Europ. J. Cancer Vol. 16 pp. 707–711

0014-2964/80/0501/0707 $02.00/0

5-Substituted Quinazoline Antifolates

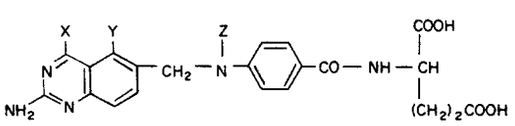
TERENCE R. JONES

Department of Biochemical Pharmacology, Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, London SW3 6JB, England

Abstract—*Two new 5-substituted-2-amino-4-hydroxy-quinazoline antifolates are described. The presence of the 5-substituent reduced the inhibitory effect upon thymidylate synthetase. In contrast, the 2,4-diaminoquinazoline series, the same 5-substituents improved the inhibition.*

INTRODUCTION

THYMIDYLATE synthetase (EC 2.1.1.45), the enzyme catalysing the reductive methylation of 2′-deoxyuridylate to thymidylate, with the concomitant conversion of 5,10-methylene tetrahydrofolate to dihydrofolate, has long been a target of cancer chemotherapists. However, the only drugs available which act significantly against this enzyme are 5-fluorouracil, its riboside and deoxyriboside which all require metabolic activation to 5-fluorodeoxyuridylate for their effect. One of the major determinants of clinical resistance to these agents is the deletion of the activating enzymes [1]. There is, accordingly, a good case for developing a structural analogue of folic acid which will inhibit thymidylate synthetase without requiring metabolic activation. The reports [2–4] that certain 2-amino-4-hydroxyquinazoline antifolates (Fig. 1, compounds 1 and 2) were effective inhibitors of the enzyme prompted an interest in this laboratory. The molecule bearing a 10-methyl group (compound 1) proved [2] to be a better inhibitor of thymidylate synthetase than the unsubstituted compound (2). The structure of the natural substrate, 5,10-methylene tetrahydrofolate, suggests that the 5 and 10 positions in a folate-like molecule will become juxtaposed upon binding to the enzyme, and, for inhibition in the 2-amino-4-hydroxyquinazoline series, the methyl substituent might thus be better located at 5. This paper reports the synthesis of the 5-methyl compound (3), also the 5-chloro analogue (4). The known [5, 6] 5-unsubstituted quinazoline (1) was made for purposes of comparison. The synthetic route to these compounds also allowed the convenient preparation of the three known 2,4-diaminoquinazolines (5, 6, 7) which were required for comparative biochemical assessment. These were first prepared by Davoll and Johnson [5] and studied by Hutchison [7] for their effects upon cells in culture and for their antileukaemic activity in mice. In the course of the present work, Hynes *et al.* [8] reprepared them as part of their studies on quinazoline inhibitors of dihydrofolate reductase (EC 1.5.1.4).



Compound	X	Y	Z
1	OH	H	CH_3
2	OH	H	H
3	OH	CH_3	H
4	OH	Cl	H
5	NH_2	H	H
6	NH_2	CH_3	H
7	NH_2	Cl	H

Fig. 1. Structures of quinazoline antifolates.

Accepted 8 November 1979.

CHEMISTRY

The compounds (2–7) were made by the reductive condensation of the appropriately substituted 6-cyanoquinazoline with diethyl *p*-aminobenzoylglutamate as described by Davoll and Johnson [5]. A slight modification concerned the isomers (8) and (9) (Fig. 2) obtained by nitration of 6-chloro-2-methylbenzonitrile. The earlier workers did not separate these isomers but separated the derived mixture of 2,4-diaminoquinazolines (10) and (11) (Fig. 2). I preferred to separate

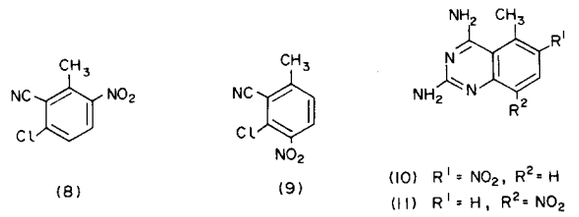


Fig. 2. Structures of chemical intermediates.

