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SHORT COMMUNICATION

2-Amino-4-hydroxyquinazolines as Inhibitors of Thymidylate Synthetase

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SUMMARY

This paper describes a 2-amino-4-hydroxyquinazoline analogue of folic acid which is much stronger than methotrexate as a thymidylate synthetase inhibitor but weaker as a dihydrofolate reductase inhibitor. It also identifies the requirements of certain structural moieties for this activity.

It has been reported by Hutchison and co-workers[1] (1) that the 2,4-diamino- and 2-amino-4-hydroxyquinazoline analogues of folic acid may be as active as, or more active than, methotrexate (amethopterin) as inhibitors of the dihydrofolate reductase (5,6,7,8-tetrahydrofolate: NADP oxidoreductase, EC1.5.1.3) from L1210 leukemic cells. Baker and Coward had reported earlier (2) that modifying the substituents of the pyrimidinol nucleus changed the relative inhibitory activity of these derivatives toward dihydrofolate reductase as compared with that toward thymidylate synthetase (methylenetetrahydrofolate:dUMP *C*-methyltransferase EC2.1.1.b). Borsa and Whitmore (3) have proposed, on the basis of work with L-cells *in vitro*, that compounds with high inhibitory activity toward thymidylate synthetase should be more effective antitumor agents than those with high inhibitory activity toward dihydrofolate reductase, and that dihydrofolate reductase inhibition tends to counteract the effect of thymidylate synthetase inhibition. We report here on the comparative inhibitory activities of a group of 2-amino-4-hydroxyquinazoline analogues of folic acid in several systems, including those against dihydrofolate reductase and thymidylate synthetase.

The 2-amino-4-hydroxyquinazolines (4) (compounds I–IV, Table 1), the 2,4-diaminoquinazoline (V), and the pyrimidine compound (VI) were synthesized by Dr. John Davoll and co-workers.[2] Methotrexate was purchased from Lederle Laboratories, Pearl River, N. Y. Pigeon liver dihydrofolate reductase was prepared from pigeon liver acetone powder, obtained from Pentex, Kankakee, Ill., by the method of Baker *et al.* (5). Dihydrofolate reductase from *Streptococcus faecalis* R (ATCC 8043) was prepared by the method of Hillcoat and Blakley (6) from rapidly growing cells grown on Difco Micro Inoculum broth. Thymidylate synthetase was prepared by the method of Friedkin (7) from strain B *Escherichia coli* cells obtained from General Biochemicals, Chagrin Falls,

[1] D. J. Hutchison, F. M. Sirotnak and A. M. Albrecht, personal communication.

[2] Parke, Davis and Company, Chemistry Department, Hounslow, Middlesex, England.

Ohio, except that the cells were sonically disrupted instead of ground with alumina.

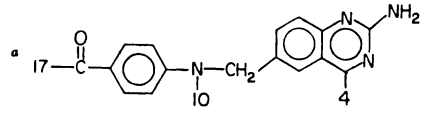
Dihydrofolate reductase activity from the two sources indicated was determined spectrophotometrically by the procedure of Friedkin *et al.* (8), at an enzyme concentration which gave a change in absorbance at 340 mμ of 0.025/min at 26°. Inhibitory activity was titrated by stepwise addition of the test compounds until 50% of the original enzyme activity remained. Thymidylate synthetase activity was also determined spectrophotometrically, by the method of Friedkin (7), and the inhibitory activities of the test compounds toward this enzyme were determined in the same way as for dihydrofolate reductase inhibition. These data are summarized in the last three columns of Table 1.

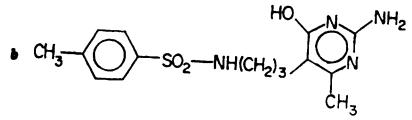
The experimental method described by Capps *et al.* (9) was used for studies of the inhibition of growth of *S. faecalis* R by the test compounds. The data are shown in columns 4–6 of the table. Ignoring permeability factors, the *S. faecalis* inhibition data in column 4 represent the over-all activity of the inhibitors against the test organism, while the data in column 5 represent the inhibitory power remaining after the inhibition of the reductases was reversed. The data

TABLE 1

Relation of structures among 2-amino-4-hydroxyquinazoline antifolates to their inhibition of growth of S. faecalis R and thymidylate synthetase and dihydrofolate reductase activities from mammalian and bacterial sources

1. Inhibitor	2. Basic ring structure[a, b]	3. Substituents			Concentration inhibiting growth of *S. faecalis* R 50%			Concentrations inhibiting enzyme activity 50%		
		4	10	17	4. With PteGlu[c]	5. With 5-CHO[d]	6. With 5-CHO + thymidine[e]	7. Thymidylate synthetase[f]	Dihydrofolate reductase[g] 8. Pigeon liver	Dihydrofolate reductase[g] 9. *S. faecalis* R
					mμM	mμM	mμM	μM	μM	μM
MTX	Pteridinyl	NH_2	CH_3	Glu	0.44	1.3	2.4	70	0.018	0.00029
I	Quinazolinyl	OH	H	Glu	1.3	1.7	39	0.75	0.25	0.27
II	Quinazolinyl	OH	CH_3	Glu	0.16	0.29	580	0.098	0.20	0.17
III	Quinazolinyl	OH	H	Asp	15	28	110	4.5	0.25	0.57
IV	Quinazolinyl	OH	CH_3	Asp	0.27	1.2	2.7	5.7	0.82	0.0014
V	Quinazolinyl	NH_2	CH_3	Glu	0.11	0.43	1.1	2.1	0.015	0.00034
VI	Pyrimidinyl				65,000			>490		

[a]  Quinazolinyl moiety. Substituents are numbered as in methotrexate (MTX).

[b]  This compound was synthesized by J. Dickinson,[3] and is referred to as compound XII by Baker and Coward (2).

[c] Pteroylglutamic acid, 0.91 mμM.

[d] *l*-Leucovorin, 0.84 mμM.

[e] *l*-Leucovorin, 0.84 mμM, plus thymidine, 41.0 μM.

[f] Substrate, 0.28 mM *dl*, L-tetrahydrofolic acid; dUMP, 0.042 mM.

[g] Substrate, 33 μM dihydrofolic acid; 67 μM NADPH.

in column 6 show the extent to which this remaining inhibition was reversed by the presence of thymidine and therefore indicate the possible effectiveness of these compounds as thymidylate synthetase inhibitors. Compound II is by far the most remarkable in this respect. This compound was also an extremely active inhibitor of thymidylate synthetase from *E. coli* (column 7), being much more active than any other in this series. Compare this especially with the large but still inadequate amount of VI, the compound recommended by Baker and Coward (2) as a thymidylate synthetase inhibitor.

The importance of the hydroxyl group at position 4 of compound II to its inhibitory activity against thymidylate synthetase is indicated by comparison with compound V, which is identical except for the presence of an amino instead of a hydroxyl group at position 4. The latter was 500 times more potent than the former as an inhibitor of dihydrofolate reductase from *S. faecalis* R, but 21 times less potent than the former as an inhibitor of thymidylate synthetase. Methotrexate was 586 times more active than compound II against dihydrofolate reductase and 714 times less active against thymidylate synthetase.

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