Quinazolines as Inhibitors of Xanthine Oxidase

D. G. PRIEST [x], J. B. HYNES, C. W. JONES, and W. T. ASHTON*

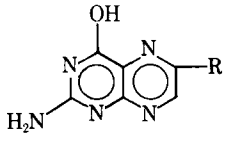
Abstract □ A series of 23 quinazolines and 5,6,7,8-tetrahydroquinazolines was evaluated for inhibition of bovine milk xanthine oxidase. The synthesis of four of these compounds has not been reported previously. Several 2-amino-4-hydroxyquinazolines, as well as 2-amino-4-mercaptoquinazoline, were effective inhibitors of this enzyme. 2-Amino-4-hydroxy-6-hydroxymethylquinazoline was a threefold better inhibitor than its pteridine counterpart, while 2-amino-4-hydroxyquinazoline-6-carboxaldehyde was the most potent compound studied.

Keyphrases □ Quinazolines—synthesis, evaluated as xanthine oxidase inhibitors □ Tetrahydroquinazolines—synthesis, evaluated as xanthine oxidase inhibitors □ Xanthine oxidase—synthesis of four and evaluation of 23 quinazolines and 5,6,7,8-tetrahydroquinazolines as inhibitors

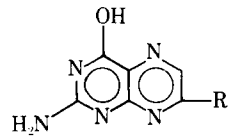
Suitably substituted members of various heterocyclic ring systems have been shown to be inhibitors of and/or substrates for xanthine oxidase. In addition to numerous purine derivatives (1), several pteridines were of interest in this regard. For example, 2-amino-4-hydroxy-6-hydroxymethylpteridine (I*a*) and 2-amino-4-hydroxypteridine-6-carboxaldehyde (I*b*) were shown to be good inhibitors, having approximately equal potency against this enzyme (2). On the other hand, 2-amino-4-hydroxypteridine (II*a*) was oxidized at essentially the same rate as xanthine, yielding 2-amino-4,7-dihydroxypteridine (II*b*), an effective inhibitor (3). In addition to other pteridines (4), certain 1*H*-pyrazolo[4,3-*c*]pyridines were recently reported to display modest inhibitory action against xanthine oxidase (5).

From a clinical standpoint, however, the most important agent acting on this enzyme is allopurinol (III*a*), which is currently employed in various conditions characterized by hyperuricemia (6). The compound is not only an inhibitor of xanthine oxidase but is also a substrate that is converted to oxypurinol (III*b*), another effective inhibitor (7, 8).

In general, the known heterocyclic inhibitors of xanthine oxidase consist of two fused unsaturated rings, each containing at least one hetero nitrogen atom. An exception is that one pyrazine derivative, 2-amino-3-pyrazinecarboxylic acid, is also a moderately effective inhibitor (4). However, since the removal of either the amino group or the carboxyl function results in total loss of activity (4), it seems more logical to consider this compound as a quasicyclic analog of II*a*. It was of interest, therefore, to determine the effects of quinazolines upon xanthine oxidase. These compounds may be regarded as analogs of either purines or pteridines which contain only one heterocyclic ring.



I*a*: R = CH_2OH
I*b*: R = CHO



II*a*: R = H
II*b*: R = OH

EXPERIMENTAL

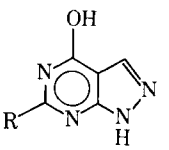
Melting points were taken with a capillary apparatus[1] and are uncorrected. Analytical samples were vacuum dried at 100° prior to analysis. TLC was performed using silica media[2]. UV absorption measurements were made using a recording spectrophotometer[3].

Enzymology—Ammonium sulfate suspensions of highly purified bovine milk xanthine oxidase[4] were used without further purification. The specific activities of the two preparations employed were 0.1 and 0.56 unit/mg protein (no signifcant kinetic differences were observed with these two preparations). Velocities were determined from absorbance changes at 293 nm in a cell chamber maintained at 28° by circulating water. Initial velocities were taken as the tangent to time plots at zero time. Reactions were usually initiated by the addition of 25 μl of enzyme solution containing 1.0 mg/ml protein to 3.0-ml reaction cells.

The substrate, xanthine, as well as each inhibitor was prepared at a final concentration of 10 μM in 0.05 *M* potassium phosphate, pH 8.0. Each inhibitor was tested separately at 10-μM concentration for possible substrate activity which could interfere with the spectrophotometric assay. In no case was there a significant absorbance change in the absence of xanthine. To test more rigorously for substrate activity, 5 ml of a 100 μM solution was allowed to react overnight in the presence of 25 μl of the xanthine oxidase solution. A compound was assumed to be a substrate if a significant spectral change was observed in the 230–350-nm region.