the benzenes, to afford 6-chloro-2-methyl-3-nitrobenzonitrile (8) (m.p. 91.5°C) and 2-chloro-6-methyl-3-nitrobenzonitrile (9) (m.p. 107.5°C). The assignment of structures (in confirmation of the findings of Davoll and Johnson [5]) rested on the observation that the higher-melting isomer, on prolonged treatment with hydrogen and a palladium catalyst afforded 5-amino-2-methylbenzonitrile. Reaction of the lower-melting isomer with guanidine carbonate then yielded the desired quinazoline (10).

Fear has been voiced that 2-amino-4-hydroxyquinazolines derived from 2,4-diamino intermediates may not be pure [3]. However, in present hands the hydrolysis, in boiling acid, of the 2,4-diaminoquinazoline-6-carbonitriles to the 4-hydroxy counterparts went to completion. This was shown by quantitative TLC studies whereby each product was found to be homogeneous in a system which allowed detection of the starting material at the 1% level. The freedom of each derived 2-amino-4-hydroxy-quinazoline from its 2,4-diamino analogue was thus assured to at least this same level.

BIOLOGICAL RESULTS

Each of the folate analogues was dissolved in water with the addition of two equivalents of sodium hydroxide and assayed against thymidylate synthetase and dihydrofolate reductase. The results are shown in Table 1. Further, more detailed, biochemical studies are published separately [9].

As can be seen, in the 2-amino-4-hydroxy series, the 5-methyl compound (3) was a much less effective inhibitor of thymidylate synthetase than the parent quinazoline (2). This is also true for the 5-chloro compound (4). However, in the 2,4-diamino series the introduction of the 5-methyl, and to a lesser extent the 5-chloro, substituent improved the inhibition of thymidylate synthetase. A similar result, though less pronounced was obtained upon introducing a 5-methyl group into the corresponding aspartate-containing 2,4-diaminoquinazoline [4]. The 2,4-diamino compounds were effective inhibitors of dihydrofolate reductase, confirming the observations of Hynes *et al.* [8]. As expected, the 2-amino-4-hydroxy compounds were moderate inhi-

Table 1. Properties of quinazoline analogues of folic acid

Compound	m.p. (°C)	Elemental analysis Found % C	H	N	Required (%) C	H	N	Thymidylate* Synthetase I_{50} (μM)	Dihydrofolate† reductase I_{50} (nM)
2	dec > 230‡	56.88	4.91	15.68	57.40	4.82	15.94	0.29§	250‖
3	dec > 230	58.12	5.26	15.14	58.27	5.11	15.45	22	1100
4	dec > 230	53.08	4.39	14.53	53.22	4.25	14.78	5.5	630
5	dec > 220¶	57.38	5.15	19.05	57.52	5.06	19.17	100	6.5**
6	dec > 220††	58.52	5.26	18.43	58.40	5.35	18.58	0.25	6.8§§
7	dec > 215‖‖	53.54	4.76	17.97	53.33	4.48	17.77	4.3	6.7¶¶

*Enzyme from Yoshida ascites sarcoma cells [9].

†Rat liver enzyme [9].

‡Acharya and Hynes [6] reported m.p. 232°C dec, Davoll and Johnson [5] reported m.p. 225–240°C for the monohydrate.

§O. D. Bird *et al.* [2] reported 0.75 μM for *E. coli* enzyme.

‖Hynes *et al.* [8] reported 23 nM (enzyme from rat liver), O. D. Bird *et al.* [2] reported 0.25 μM (enzyme from pigeon liver).

¶Hynes *et al.* [8] reported m.p. > 230°C dec for the monohydrate, Davoll and Johnson [5] reported m.p. 24–242°C for the anhydrous material.

