Potential Antileprotic Agents. 3. Inhibition of Mycobacterial Dihydrofolic Reductase by 2,4-Diamino-5-methyl-6-alkylquinazolines

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In the previous communication[1] of this series some 2,4-diamino-6-substituted pteridine compounds were investigated for their ability to inhibit (*via* disruption of folate biosynthesis) the growth of a leprosy related mycobacterium, *M. sp.* 607. The compounds were effective inhibitors of the dihydrofolic reductase isolated from the organism but failed to inhibit cell growth. We have continued to explore structure–activity effects in related fused bicyclic pyrimidine systems. In this manuscript we are reporting the synthesis and antimycobacterial action of a series of 2,4-diamino-5-methyl-6-alkylquinazolines.

The synthetic route to these compounds commenced with 2-methyl-3-nitrobenzoic acid (I) which was catalytically reduced to the amine. Subsequent diazotization, treatment with KI, and esterification gave the 3-iodo-*o*-toluate (II) in 25% yield from I. The alkyl side chain was introduced *via* coupling[2] with appropriate cuprous acetylides in pyridine to afford the acetylenic esters III in 79–92% yield. Hydrogenation and saponification yielded the 3-alkyl-o-toluic acids IV.

Nitration of the alkyl acids IV according to the procedure of Kerfanto and Raphalen[3] for 2,3-dimethylbenzoic acid gave a mixture of isomeric nitro acids. Separation of the desired ortho isomers V was facilitated by their lower pK_a's, which allowed selective precipitation upon acidification of alkaline solutions.[3] Catalytic reduction afforded the amino acids VI. Treatment with KOCN–HOAc–H_2O[4] gave the intermediate ureido acids VII which were cyclized to the dihydroxyquinazolines VIII with hot Ac_2O. Only the methyl and dimethyl acids VIIa,b could be cyclized in alkali,[4] while direct fusion of amino acids VI with urea gave little or no quinazoline products. Chlorination of VIII with $POCl_3$ followed by ammonolysis of the dichloro compounds IX completed the synthesis of the diaminoquinazolines X. The physical properties and bioassay data are shown in Table I.

The compounds were found to be moderate to strongly effective growth inhibitors of *M. sp.* 607. The activity peaked at the intermediate chain lengths, with the 6-*n*-propyl analog Xc being the most active on a molar basis with a minimum inhibitory concentration (MIC) of 1.8 nmol/ml. The methyl (Xb) and hexyl compounds (Xg) were significantly less effective with MIC's of 16 and 15.5, respectively, while 2,4-diamino-5-methylquinazoline (Xa) was fairly ineffective (MIC of 115). Activity of Xa–g against some other nonmycobacterial organisms was not outstanding (Table I).

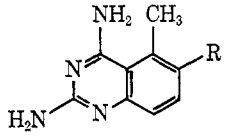
When the 6-propyl analog Xc was used in combination with diaminodiphenyl sulfone (DDS)[5] against *M. sp.* 607, a pronounced synergistic effect was observed. The optimal MIC requirement of quinazoline was reduced by eightfold and DDS by 20-fold for mycobacterial growth inhibition (Figure 1). The plot of changing values of MIC's for both drug components according to the method of Rosenoer[6] established that true potentiative synergism was in effect.

The method of synthesis precluded preparation of the 6-ethyl, isopropyl, and branched butyl analogs which could be even more potent than Xc. Development of alternate processes for the synthesis of such compounds and others with more complex side chains is under investigation. The present compounds are also being prepared in quantity for chronic toxicity measurement and examination against *Mycobacterium leprae* in the mouse footpad assay.[7] Further details of sulfa combination experiments and mouse footpad assays will be reported elsewhere.

Experimental Section

Methyl 2-Methyl-3-iodobenzoate (II). A mixture of 100 g (0.55 mol) of 2-methyl-3-nitrobenzoic acid, 1.0 g of 10% Pd/C, and 50 ml of DMF was hydrogenated at 3 atm of pressure, taking up the theoretical amount of H_2 in 6 hr. The catalyst was removed by filtration and the filtrate was diluted to 500 ml (DMF), cooled to 5°, and slowly treated with 276 ml of 6 *N* HCl. Then at −5° a solution of 40 g (0.58 mol) of $NaNO_2$ in 200 ml of H_2O was added over 30 min. After stirring at −5° for 30 min, 91.5 g (0.55 mol) of KI was added portionwise and the mixture was stirred at −5° for an additional 30 min and then at room temperature for 18 hr. The mixture was extracted with Et_2O (3 × 250 ml) and washed with 10% $NaHSO_3$ (2 × 250 ml) and then with two 500-ml portions of H_2O. The organic solution was dried ($MgSO_4$) and evaporated under reduced pressure to afford 118 g (81%) of pale pink solid.