Chemistry—Compounds I*a*[5], II*b*[4], III*a*[4], IV[6], V, XXVII[4], and XXVIII[4] were obtained commercially. The synthetic procedures for the preparation of the remaining compounds, with the exception of those presented here, were described previously (9–11).

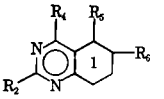
2-Amino-4-hydroxy-6-chloroquinazoline (XVI) and 2,6-Diamino-4-hydroxyquinazoline (XX)—Compounds XVI and XX were prepared by refluxing the corresponding 2,4-diaminoquinazolines (10, 11) in 2 *N* HCl for 2 and 3.5 hr, respectively. After



III*a*: R = H
III*b*: R = OH

[1] Mel-Temp, Laboratory Devices.
[2] SAF, Gelman Instrument Co.
[3] Acta C III, Beckman Instruments.
[4] Sigma Chemical Co.
[5] Raylo Chemicals Ltd.
[6] Aldrich Chemical Co.

Table I—Inhibition of Xanthine Oxidase by Selected Quinazolines and Related Compounds



Compound	R_2	R_4	R_5	R_6	Ring 1[a]	Velocity[b]	I_{50}, μM[c]	Substrate[d]
IV	H	H	H	H	Ar	4.95 ± 0.76	—	(+)
V	H	OH	H	H	Ar	6.77 ± 0.48	—	(+)
VI	H	SH	H	H	Ar	2.37 ± 0.43	26.0	(−)
VII	H	NH_2	H	H	Ar	4.69 ± 0.09	—	(+)
VIII	OH	OH	H	H	Ar	6.45 ± 0.80	—	(−)
IX	NH_2	NH_2	H	H	Ar	5.40 ± 0.36	—	(−)
X	OH	NH_2	H	H	Ar	4.68 ± 0.48	—	(−)
XI	NH_2	OH	H	H	Ar	0.50 ± 0.00	0.9	(−)
XII	CH_3CONH	OH	H	H	Ar	1.15 ± 0.63	1.0	(−)
XIII	NH_2	SH	H	H	Ar	0	0.1	(−)
XIV	SH	NH_2	H	H	Ar	3.65 ± 0.44	—	(−)
XV	NH_2	OH	Cl	H	Ar	0.53 ± 0.05	1.0	(−)
XVI	NH_2	OH	H	Cl	Ar	0.45 ± 0.14	0.7	(−)
XVII	NH_2	OH	CH_3	H	Ar	3.72 ± 0.36	—	(−)
XVIII	NH_2	OH	H	CH_3	Ar	0.47 ± 0.05	1.2	(−)
XIX	NH_2	OH	H	(7)CF_3	Ar	0.40 ± 0.08	0.6	(−)
XX	NH_2	OH	H	NH_2	Ar	1.38 ± 0.05	6.5	(−)
XXI	NH_2	OH	H	CN	Ar	0.37 ± 0.09	1.0	(−)
XXII	NH_2	OH	H	CH_2OH	Ar	0	0.3	(−)
XXIII	NH_2	OH	H	CHO	Ar	0	0.07	(+)
XXIV	NH_2	OH	H	COOH	Ar	2.52 ± 0.19[e]	9.7	(−)
XXV	NH_2	OH	H	H	H_4	4.80 ± 0.17	—	(−)
XXVI	NH_2	OH	H	CH_3	H_4	5.06 ± 0.58	—	(−)
XXVII	Isocytosine					4.37 ± 0.81	—	(−)
XXVIII	Purine					3.03 ± 0.73	—	(+)
I*a*	2-Amino-4-hydroxy-6-hydroxymethylpteridine					0.16 ± 0.04	0.9	(−)
II*b*	2-Amino-4,7-dihydroxypteridine					1.25 ± 0.68	2.5	(−)
III*a*	Allopurinol					0	1.7[f]	(+)

[a] Ar = aromatic; H_4 = 5,6,7,8-tetrahydro. [b] Control velocity = 5.07 ± 0.28; this represents the absorbance change (×200) over 1.7 min using 10 μM inhibitor and 10 μM xanthine in 0.05 M potassium phosphate buffer (pH 8.0). [c] Compounds that showed significant inhibition at 10 μM were also assayed at other concentrations to determine the level producing 50% of the control velocity (I_{50}). [d] Concentration = 100 μM; incubation time = 12 hr. [e] A final 10% dimethyl sulfoxide concentration was necessary to maintain the inhibitor in solution. (Control velocity = 3.74 ± 0.19.) [f] This value may be compared with the 0.87 μM value obtained using 8.1 μM hypoxanthine in tromethamine buffer (pH 7.4) containing 10% dimethyl sulfoxide (Ref. 1).

neutralization with concentrated ammonium hydroxide, each compound was recrystallized from dimethylformamide–water containing excess ammonium hydroxide. Compound XVI was obtained in 31% yield, mp 370–375° dec. [TLC, homogeneous in dimethylformamide–acetonitrile (1:1)].