**Hynes *et al.* [8] reported 34 nM (enzyme from rat liver).

††Hynes *et al.* [8] reported m.p. 201–203°C for a hydrate.

§§Hynes *et al.* [8] reported 69 nM (enzyme from rat liver).

‖‖Hynes *et al.* [8] reported m.p. 228–235°C dec for a hydrate, Davoll and Johnson [5] reported m.p. 220–222°C for the hemihydrate.

¶¶Hynes *et al.* [8] reported 75 nM (enzyme from rat liver).

bitors of the reductase, the 5-substituents causing slightly decreased binding.

EXPERIMENTAL SECTION

Melting points were determined on a Kofler block and are corrected. Elemental analyses were determined by Butterworth Laboratories Ltd., Teddington, Middlesex; good results for the folate analogues were obtained only by the use of both a catalytic additive and an oxygen donor. NMR spectra were taken on a Perkin–Elmer R12B 60 MHz spectrometer (solutions in $CdCl_3$) with TMS as internal standard). Infra-red spectra were taken on a Perkin–Elmer Model 257 spectrometer. Column chromatography was performed on silica gel (Merck, Art 7734). TLC was performed on Polygram PG23 precoated silica plates (Macherey–Nagel & Co.). CMA refers to the development solvent chloroform:methanol:acetic acid (75:20:5). Centrifugation was done in a bench centrifuge. Melting points and microanalyses of the folate derivatives are given in Table 1.

6-*Chloro*-2-*methyl*-3-*nitrobenzonitrile* (8)

The crude product from the nitration of 6-chloro-2-methyl-benzonitrile [5] was applied, 10 g at a time dissolved in a mixture of ether (10 ml) and toluene (6 ml), to a column of silica (1 kg) and the column was washed with ether. The product (7.5 g) eluted first. After removal of the solvent, crystallisation from ethanol gave white microneedles, m.p. 91.5°C. NMR τ 7.20 (s,3,CH_3), 2.41 (d,1,H_5), 1.86 (d,1,H_4). Found: C, 49.08; H, 2.76; N, 14.36. $C_8H_5ClN_2O_2$ requires C, 48.87; H, 2.56; N, 14.25%. There followed 2-chloro-6-methyl-3-nitrobenzonitrile (9) (2.5 g) which after similar work-up formed white microneedles, m.p. 107.5°C. NMR τ 7.30 (s,3,CH_3), 2.55 (d,1,H_5), 1.98 (d,1,H_4). Found: C, 49.14; H, 2.66; N, 14.28. $C_8H_5ClN_2O_2$ requires C, 48.87; H, 2.56; N, 14.25%.

5-*Amino*-2-*methylbenzonitrile*

The nitro compound (9) (0.984 g, 5 mmole) in ethanol (30 ml) was hydrogenated at room temperature and pressure for 71 hr using a 5% palladium on charcoal catalyst (0.5 g). After removal of the catalyst and solvent the residue was treated with dilute sodium hydroxide and ether. The organic layer was separated, washed with water and evaporated to yield the crude product (0.39 g, 59%). After two crystallisations from petroleum ether (b.p. 60–80°C) it had m.p. 89.5°C (lit [10] 88°C). NMR τ 7.59 (s,3,CH_3), 6.34 (s,2,NH_2), 3.22 (part-hidden doublet,1,H_4), 3.16 (broad-singlet,1,H_6), 2.93 (d,I,H_3). Infra-red 2230 cm^{-1} ($\gamma C \equiv N$), 870 and 833 cm^{-1} (1,2,4-trisubstitution). Found: C, 73.08; H, 5.95; N, 21.15. Calc. for $C_8H_8N_2$: C, 72, 70; H, 6.10; N, 21.20%.