Table I. Physical and Antibacterial Data for 2,4-Diamino-5-methyl-6-substituted Quinazolines



No.	R	Mp, °C	Yield, %	Formula[a]	MIC,[b,c] nmol/ml (μg/ml)
Xa	H	204–208[d]	35		115 (20.0)
Xb	CH_3	260–265	79	$C_{10}H_{12}N_4$	16 (3.0)
Xc	n-C_3H_7	210–214	73	$C_{12}H_{16}N_4 \cdot HCl$	1.8 (0.4)
Xd	n-C_4H_9	284–289	90	$C_{13}H_{18}N_4 \cdot HCl$	3.0 (0.7)
Xe	n-C_5H_{11}	282–288	54	$C_{14}H_{20}N_4 \cdot HCl$	7.1 (1.75)
Xf	i-C_5H_{11}	270–273	89	$C_{14}H_{20}N_4 \cdot HCl$	8.2 (2.0)
Xg	n-C_6N_{13}	274–283	71	$C_{15}H_{22}N_4 \cdot NCl$	15.5 (4.0)

[a] All compounds with formulas were analyzed for C, H, and N; values ±0.4% of theory. [b] Minimum inhibitory concentration for growth inhibition of *Mycobacterium* 607; μg/ml expressed as free base form. [c] The compounds were also evaluated against *Staphylococcus aureus, Streptococcus faecalis, Corynebacterium acnes, Pseudomonas aeruginosa, Erysipelothrix insidiosa, Escherichia coli, Proteus vulgaris,* and *Salmonella chloraesuis.* Weak activity (MIC 50–100 μg/ml) was observed for Xd–g; Xa–c were generally inactive (>100). Exceptions were Xg, active at 10 μg/ml against *S. aureus* and *S. faecalis.* [d] G. H. Hitchings, E. A. Falco, and K. W. Ledig, U. S. Patent 2,945,859 (July 19, 1960) [*Chem. Abstr.*, 54, 24821*a* 1960)] reported mp 212–213°.

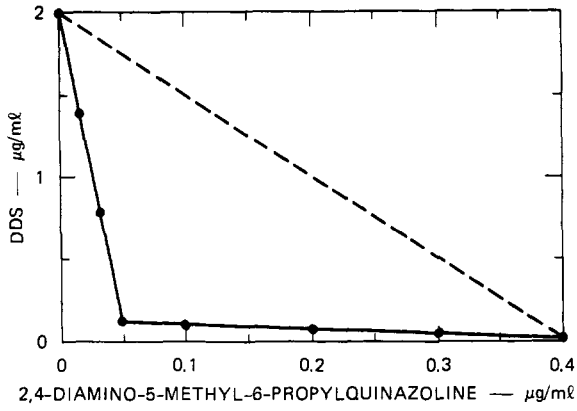
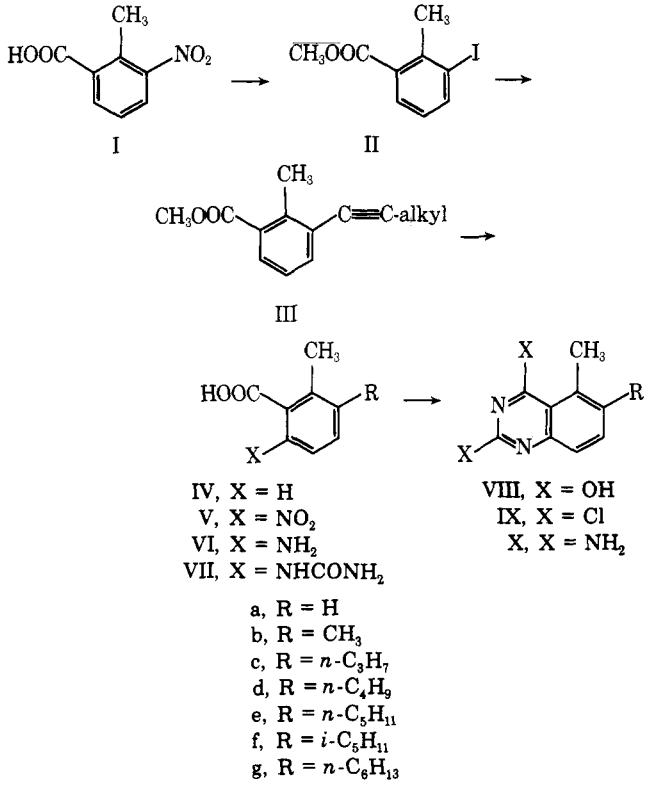


Figure 1. Synergy plot of 2,4-diamino-5-methyl-6-propylquinazoline and DDS.