Anal.—Calc. for $C_8H_6N_3O$: C, 49.12; H, 3.09; N, 21.48. Found: C, 49.10; H, 3.16; N, 21.26.

Compound XX was obtained in 42% yield, mp > 300° dec. [TLC, homogeneous in dimethylformamide–acetonitrile (1:1)].

Anal.—Calc. for $C_8H_8N_4O$: C, 54.53; H, 4.57; N, 31.80. Found: C, 54.46; H, 4.45; N, 31.69.

2-Amino-4-hydroxy-6-hydroxymethylquinazoline (XXII)—A 0.47-g (2.5-mmole) sample of 2,4-diaminoquinazoline-6-carboxaldehyde (10) in 100 ml of 5% aqueous acetic acid was shaken in the presence of 100 mg of 10% palladium-on-charcoal under 3.5 atm of hydrogen for 3 hr at ambient temperature. At this point, the hydrogen uptake had ceased and TLC (dimethylformamide) showed no aldehyde present. The solution was filtered through diatomaceous earth[7] and then evaporated to dryness *in vacuo*. After stirring with 5% NH_4OH for 1 hr, the solid product was separated by filtration, washed with water and acetone, and dried *in vacuo*. A cream-colored solid, 0.31 g (65% yield), was obtained, mp 248.5–250.5° dec. (TLC showed a trace impurity at the origin which was not removed by recrystallization from a variety of solvent combinations).

A 0.20-g sample of this material was refluxed in 3 ml of 2 N HCl for 2.5 hr. After neutralization with concentrated ammonium hydroxide, the product was isolated by filtration, washed successively with water and acetone, and dried *in vacuo*. A white powder, 0.144 g (72% yield), was obtained, mp > 360° dec. (TLC showed a trace of impurity at the origin in dimethylformamide). This material was extracted with ethanol made basic with concentrated ammonium hydroxide and filtered, and then the filtrate was concentrated until precipitation began. The resulting solid was separated by filtration, washed with absolute ethanol, and dried *in vacuo* (TLC, virtually homogeneous in dimethylformamide).

Anal.—Calc. for $C_9H_9N_3O_2 \cdot 1.5H_2O$: C, 49.54; H, 5.50; N, 19.26. Found: C, 49.29; H, 5.63; N, 19.25.

2-Amino-4-hydroxyquinazoline-6-carboxylic Acid Hydrochloride (XXIV)—To a heated suspension of 2.78 g (15 mmoles) of 6-cyano-2,4-diaminoquinazoline (10) in 37.5 ml of ethylene glycol was added dropwise 15 ml of 40% NaOH. After refluxing for 4 hr, the solution was treated with activated charcoal and then filtered through diatomaceous earth[7], the filter cake being washed with additional ethylene glycol. The filtrate was reheated, made strongly acidic with 2 N HCl, diluted further with water at the boiling point, and then cooled. The precipitate was collected on a filter and washed with water, methanol, and acetone. This solid was dissolved in boiling water made basic with concentrated ammonium hydroxide. The solution, which contained a small amount of insoluble material, was treated with charcoal, filtered through diatomaceous earth, and then reheated and made distinctly acidic as before. Crystallization occurred upon cooling; the product was collected on a filter, washed with water, methanol, and acetone; and finally dried *in vacuo*. White crystals, 2.2 g (61% yield), were obtained, mp > 450° gradual dec. (TLC, homogeneous in dimethylformamide).

Anal.—Calc. for $C_9H_7N_3O_3 \cdot HCl$: C, 44.73; H, 3.34; N, 17.39. Found: C, 44.66; H, 3.39; N, 17.21.

RESULTS AND DISCUSSION

The enzyme data obtained in this study are presented in Table I. Quinazoline (IV) and its 4-OH (V) and 4-NH_2 (VII) derivatives serve as substrates for this enzyme and are ineffective as inhibitors of xanthine oxidation. However, 4-mercaptoquinazoline (VI) is a weak inhibitor with apparently no substrate activity. As expected, purine (XXVIII) also serves as a substrate. Of the 2,4-disubstituted quinazolines, VIII–XIV, only those possessing a 2-NH_2 and either OH or SH in position 4 show significant inhibitory action. 2-Amino-4-mercaptoquinazoline is an exceptionally

[7] Celite.

potent inhibitor and acetylation of the 2-NH_2 group of XI to afford XII does not alter activity significantly.