2,4-*Diamino*-5-*methyl*-6-*nitroquinazoline* (10)

The chloronitrile (8) (5.97 g) and guanidine carbonate (5.46 g) were heated in refluxing 2-ethoxyethanol (43 ml) for 3.5 hr. After cooling, the rust-coloured product was collected and washed with ethoxy-ethanol, then ether and dried at 100°C (4.21 g, 63%) m.p. 290°C. Found: C, 49.22; H, 4.08; N, 31.83. Calc. for $C_9H_9N_5O_2$: C, 49.32; H, 4.14; N, 31.96%.

2-*Amino*-4-*hydroxy*-5-*methylquinazoline*-6-*carbonitrile* (12)

2,4-Diamino-5-methylquinazoline-6-carbonitrile [5] (2.00 g) and 2N HCl (aq.) (200 ml) were boiled for 4.5 hr. The mixture was filtered (charcoal) and the solution basified with aqueous ammonia. The gelatinous product was filtered off, washed with water and recrystallised from DMF–H_2O to give tan-coloured crystals (0.61 g, 30.4%), m.p. >350°C. Found: C, 58.40; H, 4.24; N, 27.09. $C_{10}H_8N_4O$, 0.25 H_2O requires: C, 58.67; H, 4.19; N, 27.37%. TLC: the product (10 μg) and the starting material (0.1 μg) were applied separately to a 20 cm plate and also together, in these quantities, to form a mixture spot. The solutions were in DMSO (10 mg/ml and 10 mg/100 ml, respectively). The 1 μl spottings were made using a SMI micro/pettor (from Alpha Laboratories Ltd., Greenford, Middlesex). The DMSO was removed from the plate *in vacuo* (desiccator) and the plate then developed in CMA. The product (R_f 0.71) was free from starting material (R_f 0.31).

N-(p-(((2-*amino*-4-*hydroxy*-5-*methyl*-6-*quinazolinyl*)*methyl*)*amino*)*benzoyl*)-L-*glutamic acid* (3)

The nitrile (12) (0.400 g, 2 mmole) and diethyl *p*-aminobenzoyl-L-glutamate [11] (0.645 g, 2 mmole) were dissolved in warm 75% HOAc (aq.) (35 mL). Raney nickel catalyst [12] was added and the mixture hydrogenated at room temperature and pressure. Theoretical uptake took place during 26 hr. The filtrate was evaporated and the residue

triturated with 2N Na_2CO_3 (aq.). The resulting solid was filtered, washed, dried and then chromatographed on a column of silica, eluting with ethanol. The intermediate ester thus obtained amounted to 0.240 g (23.6%). It was a single spot on TLC (R_f 0.44 in ethanol). It (0.110 g) was dissolved in a mixture of ethanol (2 ml) and 1N NaOH (aq.) (3 ml) and left for 2 hr. The pH was reduced to 5.8 (dil. HCl) and a slight precipitated impurity removed by centrifugation. The supernatant was brought to pH 4.2 and the gelatinous product was freed from inorganic ions by three cycles of the series: aqueous suspension–centrifugation–decantation. The solid was collected and dried at 90°C *in vacuo* to give an off-white powder (0.015 g, 15.3%). Ultra-violet (in 0.1N NaOH) λ max (ε): 297 nm (sh) (23,700), 284 (25,200), 232 (41,500). The product was pure by TLC (a single spot of R_f 0.37 in CMA).

2 - *Amino* - 5 - *chloro* - 4 - *hydroxyquinazoline* - 6 - *carbonitrile* (13)

This was prepared, as for (12), from the corresponding 2,4-diamino-compound [5] in 38.7% yield. It crystallised from H_2O–DMF as a pale brown powder, m.p. > 350°C. Found: C, 49.04; H, 2.40; N, 25.38. $C_9H_5ClN_4O$ requires C, 49.01; H, 2.28; N, 25.40%. Analysed as for (12) it was homogenous by TLC: (Product R_f 0.74, starting material R_f 0.48).