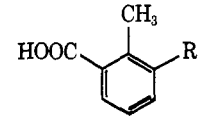


A mixture of 140 g of the crude iodo acid, 10 g of *p*-TsOH, and MeOH (1 l.) was refluxed for 15 hr and then evaporated *in vacuo*. The residue was taken up in 250 ml of Et_2O and washed twice with 200-ml portions of 1 *N* NaOH and then with 250 ml of H_2O. The organic extract was dried ($MgSO_4$) and evaporated at reduced pressure. The residue was distilled, bp 107° (0.45 mm), to give 90 g (61%) of yellow liquid: nmr (CCl_4) 7.97 and 7.75 ppm (4-H and 6-H, each pair of doublets), 6.86 (5-H, multiplet), 3.82 (ester CH_3, singlet), 2.67 (Ar-CH_3, singlet). *Anal.* ($C_9H_9IO_2$) C, H.

Methyl 2-Methyl-3-(1-alkynyl)benzoates (III). These esters were prepared in 70–80% yields from 40 g (0.14 mol) of II, 600 ml of anhydrous pyridine, and 0.166 mol of cuprous 1-alkyne according to the procedure of Stephens and Castro.[2] Ir spectra clearly showed the -C≡C- band at 4.46 μ and absence of II was confirmed by ir and tlc on silica gel.

2-Methyl-3-*n*-butylbenzoic Acid (IVd). A mixture of 23.9 g of III (alkyl = C_2H_5), 0.2 g of PtO_2, and 130 ml of absolute EtOH was hydrogenated at 3 atm of pressure, taking up the theoretical amount of H_2 in 18 hr. The catalyst was removed and filtrate was evaporated *in vacuo* to afford a theoretical yield of the saturated ester. The ester was saponified with 175 ml of 10% NaOH–175 ml of MeOH at reflux for 20 hr. Removal of MeOH and acidification (6 *N* HCl) gave a white precipitate (21.6 g, 95%) which was recrystallized (EtOH–H_2O). Physical data for IVd and the other acids, similarly prepared, are listed in Table II.

Table II. 2-Methyl-3-alkylbenzoic Acids



No.	R	Mp, °C	Yield, %	Formula[a]
IVc	*n*-C_3H_7	93.5–95.5	92	$C_{11}H_{14}O_2$
IVd	*n*-C_4H_9	80–82	95	$C_{12}H_{16}O_2$
IVe	*n*-C_5H_{11}	77–78	82	$C_{13}H_{18}O_2$
IVf	*i*-C_5H_{11}	75–76.5	100	$C_{13}H_{18}O_2$
IVg	*n*-C_6H_{13}	79–81	97	$C_{14}H_{20}O_2$

[a] All compounds were analyzed for C and H; values ±0.4% of theory.

2-Methyl-3-alkyl-6-nitrobenzoic Acids (V). These *o*-nitro acids were prepared by the treatment of a mixture of 1.6 g (0.11 mol) of IV dissolved in 150 ml of concentrated H_2SO_4 with 11.4 g (0.11 mol) of KNO_3 dissolved in 53 ml of concentrated H_2SO_4, with 11.4 g (0.11 mol) of KNO_3 dissolved in 53 ml of concentrated H_2SO_4, and a crude isomer separation was carried out according to the procedure of Kerfanto and Raphalen.[3] The crude acids were recrystallized twice from C_6H_6–cyclohexane to afford the *o*-nitro acids still contaminated with some meta isomer. Repeated crystallization or chromatography of the methyl esters was not effective for obtaining pure analytical samples. Nmr spectra (Ar protons, doublets centered at 7.29 and 7.90 ppm, ortho coupled, J = 9 Hz) and ir spectra (characteristic set of bands 11.5–14.5 μ) were used to indicate when the percentage of ortho isomer was suitable for direct use in the next step.

