	COOH	$C_{13}H_9N_3O_3$	250-252	C, H, N	2-PrOH	A	0
4	8-Cl	COOH	$C_{12}H_6N_3ClO_3$	260-262	C, H, N, Cl	pyridine	A	91
5	7-CH_3	COOH	$C_{13}H_9N_3O_3$	246-249	C, H, N	pyridine	A	32
6	8-COOH	Cl	$C_{12}H_6N_3ClO_3$	290-295	C, H, N, Cl	pyridine	C	0[b]
7	8-CO_2CH_3	Cl	$C_{13}H_8N_3ClO_3$	241-245	C, H, N, Cl	DMF	B	6[b]
8	7-COOH	Cl	$C_{12}H_6N_3ClO_3$	299-302	C, H, N, Cl	pyridine	C	0[b]
9	7-CO_2CH_3	Cl	$C_{13}H_8N_3ClO_3$	260-262	C, H, N, Cl	DMF	B	0[b]
10	7,8-(-CH=CHCH=CH-)	COOH	$C_{16}H_9N_3O_3$	264-266	C, H, N	pyridine	A	36
11[d]								

[a] Cromolyn sodium in this assay had an ED_{50} = 1-2 mg/kg, iv. [b] Tested at 5 mg/kg, ip. [c] Doxantrazole in this PCA assay had an ED_{50} = 1 mg/kg, iv, and 5 mg/kg, po. At 5 mg/kg, ip, doxantrazole inhibited the PCA test 100%. [d] Cited in ref 1. ED_{50} = 0.05 mg/kg, iv, and 0.7 mg/kg, po.

Cromolyn sodium is inactive orally.[2]

We report the synthesis and substitution effects on the antiallergy activity of a structurally related series of 10-oxo-10*H*-pyridazino[6,1-*b*]quinazoline-2-carboxylic acids.

Chemistry. The 10-oxo-10*H*-pyridazino[6,1-*b*]-quinazoline-2-carboxylic acids were prepared in one step from 6-chloropyridazine-3-carboxylic acid[3] and the appropriately substituted anthranilic acid by refluxing the mixture in glacial acetic acid (method A).[4] The 7- and 8-(carboxymethyl) analogues (**7** and **9**) were prepared from dimethyl 4-aminoisophthalate[5] or dimethyl 3-aminoterephthalate[6] and 3,6-dichloropyridazine using a fusion method (method B).[7] The corresponding carboxylic acid analogues (**6** and **8**) were obtained by acid hydrolysis of the ester intermediates (method C).

Discussion

The substituted 10-oxo-10*H*-pyridazino[6,1-*b*]-quinazoline-2-carboxylic acids were evaluated for their antiallergy activity by their ability to inhibit passive cutaneous anaphylaxis (PCA) in rats as described under Experimental Section.[8] Those agents which showed significant inhibition at 0.5 mg/kg, iv, were also evaluated for potential oral efficacy compared with 2-methyl-11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxylic acid (**11**). Substitutions which yielded active analogues of the pyrido[2,1-*b*]quinazoline series were also studied in the pyridazino[6,1-*b*]quinazoline series of compounds.

The most active analogues of the series were **1**, **4**, and **10** (Table I). Unlike **11**, compounds **1**, **4**, and **10** were orally inactive at 1 mg/kg. Interestingly, **4** was slightly more active in the pyridazino[6,1-*b*]quinazoline series than the direct analogue, 2-chloro-11-oxo-11*H*-pyrido[2,1-*b*]-quinazoline-8-carboxylic acid. The isomeric 2-chloro-8-carboxy analogue, **6** was inactive in the pyridazino series. Tilley[9] reported that the 2-methyl-8-carboxy and 8-methyl-2-carboxy analogues of the pyrido[2,1-*b*]quinazoline series possessed about equal activity. This may indicate that the pyrido and benzene ring portions of the pyrido-[2,1-*b*]quinazoline ring nucleus are about equivalent in regards to their acceptance by the drug receptor binding area. The binding is generally directed by the carboxylic acid, amide carbonyl, and tricyclic nucleus. However, in the pyridazine[6,1-*b*]quinazoline series, the benzene and pyridazine rings are not equivalent, since analogue **4** is preferred over the inactive **6**.

Generally, the pyridazino[6,1-*b*]quinazoline series of antiallergy agents reported here did not possess oral potency comparable to the pyrido[2,1-*b*]quinazoline series we previously reported.

Experimental Section

Rat Reaginic Passive Cutaneous Anaphylaxis (PCA). The PCA test[8] 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% Evans blue. Thirty 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

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physiological saline and given 10–15 min before intravenous antigen challenge. For intravenous dosing, the compounds were dissolved in the saline/ovalbumin/Evans 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 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:

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

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

Melting points were taken in open capillary tubes on a Mel-Temp 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, N, and Cl 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. 8-Chloro-10-oxo-10*H*-pyridazino[6,1-*b*]-quinazoline-2-carboxylic Acid (4). A mixture of 5-chloroanthranilic acid (5.4 g, 31.6 mmol) and 6-chloro-3-pyridazinecarboxylic acid[3] (5.0 g, 31.6 mmol) was heated in refluxing glacial acetic acid[4] (50 mL) for 80 h. The mixture was cooled and the precipitate which formed was collected, giving 4.73 g (54.6%) of crude 4, mp 253–300 °C dec. Recrystallization of this material from pyridine gave 1.3 g (15%) of the analytical sample, mp 260–262 °C.