N - (p - (((2 - *amino* - 5 - *chloro* - 4 - *hydroxy* - 6 - *quinazolinyl*)*methyl*)*amino*)*benzoyl*) - L - *glutamic acid* (4)

As for the diester of (3), the diester of (4) was prepared from the nitrile (13) (0.441 g, 2 mmole) and diethyl *p*-aminobenzoyl-L-glutamate (0.645 g, 2 mmole) suspended in glacial acetic acid (40 ml). After 24 hr the catalyst only was in suspension and 83% of theoretical uptake had occurred. Following the work-up and chromatography detailed above, the diester was obtained pure as a white solid (0.093 g, 8.8%). It was homogeneous on TLC (R_f 0.56 in ethanol). A solution of it (0.092 g) in a mixture of ethanol (2 ml) and 1N NaOH (aq.) (4 ml) was left for 1.75 hr. The pH was brought to 5.8 (dil. HCl) and a slight precipitate removed by centrifugation. Upon reducing the pH of the supernatant to 4.1 the white gelatinous product was obtained. It was washed, collected and dried as for (3). The off-white powder weighed 0.032 g (38.9%) u.v. (in 0.1N NaOH) λ max (ε): 344 nm (sh) (3,300), 285 (24,700), 235 (36,200). TLC analysis gave a single spot of R_f 0.20 in CMA.

Acknowledgements—This investigation was supported by CRC Grant 973/73 to the Institute of Cancer Research: Royal Cancer Hospital.

REFERENCES

1. K. R. Harrap, Resistance to antitumour agents. In *Scientific Foundations of Oncology*. (Edited by T. Symington and R. L. Carter), p. 649. Heinemann, London (1976).
2. O. D. Bird, J. W. Vaitkus and J. Clarke, 2-Amino-4-hydroxyquinazolines as inhibitors of thymidylate synthetase. *Mol. Pharmacol.* **6,** 573 (1970).
3. S. C. Carlin, R. N. Rosenberg, L. VandeVenter and M. Friedkin, Quinazoline antifolates as inhibitors of growth, dihydrofolate reductase, and thymidylate synthetase of mouse neuroblastoma cells in culture. *Mol. Pharmacol.* **10,** 194 (1974).
4. R. W. McCuen and F. M. Sirotnak, Thymidylate synthetase from *Diplococcus pneumoniae*. Properties and inhibition by folate analogues. *Biochim. biophys. Acta (Amst.)* **384,** 369 (1975).
5. J. Davoll and A. M. Johnson, Quinazoline analogues of folic acid. *J. chem. Soc.* (**C**), 997 (1970).
6. S. P. Acharya and J. B. Hynes, A new synthetic route to quinazoline analogues of folic acid. *J. heterocycl. Chem.* **12,** 1283 (1975).
7. D. J. Hutchison, Quinazoline antifolates: biologic activities. *Cancer Chemother. Rep.* **52,** 697 (1968).
8. J. B. Hynes, D. E. Eason, C. M. Garrett, P. L. Colvin, Jr., K. E. Shores and J. H. Freisheim, Quinazolines as inhibitors of dihydrofolate reductase. 4. Classical analogues of folic and isofolic acids. *J. med. Chem.* **20,** 588 (1977).

9. A. H. CALVERT, T. R. JONES, P. J. DADY, B. GRZELAKOWSKA-SZTABERT, R. M. PAINE, G. A. TAYLOR and K. R. HARRAP, Quinazoline antifolates with dual biochemical loci of action. Biochemical and biological studies directed towards overcoming methotrexate resistance. *Europ. J. Cancer* **16,** 713 (1980).
10. W. LANDSBERGER, Einige Abkömmlinge des *o*-Tolunitrils. *Ber. dtsch. chem. Ges.* **31,** 2880 (1898).
11. F. E. KING, R. M. ACHESON and P. C. SPENSLEY, Benziminazoles related to pteroic and pteroylglutamic acids. *J. chem. Soc.* 1401 (1949).
12. R. MOZINGO, Catalyst, Raney nickel, W-2. In *Organic Syntheses,* (Edited by E. C. Horning) Collective Vol. 3, p. 181. John Wiley, New York (1955).