5-Methyl-6-*n*-butyl-2,4-quinazolinedione (VIIId). Method A. A mixture of 4.4 g of 2-methyl-3-*n*-butyl-6-nitrobenzoic acid (Vd), 50 mg of 5% Rh/C, and 100 ml of absolute EtOH was hydrogenated at 3 atm of pressure taking up the theoretical amount of H_2 in 17 hr. The catalyst was removed by filtration and the solvent was removed *in vacuo* to give 3.8 g (98%) of VId as a white solid. A stirred mixture of 3.8 g (18.4 mmol) of VId, 40 ml of MeOH, 10 ml of H_2O, and 1.37 ml (23.8 mmol) of HOAc was warmed to 35° and then allowed to cool to room temperature. To this mixture was added, dropwise, a solution of 1.88 g (23.2 mmol) of KOCN dissolved in 6 ml of H_2O. After stirring at room temperature for 2 hr, the white solid was collected and washed with a little MeOH (4.57 g). The K salt was dissolved in 200 ml of hot H_2O and filtered, and the cooled filtrate was acidified to pH 2 (6 *N* HCl). The white precipitate was collected, washed with H_2O, and dried to afford 3.48 g (76%) of the ureido acid VIId. Treatment with 75 ml of Ac_2O at reflux for 4.5 hr, followed by evaporation and trituration with Et_2O gave VIIId as a white solid (2.0 g, 63%). Compounds VIIIc-g were prepared in this manner in 40–70% yields. Repeated crystallization did not give analytically pure materials, but direct treatment with $POCl_3$ (next step) apparently removed major contaminants.

Method B. Compounds VIIIa,b were obtained by cyclization of the ureido acids VIIa,b in NaOH media at 40–50° for 2 hr *via* the procedure of Lange and Sheibley.[4] Insolubility of the K salts of VIIc-g in alkali prevented the use of this method; however, a trace amount of VIIg, analytically pure, was obtained by method B: VIIIa, mp 272–278° ($C_9H_8N_2O_2$); VIIIb, mp >300° ($C_{10}H_{10}N_2O_2$); VIIIg, mp 260–266° ($C_{15}H_{20}N_2O_2$); all analyzed for C, H, and N.

2,4-Dichloro-5-methyl-6-alkylquinazolines (IX). A mixture of 2.0 g of 5-methyl-6-alkyl-2,4-quinazolinedione (VIII), 16 ml of $POCl_3$, and 0.9 ml of Et_3N was stirred at reflux for 5.5 hr. The dark mixture was evaporated *in vacuo* and the resulting heavy syrup was dried for 1 hr at <1 mm, then dissolved in 50 ml of $CHCl_3$, and added to 50 ml of ice H_2O. The mixture was shaken and the organic layer removed. The aqueous portion was extracted twice with $CHCl_3$ (2 × 25 ml) and the combined extracts were dried (Na_2SO_4). The solvent was removed under reduced pressure to afford the crude dichloro compounds as dark syrups: ir no OH (3.0 μ) or C=O (5.9–6.0), strong 6.50 (ClC=N).

2,4-Diamino-5-methyl-6-*n*-butylquinazoline (Xd). A mixture of 2.3 g (8.5 mmol) of crude IXd and 50 ml of absolute EtOH (saturated with dry NH_3) was heated at 160–165° in a Parr bomb for 17 hr. The solvent was removed *in vacuo* and the residue added to 26 mmol of NaOEt in 200 ml of absolute EtOH. The

mixture was stirred at reflux for 1.75 hr and evaporated *in vacuo* to dryness, and the residue was suspended in 25 ml of ice H_2O. The cream-colored solid was collected by filtration and washed (ice H_2O) to afford 1.8 g (90%). An analytical sample of the mono HCl salt was prepared by dissolving the free base in absolute EtOH and adding 1 equiv of HCl in absolute EtOH. The precipitate was collected and washed (absolute EtOH) to give white crystals. Physical data for the diaminoquinazolines are shown in Table I.

Acknowledgment. This work was supported by the National Institute of Allergy and Infectious Diseases, National Institutes of Health, under Contract No. NIH-70-2286 and Grant No. AI-10-688.

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Thymidine 5′ Variants as Inhibitors of Thymidylate Kinase

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Received December 10, 1973

Thymidine 5′-triphosphate, the product of the phosphorylation of thymidine 5′-monophosphate by thymidylate kinase, is essential for DNA synthesis. In a search for inhibitors of DNA synthesis *in vivo*, which would, unlike nucleotide analogs themselves, be capable of penetrating cell membranes, the synthesis of thymidine analogs not containing the phosphate moiety was undertaken. Recently the inhibition of thymidylate kinase by 5′-deoxy-5′-fluorothymidine has been demonstrated.[1] In this note the preparation of two novel series of 5′-thymidine derivatives is described and their activity against thymidylate kinase, compared with **1**, is reported.