Method B. 8-(Carboxymethyl)-2-chloro-10-oxo-10*H*-pyridazino[6,1-*b*]quinazoline (7). Dimethyl 4-aminoisophthalate[5] (4.2 g, 20 mmol) and 3,6-dichloropyridazine (3.0 g, 20 mmol) were heated at 155–160 °C as a melt for 0.5 h. The resulting dark solid was recrystallized from pyridine, giving 2.6 g (45%) of 7 as a red powder. The crude 7 was further recrystallized from pyridine to give 0.4 g (7%) of the analytical sample as yellow crystals, mp 241–245 °C.

Method C. 2-Chloro-10-oxo-10*H*-pyridazino[6,1-*b*]-quinazoline-8-carboxylic Acid (6). A mixture of 2.6 g (9.0 mmol) of 7 and 150 mL of 6 N HCl was refluxed for 18 h. The solution was cooled and the gray solid which formed was collected. The crude 6 was recrystallized from pyridine, giving 1.32 g (53%) of a yellow solid, mp 287–290 °C. Further recrystallizations from pyridine yielded 0.63 g (25%) of the analytical sample, mp 290–295 °C.

Book Reviews

Handbook of Experimental Pharmacology. Volume 25. Supplement. Bradykinin, Kallidin and Kallikrein. Edited by E. G. Erdös. Springer-Verlag, New York. 1979. xxii + 817 pp. 17 × 24.5 cm. $198.00.

This encyclopedic volume on bradykinin, kallidin, and kallikrein, which was published in 1979, nearly 1 decade following the previous "handbook" on this subject, is truly a supplement to the previous volume; it is not a revised edition of the 1970 text. This supplement reflects the ever increasing interest, knowledge, and range of experimental and therapeutic applications, all related to the kallikrein–kinin system. Among the many areas of experimental or clinical studies, the following are emphasized: (1) how kallikreins and kinins can affect (a) coagulation of blood, (b) activation of complement, (c) formation of angiotensin II, (d) release or modulation of effect of mediators such as prostaglandins, histamine, catecholamines, etc.; (2) how can inhibitors of kallikreins or of kininase II (identical to "angiotensin-converting enzyme") find cardiovascular applications. The former can be tried in extracorporeal circulatory systems and the latter have demonstrated efficacy as antihypertensive agents.

Twenty-four eminent authors including the editor have contributed to the vast scope of the text in this supplement volume. A total of about 3250 references have been cited in the 17 chapters. In Chapter 1, H. Z. Movat reviews in great detail "The Plasma Kallikrein–Kinin System and Its Interrelationship With Other Components of Blood". L. M. Greenbaum discusses "Kininogenases of Blood Cells (Alternate Kinin Generating Systems)" in Chapter 2. F. Fiedler presents a chapter on "Enzymology of Glandular Kallikreins" (Chapter 3). "Kallikrein Inhibitors" are reviewed in Chapter 4 by R. Vogel.

The ensuing seven chapters deal with kinins. Thus, in Chapter 5, J. M. Stewart summarizes and discusses "Chemistry and Biologic Activity of Peptides Related to Bradykinin". J. J. Pisano discusses "Kinins in Nature" in Chapter 6. "Bradykinin Receptors" are reviewed by C. E. Odya and T. L. Goodfriend in Chapter 7. The current status of "Bradykinin Radioimmunoassay" is described in Chapter 8 by R. C. Talamo and T. L. Goodfriend. W. G. Clark discusses "Kinins and the Peripheral and Central Neurons Systems" in Chapter 9. The "Effects of Kinins on Organ Systems" are reviewed by A. R. Johnson in Chapter 10. Chapter 11 presents the "Release of Vasoactive Substances by Kinins", authored by N. A. Terragno and A. Terragno.

The twelfth chapter entitled "Kininases" is written by the editor E. G. Erdös. Kininases I and II, as well as other kininases, are discussed with reference to their distribution, specificity, properties, inhibition, etc.

Three chapters are allocated to glandular, renal, and urinary kallikreins. K. D. Bhoola, M. Lemon, and R. Matthews review "Kallikrein in Exocrine Glands" in Chapter 13 (pancreatic, salivary, and submandibular kallikreins). "Renal and Urinary Kallikreins" are discussed by P. E. Ward and H. S. Margolius in Chapter 14. I. H. Mills reports on "Renal Kallikrein and Regulation of Blood Pressure in Man" in Chapter 15. R. W. Colman and P. Y. Wong review the "Kallikrein–Kinin System in Pathologic Conditions" (including deficiency of proteins necessary for kinin formation and disorders leading to increased kinin formation) in Chapter 16. The last chapter (17) is a survey of the Russian literature on "Bradykinin, Kallidin, and Kallikreins" by T. S. Paskhiva and A. P. Levitskiy.

The price of this supplement may, in some instances, tend to restrict its acquisition by some individual researchers, but it should be acquired by all libraries in chemical and life science oriented research centers. This valuable supplement (1979) is recommended very highly as a reference text to chemists, biochemists, enzymologists, physiologists, pharmacologists, and medical investigators with more than a passing interest in the kinin system. It is quite likely that research investigations of the kallikrein–kinin system will continue at an unabated pace, and volumes like the present one will always be needed to summarize the status of the field at regular intervals.

The Squibb Institute for Medical Research **Bernard Rubin**