A series of derivatives of XI was selected to test the effect of substitution in the benzenoid nucleus on inhibitory potency. The 4-mercapto analogs were not prepared for this study in view of the extreme difficulty encountered in the synthesis of the unsubstituted compound XIII (9). The addition of a chlorine in the 5-position (XV) did not alter activity, while the inclusion of a 5-CH_3 (XVII) was highly deleterious. Conversely, the 6-Cl (XVI) and 6-CH_3 (XVIII) derivatives had similar potencies to XI. The presence of a 6-CN (XXI) or 7-CF_3 (XIX) caused little change in the I_{50}'s, while the 6-NH_2 derivative (XX) was some sixfold less inhibitory than the parent compound, XI. Compounds XXII–XXIV were synthesized as analogs of the pteridines, I*a* and *b*. Both the 6-CH_2OH (XXII) and 6-CHO (XXIII) derivatives were excellent inhibitors, with XXII being three times more potent than its pteridine counterpart, I*a*. In addition, XXIII was fourfold more inhibitory than XXII (which was apparently not the case with respect to I*a* and I*b*) and also served as a slow substrate, apparently being converted into the 6-COOH derivative (XXIV) based upon spectrophotometric observations. As was the case in the pteridine series (2), the introduction of a 6-COOH group (XXIV) yielded a substantially less inhibitory compound.

The 5,6,7,8-tetrahydroquinazolines (XXV and XXVI) were examined together with isocytosine (XXVII) to determine whether a second ring fused to the 2-amino-4-hydroxypyrimidine moiety was required for good activity and, if so, whether this ring needed to be unsaturated. None of these compounds showed inhibitory effects at 10 μM, so this structural feature appears to be required for good inhibition.

CONCLUSION

From the results discussed, it is apparent that suitably substituted quinazolines, but not related 5,6,7,8-tetrahydroquinazolines, can act as potent inhibitors of xanthine oxidase, rivaling or even surpassing their highly potent pteridine analogs. Furthermore, structure–activity considerations indicate that the quinazolines more closely resemble the pteridines with regard to inhibition of this enzyme than the analogous purines or pyrazolo[3,4-*d*]pyrimidines such as III*a*. For example, 2,4-dihydroxyquinazoline (VIII) is far less inhibitory than the reported (1) activity for III*b*, while XIII is substantially more inhibitory than its purine counterpart 2-amino-6-mercaptopurine (1).

REFERENCES

(1) B. R. Baker, W. F. Wood, and J. A. Kozma, *J. Med. Chem.*, **11**, 661(1968).

(2) H. G. Petering and J. A. Schmitt, *J. Amer. Chem. Soc.*, **72**, 2995(1950).

(3) D. G. Priest and J. R. Fisher, *Arch. Biochem. Biophys.*, **146**, 391(1971).

(4) J. J. McCormack and D. M. Valerino, in "Chemistry and Biology of Pteridines," International Academic Printing Co., Ltd., Tokyo, Japan, 1970, p. 381.

(5) S. S. Parmar, C. Dwivedi, and B. Ali, *J. Pharm. Sci.*, **61**, 179(1972).

(6) G. B. Elion, A. Kovensky, G. H. Hitchings, E. Metz, and R. W. Rundles, *Biochem. Pharmacol.*, **15**, 863(1966).

(7) G. B. Elion, *Ann. Rheum. Dis.*, **25**, 608(1966).

(8) T. Spector and D. G. Johns, *J. Biol. Chem.*, **245**, 5079(1970).

(9) W. T. Ashton, F. C. Walker, III, and J. B. Hynes, *J. Med. Chem.*, **16**, 694(1973).

(10) J. Davoll and A. M. Johnson, *J. Chem. Soc. C*, **1970**, 997.

(11) A. Rosowsky, J. L. Marini, M. E. Nadel, and E. J. Modest, *J. Med. Chem.*, **13**, 882(1970).

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Chemical Constituents of *Piper sylvaticum* (Roxb) and *Piper boehmerifolium* (Wall)

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Abstract □ A phytochemical investigation of the roots and stems of *Piper sylvaticum* (Roxb) resulted in the isolation and identification of piperine, piperlonguminine, *N*-isobutyldeca-*trans*-2-*trans*-4-dienamide, and β-sitosterol. In addition, two unidentified compounds were detected. The stems of *P. boehmerifolium* (Wall) included piperine, but further investigation was not attempted.

Keyphrases □ *Piper sylvaticum* (Roxb)—phytochemical investigation of roots and stems, isolation and identification of piperine, piperlonguminine, *N*-isobutyldeca-*trans*-2-*trans*-4-dienamide, and β-sitosterol □ *Piper boehmerifolium* (Wall)—phytochemical investigation of stems, isolation and identification of piperine

Piper sylvaticum and *P. boehmerifolium* are members of the Piperaceae and are distinguished by perennial roots and branches creeping on the ground or rooting like ivy on trees. These plants are abundantly found in the upper and lower parts of Assam in India (1). In the Ayurvedic system of medicine in India, these roots have been used for their laxative, anthelmintic, and carminative properties and in bronchitis, diseases of the spleen, and the treatment of tumors (2). Although no phytochemical studies on