In the first series the readily available carboxylic acid **2**[2] was converted to the esters **3a–d.** Treatment of **3a** with *d* 0.88 ammonia gave the amide **4.** Acetylation gave **5,** which was smoothly dehydrated with phosphorus oxychloride in methylene dichloride to the nitrile **6.** Deacetylation gave the nitrile **7,** which was cyclized by treatment with ammonium azide in DMF to the tetrazole **8.**

The chemical shift values of the H-4′ proton of each member of the above series lay in the expected electronegativity order for each different functional group at the 4′ position of the carbohydrate moiety. For instance, the H-4′ proton in the tetrazole **8** was at lowest field (δ 5.14) followed by that in the nitrile **7** (δ 4.65) and in the amide **4** (δ 4.20). This empirical observation was maintained in other similar series of 5′ variants of nucleosides described elsewhere.†

In the second series, 5′-azido-5′-deoxythymidine[3] (**9**) was cyclized with dimethylacetylene dicarboxylate to give the triazole **10** in high yield. This was converted by direct treatment with *d* 0.88 ammonia, *n*-propylamine, ethanolamine, and benzylamine to the bisamides **11, 12, 13,** and **14,** respectively. Treatment with aqueous hydrazine gave the bishydrazide **15.** Reaction of **9** with ethyl propiolate gave a major triazole isomer **16a.** The minor isomer **16b** was isolated in a very low yield by preparative tlc. The structures of the two isomers were established by comparison of the chemical shift values for the triazole ring protons with those available from a known pair of 4- and 5-phenyl-1,2,3-triazolylglucose derivatives.[4] In the case of the 4-phenyl isomer the H-5 proton occurred at δ 8.01 and for the 5-phenyl isomer the H-4 proton occurred at δ 7.70. In **16a** the ring proton was at lowest field δ 8.77 compared with δ 8.24 for the minor isomer. Thus the structure of **16a** was assigned as the 4-ethoxycarbonyl isomer and the minor isomer **16b** was considered to be 5′-(5-ethoxycarbonyl-1,2,3-triazol-1-yl)-5′-deoxythymidine. **16a** was converted directly to **17** and **18** with aqueous base and *d* 0.88 ammonia, respectively. Acetylation of **18** gave **19,** which was dehydrated with phosphorus oxychloride to the protected nitrile **20.** Deacetylation gave the nitrile **21** (Chart I).

Table I. Per Cent Inhibition of Thymidylate Kinase

Compd no.	Substrate concn, m*M*			
	1	0.5	0.25	0.1
1	95	87	83	43
2	71, 59	10		
3b	61, 40	6		
3c	41, 32	0		
3d	0, 13			
4	81, 85	47	38	16
5	68, 75	49	28	0
7	97, 95	84	80	62
8	76	58	43	23
10	14, 4			
11	1, 17			
16a	19, 5			
17	0			
18	0			
21	0			

The percentage inhibition by the analogs is given in Table I from which it can be seen that the only compound with comparable activity to 5′-deoxy-5′-fluorothymidine (**1**) is the nitrile **7.** The tetrazole **8** and the amides **4** and **5** are also active, but less so. The acid **2** has only slight activity which is diminished by esterification and the triazoles **10, 11, 16a, 17, 18,** and **21** are inactive. None of the analogs exhibited significant activity *in vivo* in the inhibition of mitosis in cultured mouse fibroblasts or in the inhibition of growth of herpes simplex type I (a DNA virus). The nitrile **7** was also tested against the virus in combination with 5-iododeoxyuridine, arabinosylcytidine, and aphidicolin[5] and no synergism was found. This lack of activity may have been due to the degradation of the analogs by intracellular nucleosidases.

Experimental Section

Melting points were determined by use of a Büchi melting point apparatus and were uncorrected. Evaporations under reduced pressure were carried out with the aid of a Rotavapor R (Büchi, Switzerland) at 30° (24 mm) unless otherwise stated. Column chromatography was carried out on silicic acid (Kieselguhr 7734, mesh 70–200, Merck). Solvents used in the chromatographic procedures are designated as follows: solvent 1, chloroform–ethanol (19:1 v/v); solvent 2, acetonitrile–water (22:3 v/v). Nuclear magnetic resonance spectra were recorded in DMSO-d_6 by use of a Varian HA100 spectrometer. Ultraviolet spectra were recorded in spectroscopic ethanol by use of a Varian Cary 16 uv spectrometer.

General Procedure. Methyl 1′-Thymin-1-yl-2′-deoxy-β-D-ri-

†J. J. Baker, A. M. Mian, and J. R. Tittensor, manuscript in preparation.